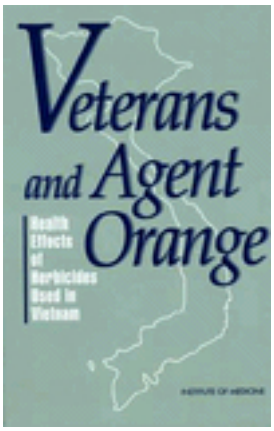

Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam

Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides, Institute of Medicine

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Veterans and Agent Orange

Health Effects of Herbicides Used in Vietnam

Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides
Division of Health Promotion and Disease Prevention
INSTITUTE OF MEDICINE

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The Institute of Medicine was chartered in 1970 by the National Academy of Sciences to enlist distinguished members of the appropriate professions in the examination of policy matters pertaining to the health of the public. In this, the Institute acts under the Academy's 1863 congressional charter responsibility to be an adviser to the federal government and its own initiative in identifying issues of medical care, research, and education. Dr. Kenneth I. Shine is president of the Institute of Medicine.

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The image adopted as a logo-type by the Institute of Medicine is based on a relief carving from ancient Greece, now held by the Staatlichemusseen in Berlin.

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Foreword

In response to decades of concern surrounding the possible long-term health consequences of exposures to herbicides and the contaminant dioxin, Congress directed the Secretary of Veterans Affairs, in Public Law 102-4 signed on February 6, 1991, to request the National Academy of Sciences (NAS) to conduct a comprehensive review and evaluation of the available scientific and medical information regarding the health effects of exposure to Agent Orange and other herbicides used during the Vietnam conflict. This report from the Institute of Medicine (IOM) Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides is hereby submitted in compliance with Public Law 102-4.

Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam reviews and evaluates the available scientific evidence regarding the association between exposure to dioxin or other chemical compounds in herbicides used in Vietnam and a wide range of health effects, and provides the committee's best assessment of this body of knowledge for the Secretary of Veterans Affairs to consider as the Department of Veterans Affairs exercises its responsibilities to Vietnam veterans. The report also describes areas in which the available scientific data are insufficient to determine whether an association exists and provides the committee's recommendations for areas in which future research is likely to be most productive.

That Congress would ask the NAS—a nongovernmental organization—to conduct this study reflects a time-honored tradition. Created by an act of Congress and signed into law in 1863 by President Abraham Lincoln, the NAS is dedicated to the furtherance of science and technology and to their

use for the promotion of general public welfare. A private, nonprofit society of distinguished scholars engaged in scientific and engineering research, the NAS has a mandate to advise the federal government on scientific and technical issues of pressing importance. Its members, drawn from universities and the private sector, are elected by their peers on the basis of exemplary professional achievement. Members, along with other leading experts, voluntarily participate in National Research Council and IOM studies and serve without compensation.

The IOM was chartered by the NAS in 1970 to serve as an adviser to the federal government on issues that affect the public's health, as well as to act independently in identifying important issues of medical care, research, and education. The IOM brings to this mission more than two decades of experience in conducting independent analyses of pressing health problems that involve federal policy decisions.

As described in more detail in Chapter 2 of this report, the NAS has a history of involvement with the Agent Orange issue. A major study in 1974 focused primarily on the possible ecological consequences of herbicides used in Vietnam, but an individually authored component of that report published eight years later reviewed its possible reproductive effects among the Vietnamese. In the early 1980s, two committees reviewed protocols for large, epidemiologic studies of the health effects in veterans. Between 1986 and 1990, an IOM committee reviewed protocols and the analytical methods of a series of epidemiologic studies of Vietnam veterans carried out by the Centers for Disease Control, though it did not contribute to the final conclusions reached in those studies. Thus, while the NAS and the IOM have been aware of the controversy surrounding the military use of Agent Orange and other herbicides in Vietnam, these past activities are quite different from the current study, of which the primary purpose is to determine whether there are health effects related to exposure to herbicides.

The 16 members of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides represent a wide range of expertise including occupational and environmental medicine, toxicology, epidemiology, pathology, clinical oncology, psychology, neurology, and biostatistics. The committee was chaired by Harold Fallon, M.D., Dean of the University of Alabama Medical School, Birmingham, and a member of the IOM. David Tollerud, M.D., M.P.H., Director of Occupational and Environmental Medicine at the University of Pittsburgh, served as vice-chair. Committee member Norman Breslow, Professor of the Department of Biostatistics of the University of Washington and also a member of the IOM, served as a liaison to the IOM Board on Health Promotion and Disease Prevention, which was responsible for overseeing this study. Biographical sketches of the other committee members and the professional staff appear in Appendix D.

All committee members were selected because they are leading authorities in their scientific fields, are well-respected by their colleagues and peers, have no conflicts of interest with regard to the matters under study, and, indeed, have taken no public positions concerning the potential health effects of herbicides in Vietnam veterans or related aspects of herbicide or dioxin exposure. The committee thus has provided a fresh analysis of this issue—which is both scientifically complex and emotionally charged—and this report reflects the committee's thorough and unbiased scientific judgments. As with all reports from the IOM, the committee's work was reviewed by an independent panel of distinguished experts.

Kenneth I. Shine
President, Institute of Medicine

Preface

The use of Agent Orange and other herbicides in Vietnam has stimulated concern and controversy ever since the U.S. began the military herbicide program in 1962. Questions regarding the effects of herbicides on health and the environment have persisted over several decades. Many veterans, who served their country in Vietnam at great personal sacrifice and hardship, face continuing uncertainty about whether a myriad of diseases and health effects are associated with exposure to the herbicides used in Vietnam. Some of these veterans and their families feel that their pain and suffering have been ignored and that these questions have not been adequately addressed.

In response to the concerns voiced by Vietnam veterans and their families, Congress called upon the National Academy of Sciences (NAS) to review the scientific evidence on the possible health effects of exposure to herbicides. The creation of the NAS Institute of Medicine's committee underscores the critical importance of approaching these questions from a scientific standpoint, yet the committee realized from the beginning that it could not conduct a credible scientific review without a full understanding of the experiences and perspectives of veterans. Thus, to supplement its standard scientific process, the committee opened several of its meetings to the public to allow veterans and other interested individuals to voice their concerns and opinions, to provide personal information about individual exposure to herbicides and associated health effects, and to educate the committee on recent research results and studies still under way. This information provided a meaningful backdrop for the numerous scientific

articles that the committee reviewed and evaluated. The committee appreciates the efforts of everyone who presented information to it and acknowledges this valuable addition to the study process.

As the study progressed, two separate but interdependent themes became evident to the committee. First, this report is a scientific investigation of the potential health effects of exposure to the herbicides that were used in Vietnam and to dioxin (2,3,7,8-tetrachlorodibenzo-*para*-dioxin; TCDD), an unintentional contaminant of some of those herbicides. This theme is discussed first in Chapter 2, which provides a context for the investigation by relating the history of national concern about TCDD and herbicides and of efforts to address this concern. Chapter 4 reviews the toxicological data (based on laboratory studies and animal investigations) on these chemicals, with a focus on the TCDD contaminant because this area has been the object of more substantial scientific research. Most of the committee's work, however, focused on the review of epidemiologic studies. Chapters 8 through 11 analyze and present the committee's conclusions regarding the relationship between herbicide/TCDD exposure and 44 specific diseases and disorders. These diseases and disorders include different forms of cancer, reproductive and developmental effects, neurobehavioral disorders, and other health effects, including chloracne and porphyria cutanea tarda. In order to understand the committee's approach to these reviews, Chapter 5 lays out the general methodological considerations that the committee used in evaluating this evidence, and Chapter 6 addresses the question of how to assess the nature of exposure to the substances in question, a critical element in evaluating the epidemiologic studies that were reviewed. Many of these studies addressed the health effects of people who were occupationally or environmentally exposed to TCDD or the herbicides in question, and many of the studies investigated more than one health outcome. Rather than summarize the methods of these studies each time they are considered, the committee's review and summary of the health effects are preceded by a complete and thorough methodologic description of all the studies under review—organized by the nature of the population exposed and by study methods—in Chapter 7.

The second theme in this report relates to the use of herbicides in Vietnam, the effects of exposure on Vietnam veterans, and the direction of future research efforts toward learning more than is currently known about these issues. The discussion of this theme also begins in Chapter 2, but the history of military operations in Vietnam, with a special focus on the herbicide program, is described in detail in Chapter 3. In addition to addressing exposure assessment in general, Chapter 6 discusses the methods that have been used to assess exposure to herbicides in studies of Vietnam veterans and summarizes what is currently known about the nature and extent of that exposure. Chapter 6 also proposes a new method of

historical exposure reconstruction in studies of Vietnam veterans, a topic that is the focus for the committee's research recommendations in Chapter 12. In addition, Chapter 12 comments on existing studies of Vietnam veterans and makes recommendations about four specific programs mandated in Public Law 102-4.

CONDUCT OF THE STUDY

The committee worked on several fronts in conducting this study, always with the goal of seeking the most accurate information and advice from the widest possible range of knowledgeable sources. Consistent with procedures of the Institute of Medicine (IOM), the committee met in a series of closed sessions and working group meetings in which members could freely examine, characterize, and weigh the strengths and limitations of the available evidence. Given the nature of the controversy surrounding this issue, the committee deemed it vital to convene open meetings as well. Three public meetings were held during the course of the study, which provided timely forums for veterans and veterans service organizations, researchers, policymakers, and other interested parties to present their concerns, review their research, and exchange information directly with the committee members.

The first open meeting was held in September 1992. To solicit broad participation, the IOM committee sent announcements to nearly 1,000 persons known to have an interest in this issue. Names were gathered from veterans service organizations, scientific organizations, labor unions, environmental groups, government agencies, and numerous other sources, and news of the meeting was circulated to approximately 1,500 media outlets nationwide. During this day-long public meeting, 25 persons made oral presentations. Because some individuals were unable to attend the public meeting, written statements were given equal weight to oral presentations; by April 15, 1993, 28 additional individuals had submitted written statements. Besides these statements, the committee received specially prepared analyses from several groups. All of this material was carefully considered by the committee over the course of the study. The oral presentations and written statements submitted to the committee are described in detail in Appendix B.

The second public meeting, a "Scientific Workshop on Exposure Assessment," took place in December 1992. The committee assembled 17 experts in various scientific fields—drawn from universities, veterans service organizations, federal agencies, and health groups—to discuss how exposure to Agent Orange, other herbicides, and TCDD is assessed in epidemiologic studies. Participants discussed records-based methods, as well as more recent biomedical research in which current dioxin levels are

measured in the blood and tissue of individuals to estimate previous levels of exposure to TCDD (see Appendix B).

A third public meeting, held in February 1993, focused on the "Vietnam Experience." The committee heard from veterans who had served in the U.S. Marines, Navy, Army, and Air Force. Some of these individuals experienced extensive combat, frequently in areas sprayed with herbicides. Others had been directly involved in spraying herbicides from aircraft or "brown water" river patrol boats. The committee also heard from the Vietnam Veterans of America on the wartime experiences of women, thousands of whom served in Vietnam, primarily as military nurses.

In addition to its formal meetings, the committee actively and continuously sought information from, and explained its mission to, a broad array of individuals and organizations with interest or expertise in assessing the effects of exposure to herbicides. These interactions included frequent meetings with representatives of veterans service organizations, congressional committees, federal agencies, and scientific organizations. The committee heard from the public through several hundred telephone calls and letters, each of which received a response from the IOM staff.

The committee also benefited from the expert advice and reviews of consultants in toxicology, environmental health, neurotoxicology, autoimmune disorders, reproductive effects, and dermatological disorders, including porphyria cutanea tarda. A list of the background papers, their authors, and the experts consulted appears in Appendix B.

During the course of the committee's work, the Environmental Protection Agency (EPA) has been in the process of carrying out an open scientific reassessment of the health risks of dioxin to guide its regulatory policy. The committee has benefited from this effort by being able to read and consider draft scientific reports prepared for the EPA by independent scientists as part of this process, and by IOM committee members' and staff's attendance at the EPA's public meetings. However, the congressional charge to the IOM committee is substantially different than the EPA's review in at least two important ways: (1) the EPA is concerned only with dioxin, whereas the IOM is concerned with all of the herbicides used in Vietnam, and (2) because of its regulatory focus, the EPA is more concerned with defining a dose-response relationship than the IOM committee felt was either necessary or feasible for Vietnam veterans.

The value of this continued, open, and wide-ranging dialogue between the IOM committee and the scientific community, veterans, policymakers, and citizens proved itself many times over and ultimately contributed to a more comprehensive report.

Most of the committee's work involved reviewing the scientific literature bearing on the association between herbicides or dioxin and various health outcomes. The committee or its staff read approximately 6,420 abstracts

of scientific or medical articles which were then entered into a computerized bibliographic data base. From these, approximately 230 epidemiologic studies were chosen for detailed review and analysis. These included studies of people exposed to the herbicides in question in occupational and environmental settings, as well as studies of Vietnam veterans. The committee relied on the original publications themselves rather than on summaries or commentaries. Such secondary sources were used to check the completeness of the review. The committee also reviewed the primary and secondary literature on basic toxicological and animal studies related to dioxin and the herbicides in question. Appendix A describes the committee's literature review strategy in detail.

Controversy has surrounded the study of Agent Orange since the first questions of herbicide-related health effects in Vietnam veterans were raised more than 20 years ago. In the course of its work, the committee heard allegations of scientific misconduct and claims of a government conspiracy to suppress information on health effects, as well as serious disagreements among scientists about the interpretation of laboratory and clinical data. The committee was not charged with investigating or resolving these controversies, and it did not attempt to do so. The committee took these issues into consideration only to the extent that they had a direct bearing on the scientific results that are the subject of this review.

We believe that the committee has produced a comprehensive, unbiased scientific review of the available evidence regarding potential health effects of exposure to herbicides in Vietnam veterans. Although the conclusions and recommendations presented here will not end the controversy surrounding this issue, it is the committee's hope that this report will crystallize the current scientific information on this important topic and prompt further research to answer the remaining questions being asked by veterans and their families, the Department of Veterans Affairs, and Congress.

The committee wishes to acknowledge that this study could not have been done without the assistance of a number of people, many of whom are listed in Appendix B. A special acknowledgment is extended to Donald Whorton and Albert Munson, both of whom served for a brief period with the committee. The work of the Institute of Medicine staff deserves high praise. Thanks are extended to the professional staff, Susan Rogers, Diane Mundt, Cynthia Abel, Catharyn Liverman, Gail Charnely, and Jane Durch, for their input, advice, and support. Thanks are also extended to Catherine Wesner, the study's project assistant, who planned travel and meeting arrangements and provided assistance with editorial changes to the manuscript; Jana Katz, the committee's student intern, who assisted with literature searches and in compiling the literature data base; Thomas Burroughs, who worked with IOM staff members in drafting several sections of the report; Zoe Schneider who aided in the preparation of the final manuscript;

Andrea Posner, who proofread the final changes in the manuscript; and Florence Poillon, who provided excellent editorial skills. Finally, the committee wishes to recognize the major contributions of the study director, Michael Stoto. It is through his expert leadership that this report has come to fruition.

Harold Fallon, *Chairman*

David Tollerud, *Vice-chairman*

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Veterans and Agent Orange

1

Executive Summary

Between 1962 and 1971, U.S. military forces sprayed nearly 19 million gallons of herbicides over approximately 3.6 million acres in Vietnam. The preparation known as Agent Orange accounted for approximately 11.2 million gallons of the total amount sprayed. Herbicides were used to strip the think jungle canopy that helped conceal opposition forces, to destroy crops that enemy forces might depend upon, and to clear tall grass and bushes from around the perimeters of U.S. base camps and outlying fire support bases. Most large-scale spraying operations were conducted using airplanes and helicopters, but considerable quantities of herbicides were sprayed from boats and ground vehicles, as well as by soldiers wearing back-mounted equipment. Spraying began in 1962 and increased greatly in 1967. After a scientific report in 1969 concluded that one of the primary chemicals used in Agent Orange, namely, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) could cause birth defects in laboratory animals, U.S. forces suspended use of this herbicide in 1970 and halted all herbicide spraying in Vietnam the next year.

As the decade wore on, concern about possible long-term health consequences of Agent Orange and other herbicides heightened, fueled in particular by reports from growing numbers of Vietnam veterans that they had developed cancer or fathered handicapped children, which they attributed to wartime exposure to the herbicides. Along with the concerns of Vietnam veterans, public awareness increased because of reports of health concerns surrounding occupational and environmental exposure to dioxin—more specifically, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), informally known

as TCDD—a contaminant of 2,4,5-T. Thousands of scientific studies have since been conducted, numerous government hearings have been held, and veterans organizations have pressed for conclusive answers, but the question of the health effects of herbicide exposure in Vietnam remains shrouded in controversy and mistrust. Indeed some veterans organizations, researchers, and public interest organizations remain skeptical that the issue has received full and impartial consideration by the Department of Veterans Affairs (DVA; formerly the Veterans Administration) and other federal agencies.

Faced with this lingering uncertainty and demands that the concerns of veterans be adequately addressed, the U.S. Congress passed Public Law 102-4, the "Agent Orange Act of 1991." This legislation directed the Secretary of Veterans Affairs to request that the National Academy of Sciences conduct a comprehensive review and evaluation of available scientific and medical information regarding the health effects of exposure to Agent Orange, other herbicides used in Vietnam, and their components, including dioxin.

In February 1992, the Institute of Medicine (IOM) of the National Academy of Sciences signed an agreement with the DVA to review and summarize the strength of the scientific evidence concerning the association between herbicide exposure during Vietnam service and each disease or condition suspected to be associated with such exposure. The IOM was also asked to make recommendations concerning the need, if any, for additional scientific studies to resolve areas of continuing scientific uncertainty and to comment on four particular programs mandated in Public Law 102-4.

To carry out the study, the IOM established the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. In conducting its study, the committee operated independently of the DVA and other government agencies. The committee was not asked to and did not make judgments regarding specific cases in which individual Vietnam veterans have claimed injury from herbicide exposure; this was not part of its congressional charge. Rather, the study provides scientific information for the Secretary of Veterans Affairs to consider as the DVA exercises its responsibilities to Vietnam veterans.

ORGANIZATION AND FRAMEWORK

The framework for this report reflects the size and complexity of the committee's task. The committee felt that an evaluation of the health effects of exposure to herbicides in Vietnam veterans would not be complete without a historical review of the Agent Orange controversy. The report begins in Chapter 2 by tracing more than two decades of public concern about the military use of herbicides during the war in Vietnam, in addition to public concern over various environmental and occupational exposures to

herbicides and dioxin that arose in parallel to veterans' concerns, and describes federal and state responses to this national dilemma.

Chapter 3 provides background information on the nature and extent of potential exposure of Vietnam veterans to herbicides, based on information about the military herbicide program. Some 3 million military personnel served in or near Vietnam, and as one historian notes, "there was no 'typical' U.S. soldier in Vietnam … Americans who served there went through many varied experiences—partly because the quality of the war varied in different areas of the country, and partly because the nature changed over time" (Karnow, 1991). Individual experiences also varied by branch of service, military occupation, rank, and type of military unit. As reflected in military records, the use of herbicides was varied as well. Starting in 1962 and peaking in the late 1960s, seven different herbicide formulations were used in varying quantities for a variety of purposes in different parts of the country; approximately 65 percent of these herbicides were contaminated by TCDD, in varying concentrations. Aerial spraying of herbicides by Operation Ranch Hand accounted for approximately 86 percent of all spraying and was well documented; other spraying by helicopters and from trucks or backpacks was poorly documented.

Chapter 4 provides toxicological background on the biologic plausibility of health effects that may occur in humans after accidental or occupational exposure to herbicides and TCDD components. This chapter describes the biological and chemical properties of the compounds in question as determined by basic research and animal studies. TCDD administered to laboratory animals interacts with an intracellular protein called the Ah receptor. This interaction appears to play a role in a number of health effects observed in animals. Because humans also have intracellular proteins that have been identified as Ah receptors, it is plausible that interactions between TCDD and these receptors could play a role in human health effects. In contrast to TCDD, the effects of the herbicides do not appear to be mediated through interactions with intracellular receptors. TCDD has also been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence in animals of, or mechanistic basis for, carcinogenicity or other health effects of any of the herbicides, although they have not been studied as extensively as TCDD.

In fulfilling its charge of judging whether each of a set of human health effects is associated with exposure to herbicides or dioxin, most of the committee's efforts concentrated on reviewing and interpreting epidemiologic

studies. The committee began its evaluation presuming neither the existence nor the absence of association. It has sought to characterize and weigh the strengths and limitations of the available evidence. These judgments have both quantitative and qualitative aspects. They reflect the nature of the exposures, health outcomes, and populations at issue; the characteristics of the evidence examined; and the approach taken to evaluate that evidence. To facilitate independent assessment of the committee's conclusions, Chapter 5 describes as explicitly as possible the methodological considerations that guided the committee's review and its process of evaluation.

In reviewing the literature, the committee discerned that the existing epidemiologic data base is severely lacking in quantitative measures of individual exposure to herbicides and dioxin. Assessment of the intensity and duration of individual exposures is a key component in determining whether specific health outcomes are associated with exposure to dioxin or other chemicals found in the herbicides used in Vietnam. Although different approaches have been used to estimate exposure in Vietnam veterans and in others exposed occupationally or environmentally, each approach is limited in its ability to determine precisely the degree and level of individual exposure. The problems associated with each of these approaches are discussed in detail in Chapter 6. New biochemical techniques that can detect small amounts of TCDD in the blood many years after exposure have some merit, especially for detecting *group* differences. However, because of common background exposure of all Americans to TCDD, poorly understood variations among individuals in TCDD metabolism, and relatively large measurement errors, *individual* TCDD serum levels are usually not meaningful. Furthermore, because not all herbicides used in Vietnam contained TCDD, serum TCDD levels are not good indicators of overall exposure to herbicides. Chloracne has been used in epidemiologic studies as a biomarker for TCDD exposure, but the data indicate that it is neither sensitive nor specific. It is usually not long lasting, is difficult to diagnose, and is not at all sensitive to exposure to herbicides that are not contaminated with TCDD.

Although definitive data are lacking, the available quantitative and qualitative evidence about herbicide exposure summarized in Chapter 6 suggests that Vietnam veterans as a group had substantially lower exposure to herbicides and dioxin than the subjects in many occupational studies. The participants in Operation Ranch Hand are an exception to this pattern, and it is likely that others among the approximately 3 million men and woman who served in Vietnam were exposed to herbicides at levels associated with health effects. Thus, in the committee's judgment, a sufficiently large range of exposures may exist among Vietnam veterans to conduct a valid epidemiologic study for certain health outcomes (see research recommendations below).

Due, in part, to the uncertain validity of exposure measurements in

many of the studies of veterans, the committee decided to review studies of other groups potentially exposed to the herbicides used in Vietnam and TCDD, especially phenoxy herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T, chlorophenols, and other compounds. These groups include chemical production and agricultural workers, residents of Vietnam, and people exposed heavily to herbicides or dioxins as a result of residing near the site of an industrial accident. The committee felt that considering studies of other groups could help address the issue of whether these compounds might be associated with particular health outcomes, even though these results would have only an indirect bearing on the increased risk of disease in veterans themselves. Some of these studies, especially those of workers in chemical production plants, provide stronger evidence about health effects than studies of veterans because exposure was generally more easily quantified and measured. Furthermore, the general level and duration of exposure to the chemicals were greater and the studies were of sufficient size to examine the health risks among those with varying levels of exposure.

Because the committee relied on many of the same epidemiologic studies when assessing potential associations with various health effects, Chapter 7 provides a framework for the methods used in the epidemiologic studies on which the committee based its report. The nature of the exposure to herbicides and herbicide components varied substantially for each; therefore, both the organization of the chapter (which is structured to reflect similarities and differences in the populations studied) and the methodologic issues that are summarized for each study emphasize exposure.

CONCLUSIONS ABOUT HEALTH OUTCOMES

Chapters 8 through 11 provide a detailed review of the epidemiologic studies evaluated by the committee and their implications for cancer, reproductive, neurobehavioral, and other health effects. The committee's specific mandate was to determine, if possible,

1. whether there is a statistical association between the suspect diseases and herbicide use, taking into account the strength of the scientific evidence and the appropriateness of the methods used to detect the association;
2. the increased risk of disease among individuals exposed to herbicides during service in Vietnam; and
3. whether there is a plausible biologic mechanism or other evidence of a causal relationship between herbicide exposure and a disease.

As detailed in Chapter 5, the committee addressed the first part of this charge by assigning each of the health outcomes under study into one of the four categories listed in Table 1-1 on the basis of the epidemiologic evidence

TABLE 1-1 Summary of Findings in Occupational, Environmental, and Veterans Studies Regarding the Association Between Specific Health Problems and Exposure to Herbicides

Sufficient Evidence of an Association

Evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between herbicides and the outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example, if several small studies that are free from bias and confounding show an association that is consistent in magnitude and direction, there may be sufficient evidence for an association. There is sufficient evidence of an association between exposure to herbicides and the following health outcomes:

Soft tissue sarcoma
Non-Hodgkin's lymphoma
Hodgkin's disease
Chloracne
Porphyria cutanea tarda (in genetically susceptible individuals)

Limited/Suggestive Evidence of an Association

Evidence is suggestive of an association between herbicides and the outcome but is limited because chance, bias, and confounding could not be ruled out with confidence. For example, at least one high-quality study shows a positive association, but the results of other studies are inconsistent. There is limited/ suggestive evidence of an association between exposure to herbicides and the following health outcomes:

Respiratory cancers (lung, larynx, trachea)
Prostate cancer
Multiple myeloma

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association. For example, studies fail to control for confounding, have inadequate exposure assessment, or fail to address latency. There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides and the following health outcomes:

Hepatobiliary cancers
Nasal/nasopharyngeal cancer
Bone cancer
Female reproductive cancers (cervical, uterine, ovarian) and breast cancer
Renal cancer
Testicular cancer
Leukemia
Spontaneous abortion
Birth defects
Neonatal/infant death and stillbirths
Low birthweight
Childhood cancer in offspring

Inadequate/Insufficient Evidence to Determine Whether an Association Exists—*continued*

Abnormal sperm parameters and infertility
Cognitive and neuropsychiatric disorders
Motor/coordination dysfunction
Peripheral nervous system disorders
Metabolic and digestive disorders (diabetes, changes in liver enzymes, lipid abnormalities, ulcers)
Immune system disorders (immune modulation and autoimmunity)
Circulatory disorders
Respiratory disorders

Limited/Suggestive Evidence of No Association

Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter, are mutually consistent in not showing a positive association between exposure to herbicides and the outcome at any level of exposure. A conclusion of "no association" is inevitably limited to the conditions, level of exposure, and length of observation covered by the available studies. *In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.* There is limited/suggestive evidence of no association between exposure to herbicides and the following health outcomes:

Skin cancer
Gastrointestinal tumors (stomach cancer, pancreatic cancer, colon cancer, rectal cancer)
Bladder cancer
Brain tumors

NOTE: "Herbicides" refers to the major herbicides used in Vietnam: 2,4-D (2,4-dichlorophenoxyacetic acid); 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and its contaminant TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin); cacodylic acid; and picloram. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

that it reviewed. The specific rationale for each of the findings summarized in this table is given in Chapters 8 through 11. The second part of the charge is addressed at the end of this section. The committee's response to the third part of the charge is summarized in general terms in Chapter 4, and specific findings for each health outcome are also given in Chapters 8 through 11.

The definitions of the categories and the criteria for assigning a particular health outcome to them are described in Table 1-1. Consistent with the charge to the Secretary of Veterans Affairs in Public Law 102-4, the distinctions between categories are based on "statistical association," not on causality, as is common in scientific reviews. The committee was charged with reviewing the scientific evidence, rather than making recommendations regarding

DVA policy, and Table 1-1 is not intended to imply or suggest any policy decisions, which must rest with the Secretary.

Health Outcomes with Sufficient Evidence of an Association

The committee found sufficient evidence of an association with herbicides and/or TCDD for three cancers: soft tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease. For cancers in this category, a positive association between herbicides and the outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. The committee regards evidence from several small studies that are free from bias and confounding, and show an association that is consistent in magnitude and direction, as sufficient evidence for an association.

Soft tissue sarcomas (STSs) are a rare but diverse group of tumors that share a common International Classification of Diseases code but have a wide variety of forms and causes. The strongest evidence for an association between STS and exposure to phenoxy herbicides comes from a series of case-control studies involving a total of 506 cases conducted by Hardell and colleagues in Sweden (Hardell and Sandstrom, 1979; Eriksson et al., 1981; Hardell and Eriksson, 1988; Eriksson et al., 1990; Wingren et al., 1990) that show an association between STS and exposure to phenoxy herbicides, chlorophenols, or both. Although these studies have been criticized, the committee feels that there is insufficient justification to discount the consistent pattern of elevated risks, and the clearly described and sound methods employed. These findings are supported by a significantly increased risk in the National Institute for Occupational Safety and Health (NIOSH) study (SMR = 9.2, CI 1.9-27.0) for the production workers most highly exposed to TCDD (Fingerhut et al., 1991), and a similar increased risk in the International Agency for Research on Cancer (IARC) cohort (SMR = 6.1, CI 1.7-15.5) for deaths that occurred between 10 and 19 years after the first exposure (Saracci et al., 1991). These are the two largest, as well as the most highly exposed occupational cohorts. Some studies in other occupational, environmental, and veterans groups showed an increased risk for STS, but the results were commonly nonsignificant possibly because of small sample sizes related to the relative rarity of STS in the population. Because of difficulties in diagnosing this group of tumors, the epidemiologic studies reviewed by the committee were inconsistent with regard to the specific types of tumors included in the analyses. The available data did not permit the committee to determine whether specific forms of STS were or were not associated with TCDD and/or herbicides. Therefore, the committee's findings relate to the class as a whole.

Non-Hodgkin's lymphoma (NHL) includes a group of malignant lymphomas, that is, neoplasms derived from lymphoreticular cells in lymph nodes, bone

marrow, spleen, liver, or other sites in the body. One large, well-conducted case-control study in Sweden by Hardell and colleagues (1981) examined NHL and Hodgkin's disease together and found an odds ratio of 6.0 (CI 3.7-9.7) based on 105 cases for exposure to phenoxy acids or chlorophenols, and these results held up under further investigation of the validity of exposure assessment and other potential biases (Hardell, 1981). A more recent case-control study by Persson and colleagues (1989) showed increased risk for NHL in those exposed to phenoxy acids (OR = 4.9, CI 1.0-27.0), based on a logistic regression analysis of 106 cases. Other studies of farmers and agricultural workers are generally positive for an association between NHL and herbicides/TCDD; however, only some are significant. All of the studies of U.S. agricultural workers reviewed showed elevated relative risks (although none were significant), and two National Cancer Institute (NCI) studies of farmers in Kansas and Nebraska (Hoar et al., 1986; Zahm et al., 1990) show patterns of increased risk linked to use of 2,4-D. The Centers for Disease Control (CDC) Selected Cancers Study found an increased risk of NHL in association with service in Vietnam; other studies of veterans, generally with small sample sizes, are consistent with an association. In contrast, studies of production workers, including the largest, most heavily exposed cohorts (Fingerhut et al., 1991; Saracci et al., 1991; Zober et al., 1990; Manz et al., 1991) indicate no increased risk. Thus, unlike most of the other cancers studied by the committee for which the data do not distinguish between the effects of herbicides and TCDD, the available epidemiologic data suggest that the phenoxy herbicides, including 2,4-D, rather than TCDD may be associated with non-Hodgkin's lymphoma.

Hodgkin's disease (HD), also a malignant lymphoma, is a neoplastic disease characterized by progressive anemia and enlargement of lymph nodes, spleen, and liver. Fewer studies have been conducted of HD in relation to exposure to herbicides or TCDD than have been conducted of STS or NHL, but the pattern of results is strikingly consistent. The 60 HD cases in the study by Hardell and colleagues (1981) were later examined by Hardell and Bengtsson (1983), who found odds ratios of 2.4 (CI 0.9-6.5) for low-grade exposure to chlorophenols and 6.5 (CI 2.7-19.0) for high-grade exposures. Persson and colleagues' study (1989) of 54 HD cases showed a large, but not statistically significant, OR = 3.8 (CI 0.5-35.2) for exposure to phenoxy acids. Furthermore, nearly all of the 13 case-control and occupational cohort studies show increased risk for HD, although only a few of these results are statistically significant. As with NHL, even the largest studies of production workers exposed to TCDD do not indicate an increased risk. The few studies of HD in Vietnam veterans tend to show elevated risks; all but one are not statistically significant.

When these three cancers (STS, NHL, and HD) are considered as a whole, it is noteworthy that the strongest evidence for an association with

exposure to phenoxy herbicides is the series of case-control studies conducted by Hardell and colleagues and the cohort studies of herbicide applicators and agricultural workers. Studies in other countries are sometimes positive, but not as consistently. Whether this reflects higher typical exposure levels in workers in the countries studied, genetic differences in susceptibility to these diseases, the fact that more intensive studies have taken place, or other risk factors is not known. With regard to STS, the study of Woods and colleagues (1987) suggests that both exposure levels and genetic differences are at play. However, although there may be differences from population to population in the increased risk associated with exposure to herbicides and TCDD, the committee regards the available evidence as sufficient to indicate that there is a statistical association between the herbicides used in Vietnam and STS, NHL, and HD.

The other two health outcomes for which the committee found sufficient evidence of an association with herbicides or TCDD are both skin conditions (see Chapter 11). Chloracne is a specific acne-like skin disorder characterized by exposure to TCDD or related chemicals (but not herbicides). Porphyria cutanea tarda (PCT), which is characterized by thinning and blistering of the skin in sun-exposed areas, is an uncommon disease in which porphyrins are abnormally metabolized. Only genetically predisposed individuals have been shown to develop PCT after TCDD exposure. Both chloracne and PCT have been shown in animal and human studies to be associated with TCDD per se. The clinical evidence for these conditions suggests that onset occurs soon after exposure to TCDD; however, the conditions subside (although perhaps slowly) after exposure ceases.

Health Outcomes with Limited/Suggestive Evidence of an Association

The committee found limited/suggestive evidence of an association for three other cancers: respiratory cancers, prostate cancer, and multiple myeloma. For outcomes in this category, the evidence must be suggestive of an association between herbicides and the outcome, but may be limited because chance, bias, or confounding could not be ruled out with confidence. Typically, at least one high-quality study indicates a positive association, but the results of other studies may be inconsistent.

Among the many epidemiologic studies of respiratory cancers (specifically cancers of the lung, larynx, and trachea), positive associations were found consistently only in those studies in which TCDD or herbicide exposures were probably high and prolonged, especially the largest, most heavily exposed cohorts of chemical production workers exposed to TCDD (Zober et al., 1990; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991) and herbicide applicators (Axelson and Sundell, 1974; Riihimaki et al.,

1982; Blair et al., 1983; Green, 1991). Studies of farmers tended to show a decreased risk of respiratory cancers (perhaps due to lower smoking rates), and studies of Vietnam veterans are inconclusive. The committee felt that the evidence for this association was limited/suggestive rather than sufficient because of the inconsistent pattern of positive findings across populations with various degrees of exposure and because the most important risk factor for respiratory cancers—cigarette smoking—was not fully controlled for or evaluated in all studies.

Several studies have shown elevated risk for prostate cancer in agricultural or forestry workers. In a large cohort study of Canadian farmers (Morrison et al., 1993), an increased risk of prostate cancer was associated with herbicide spraying, and increasing risk was shown with increasing number of acres sprayed. For the entire cohort, the relative risk for prostate cancer and spraying at least 250 acres was 1.2 (CI 1.0-1.5). When the analysis was restricted to the farmers most likely to be exposed to phenoxy herbicides or other herbicides, and those with no employees, no custom workers to do the spraying for them, age between 45-69 years, and $\geq$ 250 acres sprayed, RR = 2.2 (CI 1.3-3.8); the test for trend over increasing number of acres sprayed was significant. The mortality risk was elevated in a study of USDA forest conservationists (PMR = 1.6, CI 0.9-3.0) (Alavanja et al., 1989), and a case-control study of white male Iowans who died of prostate cancer (Burmeister et al., 1983) found a significant association (OR = 1.2) that was not associated with any particular agricultural practice. These results are strengthened by a consistent pattern of nonsignificant elevated risks in studies of chemical production workers in the United States and other countries, agricultural workers, pesticide applicators, paper and pulp workers, and the Seveso population. Studies of prostate cancer among Vietnam veterans or following environmental exposures have not consistently shown an association. However, prostate cancer is generally a disease of older men, and the risk among Vietnam veterans would not be detectable in currently published epidemiologic studies. Because there was a strong indication of a dose-response relationship in one study and a consistent positive association in a number of others, the committee felt that the evidence for association with herbicide exposure was limited/suggestive for prostate cancer.

Multiple myeloma (MM), a cancer of specific bone marrow cells, has been less extensively studied than other lymphomas, but a consistent pattern of elevated risks appears in the studies that have been conducted. Ten studies of agricultural and forestry workers provide information on MM risk in relation to herbicide or pesticide exposure. All demonstrated an odds ratio or SMR greater than 1.0; seven did so at a statistically significant level. This finding is made more specific for herbicide exposure by subanalyses in four of these studies (Burmeister et al., 1983; Cantor and Blair, 1984;

Alavanja et al., 1989; Boffetta et al., 1989) that suggest higher risks for those exposed to herbicides, and higher risks for the studies of herbicide applicators (Riikimaki et al., 1983; Swaen et al., 1992). The committee determined that the evidence for this association was limited/suggestive because the individuals in the existing studies—mostly farmers—have, by the nature of their occupation, probably been exposed to a range of potentially carcinogenic agents other than herbicides and TCDD. Multiple myeloma, like non-Hodgkin's lymphoma and Hodgkin's disease for which there is stronger epidemiologic evidence of an association, is derived from lymphoreticular cells, which adds to the biologic plausibility of an association.

Health Outcomes with Limited/Suggestive Evidence of No Association

For a small group of cancers the committee found a sufficient number and variety of well-designed studies to conclude that there is limited/suggestive evidence of no association between these cancers and TCDD or the herbicides under study. This group includes gastrointestinal tumors (colon, rectal, stomach, and pancreatic), skin cancer, brain tumors, and bladder cancer. For outcomes in this category, several adequate studies covering the full range of levels of exposure that human beings are known to encounter are mutually consistent in not showing a positive association between exposure to herbicides and the outcome at any level of exposure, and which have relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, level of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.

The data on colon cancer exemplify the situation that led the committee to say that there was evidence of no association between a cancer and exposure to herbicides and/or TCDD. Colon cancer is relatively common, so an increase in the risk of these cancers would be relatively easy to detect in occupational studies. The epidemiologic studies reviewed by the committee that address colon cancer include a mixture of occupational studies of various types, environmental studies, and studies of Vietnam veterans. Some of the studies such as the NIOSH (Fingerhut et al., 1991) and IARC (Saracci et al., 1991) cohorts are large and have relatively high exposures. The number of studies with estimated relative risks above and below 1.0 are roughly evenly distributed, and a number of studies have tight confidence intervals that include 1.0. The NIOSH study, for instance, based on 25 exposed cases, finds an odds ratio of 1.2 with a 95 percent confidence interval of 0.8 to 1.8. The IARC study finds an odds ratio of 1.1 (CI 0.8-1.5)

based on 41 cases. Thus, this pattern suggests that there is no association between herbicides/TCDD and colon cancer, at least in the situations represented in the available studies.

Health Outcomes with Inadequate/Insufficient Evidence to Determine Whether an Association Exists

The scientific data for the remainder of the cancers and other diseases reviewed by the committee were inadequate or insufficient to determine whether an association exists. For cancers in this category, the available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association. For example, studies fail to control for confounding or have inadequate exposure assessment.

This group includes hepatobiliary cancers, nasal/nasopharyngeal cancer, bone cancer, female reproductive cancers (breast, cervical, uterine, ovarian), renal cancer, testicular cancer, and leukemia. For example, there are relatively few occupational, environmental, or veterans studies of liver cancer, and most of these are small in size and have not controlled for life-style-related risk factors. One of the largest studies (Hardell et al., 1984) indicates an increased risk for liver cancer and exposure to herbicides, but another study of Swedish agricultural workers (Wiklund, 1983) estimates a relative risk that is significantly less than 1.0. The estimated relative risks from other studies are both positive and negative. As a whole, when bearing in mind the methodological difficulties associated with most of the few existing studies, the evidence regarding liver cancer is not convincing about either an association with herbicides/TCDD or the lack of an association.

The epidemiologic evidence for an association between exposure to herbicides and leukemia comes primarily from studies of farmers and residents of Seveso, Italy. The observed overall relative risk for leukemia mortality and incidence in Seveso was elevated, but not significantly. A number of studies of farmers that the committee found convincing for NHL, HD, or MM also show a consistently elevated risk of leukemia, but these results are not necessarily due to herbicide use because confounding exposures were not controlled for adequately in the analyses of these studies and because when farmers are stratified by suspected use of herbicide, the incidence of leukemia is generally not elevated. Some studies of chemical workers found an increased risk of leukemia, but the number of cases was small in all of these studies. The available data on Vietnam veterans are generally not conclusive because the exposure data are inadequate for the cohort being studied. Small sample sizes weaken the studies of the Ranch Hands or Chemical Corps, where excesses are not likely to be detected.

A number of occupational, environmental, and Vietnam veteran studies

were available for assessing the association between herbicide and TCDD exposures and reproductive outcomes. These studies generally reported no association with any of the reproductive outcomes examined by the committee —spontaneous abortion, birth defects, stillbirth, neonatal and infant death, low birthweight, childhood cancer, or altered sperm parameters and infertility. However, given the small sample sizes, the lack of consistent findings, and inadequate exposure classification in most studies, the evidence is considered inadequate for determination of an association.

Studies of neurotoxic effects of herbicides or TCDD were also inadequate for determining whether an association exists between exposures and chronic cognitive or neuropsychiatric disorders, motor/coordination dysfunction, and peripheral nervous system disorders. As a group the studies have not applied uniform operational definitions of neurobehavioral disorders. Information on individual exposure was often inadequate and complicated by exposure to multiple chemicals, and only a limited number of studies provided sufficient comparison group data. Reported abnormalities have ranged from mild and reversible to severe and chronic. While the chances of detecting subtle central nervous system disorders 20 years after exposure are small given the assessment tools currently available, the committee recognized that it may be possible for subtle changes that occurred earlier in life to manifest themselves in later adult life when compounded by the normal aging process. Therefore, while the currently available evidence is insufficient, study of the interactive effects of exposure to herbicides and TCDD with age on neurobehavioral functioning are encouraged. In addition, observations from follow-up of veterans and some environmental studies warrant further investigation of motor/sensory/ coordination problems in exposed persons.

Other health effects examined by the committee for which the evidence was determined to be insufficient included several metabolic and digestive disorders (diabetes, changes in liver enzymes, lipid abnormalities, and gastrointestinal ulcers), immune system disorders, and circulatory and respiratory disorders. Assessment of these disorders in association with herbicides and TCDD involved the medical evaluation of a wide array of critical signs and symptoms, laboratory parameters, and other diagnostic tools. Studies of these health effects were limited by poor exposure measures, generally small sample sizes, and the lack of assessment of independent risk factors for certain outcomes, such as smoking and certain circulatory and respiratory disorders, or alcohol use and ulcers.

Increased Risk in Vietnam Veterans

Although there have been numerous health studies of Vietnam veterans, most have been hampered by relatively poor measures of exposure to herbicides

or TCDD, in addition to other methodological problems. In Table 1-1, most of the evidence on which the findings are based comes from studies of people exposed to dioxin or herbicides in occupational and environmental settings, rather than from studies of Vietnam veterans. The committee found this body of evidence sufficient for reaching the conclusions about statistical associations between herbicides and health outcomes summarized in Table 1-1; however, the lack of adequate data on Vietnam veterans per se complicates the second part of the committee's charge, which is to determine the increased risk of disease among individuals exposed to herbicides during service in Vietnam. To estimate the magnitude of risk for a particular health outcome among herbicide-exposed Vietnam veterans, quantitative information about the dose-time-response relationship for each health outcome in humans, information on the extent of herbicide exposure among Vietnam veterans, and estimates of individual exposure are needed. Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the studies that have been reviewed (Chapters 8-11), the inadequate control for important confounders, and the uncertainty about the nature and magnitude of exposure to herbicides in Vietnam (Chapter 6), none of the ingredients necessary for a quantitative risk assessment are available. Thus, it is not possible for the committee to quantify the degree of risk likely to be experienced by veterans because of their exposure to herbicides in Vietnam. The available quantitative and qualitative evidence about herbicide exposure among various groups studied suggests that Vietnam veterans as a group (except those with documented high exposures, such as participants in Operation Ranch Hand) had lower exposure to herbicides and TCDD than the subjects in many occupational and environmental studies. However, individual veterans who had very high exposures to herbicides could have risks approaching those in the occupational and environmental studies.

RESEARCH RECOMMENDATIONS

The committee was also asked to make recommendations concerning the need, if any, for additional scientific studies to resolve areas of continuing scientific uncertainty concerning the health effects of the herbicides used in Vietnam. Based on its review of the available epidemiologic evidence and a consideration of the quality of exposure information available in existing studies, especially of Vietnam veterans, the committee concluded that a series of epidemiologic studies of veterans could yield valuable information if a new, valid exposure reconstruction model could be developed. The committee also sees value in continuing the existing Ranch Hand study and expanding it to include Army Chemical Corps veterans. The committee's research recommendations emphasize studies of Vietnam veterans, rather

than general toxicologic or epidemiologic studies of occupationally or environmentally exposed populations. A substantial amount of research on the toxicology and epidemiology of herbicides and herbicide components is already under way in the United States and abroad. Indeed, many of the studies on which the committee's conclusions are based have been published since 1991. Although not targeted specifically to Vietnam veterans, it is likely that this research will also contribute to the knowledge of potential health effects in this population.

Epidemiologic Studies of Vietnam Veterans

The committee makes the following recommendations regarding epidemiologic studies of Vietnam veterans.

Recommendation 1. The committee endorses continued follow-up of the Air Force Ranch Hand cohort and its comparison group, and recommends that members of the Army Chemical Corps and an appropriate comparison group be followed in a similar study. An independent, nongovernmental scientific panel should be established to review and approve a new, expanded research protocol for both study populations, and to commission and direct a common analysis of the results.

Much can be learned by reanalysis of existing data or more in-depth analysis of data expected from current research programs investigating the health of Vietnam veterans, including the Air Force Ranch Hand study and DVA studies of other highly exposed Vietnam veterans such as members of the Chemical Corps. Priorities for specific health outcomes are discussed after recommendation 6. Public perception of the federal government's interest in the outcome of these studies suggests the need for studies of the health of Vietnam veterans to be conducted by a nongovernmental organization. Ranch Hand's excellent participation rate argues that components of the Department of Defense or the DVA continue to conduct follow-up examinations of the Ranch Hand and Army Chemical Corps cohorts. However, an independent, nongovernmental scientific panel is needed to oversee the analyses of resulting data in order to satisfy the public's concern about impartiality and scientific credibility.

As discussed in Chapter 6, one of the major problems with the interpretation of existing studies is the frequent lack of appropriate measures of exposure to herbicides or TCDD; however, the committee finds that it may be possible to develop better exposure measures for Vietnam veterans. In particular, Chapter 6 proposes measures that are not dependent on serum TCDD levels (which the committee finds inappropriate for the full range of herbicide exposures) but instead recommends the use of less formal sources of historical information about base perimeter spraying and other relevant

exposures, as discussed below in Recommendation 4. Thus, the committee concludes that certain further research efforts using new measures of exposure to herbicides in Vietnam are both necessary and potentially feasible. However, each of the possible measures that the committee has considered involves some degree of nondifferential misclassification bias, and the effect of this bias on risk estimates would likely be to underestimate true effects if they existed, possibly to the point that they would not be detected. In particular, the committee recommends that the following steps be taken prior to undertaking new epidemiologic studies of Vietnam veterans, for the reasons described below.

Recommendation 2. The Department of Defense and the Department of Veterans Affairs should identify Vietnam service in the computerized index of their records.

Chapter 3 notes that Vietnam service is not a "flagged item" on the computerized index of military personnel records archived at the National Personnel Records Center, which is maintained by the General Services Administration, under an agreement with the Department of Defense, in St. Louis, Missouri. Therefore, the computerized index of the record system does not allow for searches or selection of records of individuals who have served in Vietnam. The lack of an indicator of Vietnam service complicates every epidemiologic study of veterans based on military records and leads to methodologic inconsistencies among studies in defining the population under consideration. Adding this indicator to the computerized data base would facilitate future mortality studies based on computerized records, thereby increasing accuracy and decreasing cost, and would also simplify other epidemiologic studies of health outcomes in Vietnam veterans. All servicemen and women who were stationed in Vietnam or in the Vietnam theater during the Vietnam era should be identified in the records.

Recommendation 3. Biomarkers for herbicide exposure should be developed further.

Considerable uncertainty remains about the use of current of future serum TCDD levels as indicators of past exposure to dioxin in Vietnam veterans. Further research on the toxicokinetics of TCDD (2,3,7,8-tetrachlorodibenzo*p*-dioxin) is needed to permit more accurate extrapolation from current serum TCDD measurements to past exposures. Development of new biomarkers for exposure to herbicides, per se, also would be useful.

Recommendation 4. A nongovernmental organization with appropriate experience in historical exposure reconstruction should be commissioned to develop and test models of herbicide exposure for use in studies of Vietnam veterans.

Exposure assessment has been a weak aspect of most epidemiologic studies of Vietnam veterans. The military reports and personal testimony reviewed by the committee suggest that a sufficient range of exposure to herbicides may exist among Vietnam veterans for valid epidemiologic studies of certain health outcomes, and the committee believes that it is possible to develop valid exposure reconstruction models for such studies by using the methods of historical exposure reconstruction. Historical exposure reconstruction requires substantial professional judgment, and the results might be questioned if developed by a government agency; therefore, the committee recommends that the DVA arrange for a nongovernmental organization with appropriate experience in historical exposure reconstruction to develop and test potential models of herbicide exposure for use in studies of Vietnam veterans.

Recommendation 5. The exposure reconstruction models developed according to Recommendation 4 should be evaluated by an independent, nongovernmental scientific panel established for this purpose.

Herbicide exposure reconstruction models for Vietnam veterans must be thoroughly evaluated before epidemiologic studies based on these models proceed. The committee has identified three possible approaches to such an evaluation, which are discussed in more detail in Chapter 6: (1) internal consistency checks, (2) comparisons of exposure measures based on the reconstruction model with actual serum dioxin measurements, and (3) assessments of the association between exposure reconstruction measures and health outcomes shown in occupational or environmental studies to be associated with herbicides. Scientific judgment is required in interpreting the results of such an evaluation, so the committee cannot specify explicit criteria for acceptance or rejection of the new exposure reconstruction models in advance of their development and testing. Thus, the committee recommends that an independent, nongovernmental scientific panel be established to review the results of the proposed evaluation studies and to judge the validity and feasibility of the exposure reconstruction models. This panel should have expertise in historical exposure reconstruction and in epidemiology. In order to maintain the public and scientific credibility of the study, the panel members should be nongovernmental and independent of the organization that develops the exposure reconstruction models.

Recommendation 6. If the scientific panel proposed in Recommendation 5 determines that a valid exposure reconstruction model is feasible, the Department of Veterans Affairs and other government agencies should facilitate additional epidemiologic studies of veterans.

A number of possible epidemiologic studies could provide additional information on the health effects of exposure to herbicides in Vietnam beyond

what is already known. Highest research priority should be given to those health effects for which additional study is likely to change the balance of the evidence for or against an association. This includes

a. health outcomes for which current evidence is limited/suggestive of an association (lung and respiratory cancers, multiple myeloma, and prostate cancer);
b. health outcomes for which current evidence is insufficient or inadequate to determine whether an association exists, but which, in the committee's judgment, are plausible based on animal toxicologic data (such as nasal/nasopharyngeal cancer) or for which there are known associations with related chemical compounds in humans (such as liver cancer and polychlorinated biphenyls);
c. health outcomes for which the typical age at onset has not yet been reached by members of the Vietnam veteran cohort (such as prostate cancer).

The committee also recommends that priority be given to additional research on reproductive effects that would help clarify the possible effects of herbicides. In particular, the committee believes that extensive reanalysis of the Ranch Hand reproductive data could shed additional light on these questions (see Chapter 9 and Appendix C).

Although there is sufficient evidence of an association between occupational or environmental exposures to herbicides and non-Hodgkin's lymphoma, Hodgkin's disease, and soft tissue sarcomas, the existing information on dose-response relationships is incomplete, especially with regard to Vietnam veterans. If a valid exposure reconstruction method can be developed, it might be applied to the exposure data available from existing case-control studies to provide additional dose-response evaluations. Additional refinement of the clinical and pathological definitions of soft tissue sarcomas in epidemiologic studies would also help to determine which of the specific cancers in this class are associated with herbicides or TCDD.

The committee recognizes that the recommendations for development of a historical exposure reconstruction model and its use in epidemiologic studies might seem at variance with the Centers for Disease Control (CDC), White House Agent Orange Working Group (AOWG), and Office of Technology Assessment (OTA) conclusions made in 1986 with regard to the congressionally mandated Agent Orange Study. The committee has come to a different conclusion for four reasons: First, the CDC-AOWG-OTA conclusions were based in large part on serum TCDD measurements, which the committee feels are insufficient for validating exposure to herbicides used in Vietnam, as explained in Chapter 6. Second, the arguments underlying the earlier conclusion that individuals in combat units were widely dispersed and that troop movement data are incomplete imply that exposure measurements may be imprecise, not that they are invalid. However, these

arguments do suggest that historical reconstruction of exposure will have nondifferential misclassification errors that will lead to underestimates of the relative risk of health outcomes if an association is in fact present. Third, the committee is proposing the use of more, but less formal, information on exposure than was considered in 1986. This includes the development and use of informal information on perimeter spraying, which might account for more meaningful herbicide exposure than the aerial spraying documented on the HERBS tapes. Finally, the committee does not know whether the approach it proposes will prove valid or whether new methods will identify a sufficient number of highly exposed Vietnam veterans for an epidemiologic study. In the committee's judgment, however, the likelihood that this approach will be successful is sufficient for it to be recommended.

Mandated Research Efforts

For the purposes of further research on the health effects of Vietnam service, Public Law 102-4 mandates that the DVA establish four specific programs that are subject to initiation, continuance, or discontinuation, depending on the findings of this IOM report, and the committee is charged with making recommendations about these specific mandates. The DVA has no specific plans for any of these research efforts beyond the minimal descriptions given in the law, so the committee is able to comment on them in only the broadest terms.

The committee's recommendations speak to its legislative mandate to determine "the feasibility of conducting additional scientific research on" health hazards resulting from exposure to dioxin and herbicides used in Vietnam, the research mandate in section 8 of Public Law 102-4. As previously stated, the committee feels that a series of epidemiologic studies of veterans could yield valuable information if a new, valid exposure reconstruction model can be constructed.

Section 6 of Public Law 102-4 requires the DVA to "compile and analyze, on a continuing basis, all clinical data" that (1) are obtained in connection with DVA examinations and treatment of Vietnam veterans, and (2) are likely to be scientifically useful in determining the association between disabilities experienced by these veterans and exposure to dioxin or herbicides. Such a system, called the Agent Orange Registry (see Chapter 2), currently exists. Section 7 of the law calls for the establishment of a system for the collection and storage of voluntarily contributed samples of blood and tissue of veterans who served in Vietnam. Balancing the strengths and weaknesses of stored biological samples and clinical data for research purposes, the committee feels that systems of this sort have scientific value, but only to the extent that they are components of specific, well-designed studies; see, for instance, National Research Council (1991). In the absence

of a clear study design to guide such activities, and without resolution of important design, quality control, and ethical issues regarding tissue banks, the committee does not recommend the establishment at this time of the clinical data and tissue archiving systems described in sections 6 and 7 of the law.

The final mandate in Public Law 102-4 on which the committee must comment calls for the testing of serum of Vietnam veterans who apply for medical care or file a disability compensation claim for TCDD (section 9). The purpose of this mandate is not stated in the legislation. If research purposes are contemplated, the committee's discussion about tissue archiving systems applies, and such a program would not be recommended at this time. It is also possible that this program is intended to provide information on individual exposure to dioxins or herbicides to aid in individual compensation decisions. The committee cannot make recommendations for DVA policy, but notes that the finding in Chapter 6 that individual TCDD serum levels in Vietnam veterans are usually not meaningful (because of common background exposures to TCDD, poorly understood variations among individuals in TCDD metabolism, relatively large measurement errors, and exposure to herbicides that did not contain TCDD) might apply to this mandate.

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2

History of the Controversy Over the Use of Herbicides

The United States has been involved for more than two decades in a controversy over the military use of herbicides in Vietnam during the Vietnam era. The controversy centers around both the use of herbicides in Vietnam and the purported health problems associated with exposure to herbicides, primarily Agent Orange and its contaminant 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), known scientifically as TCDD and to the general public as dioxin[1] (Young and Reggiani, 1988). The controversy is further complicated by public fears over exposure to herbicides and dioxin resulting from domestic herbicide spraying, chronic exposure to dioxin of workers in the chemical industry, accidents in chemical plants that exposed workers, and dioxin released to the environment from several sources.

This chapter reviews the use of herbicides, the early history of the controversy, the concerns that Vietnam veterans have voiced about health problems they believe are related to exposure to herbicides, the Agent Orange product liability litigation, and the response to concerns of Vietnam veterans and the public by the federal government, state governments, veterans organizations, and others. The events and issues surrounding the domestic use of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and occupational exposure to 2,4,5-T and its

[1] 2,3,7,8-TCDD is actually one specific member of the family of chemicals known as "dioxins." In other chapters of the report, TCDD is specifically used to denote 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, but because public concern focuses on "dioxin," that term is also used in this historical review.

dioxin contaminant are also addressed in this chapter. As a result of several major events relating to dioxin exposure, the public became aware of the potential health effects of exposure to dioxin in tandem with the increased concern over possible health effects of exposure to herbicides sprayed in Vietnam. Researchers studied populations (described in this chapter) that had potential health effects from exposure to herbicides and TCDD, including production workers in chemical plants, agricultural and forestry workers, pulp and paper mill workers, and residents environmentally exposed in specific areas, such as Times Beach, Missouri; Alsea, Oregon; and Seveso, Italy. For the studies introduced in this chapter, the methodological framework is described in Chapter 7, and the results are discussed in the health outcome chapters (8-11).

MILITARY USE OF HERBICIDES IN VIETNAM

The military use of herbicides in Vietnam began in 1962, was expanded during 1965 and 1966, and reached a peak from 1967 to 1969. Herbicides were used extensively in Vietnam by the U.S. Air Force's Operation Ranch Hand to defoliate inland hardwood forests, coastal mangrove forests, and, to a lesser extent, cultivated land, by aerial spraying from C-123 aircraft and helicopters. Soldiers also sprayed herbicides on the ground to defoliate the perimeters of base camps and fire bases; this spraying was executed from the rear of trucks and from spray units mounted on the backs of soldiers on foot. Navy riverboats also sprayed herbicides along riverbanks. The purpose of spraying herbicides was to improve the ability to detect enemy base camps and enemy forces along lines of communication and infiltration routes, and around U.S. base camps and fire bases. Spraying was also used to destroy the crops of the Vietcong and North Vietnamese (Dux and Young, 1980).

Herbicide Development and Testing

Experiments with chemicals for the control of vegetative growth were first conducted around the turn of this century. The practical purpose of these early compounds was to control weeds that competed with crops for available water, nutrients, and sunlight (NAS, 1974; Buckingham, 1982). It was not until the 1940s that agricultural chemical research led to the development of a number of synthetic compounds capable of regulating or suppressing plant growth. Some compounds, when applied at high doses, killed certain plants but did not harm others; these compounds were termed selective herbicides (NAS, 1974). Two of the most successful developments during that period were the discoveries of 2,4-D and 2,4,5-T. These chemicals were effective against broadleaf plants and several crops.

Throughout World War II and after, classified military research on these chemicals and nearly 1,100 other substances was conducted at the War Research Service in Fort Detrick, Maryland (MRI, 1967). Although defoliants were not introduced into the World War II conflict, the military potential of chemicals for reducing or removing heavy vegetative growth was further investigated.

The research program at Fort Detrick involved screening and evaluation of candidate defoliants (Warren, 1968). One component of the research program was organized to solicit "the best research and industrial competencies" to develop and evaluate various chemical defoliants and formulations (U.S. Army, 1964). Compounds for military consideration were also received from private companies as part of unsolicited proposals, and from individuals working in universities in other areas of chemical synthesis. The chemicals were evaluated in terms of their effectiveness at low doses, cost, availability or capability of being manufactured in large quantities, nontoxicity to man and animals, stability in storage, and corrosive properties. For chemicals that passed initial screening tests, field trials were conducted on major vegetational types using airplane dissemination equipment. Formulations and mixtures of chemicals were evaluated at various rates, volumes, and seasons of application as a basis for selection and standardization of defoliants (U.S. Army, 1964).

In addition to research and development on chemical herbicides during the 1950s, anticrop aerial spray trials for improving the delivery equipment were also conducted. In particular, U.S. military authorities were concerned about the various time lags in defoliation evidenced by different species of plants to which the herbicides were applied (U.S. Army, 1964; Huddle, 1969). The military assessment of chemical defoliants also appears to have involved questions such as the feasibility of developing techniques by which large, slow-moving, and low-flying aircraft could traverse enemy-occupied jungle terrain without being shot down; the selection of the appropriate chemicals for particular types of foliage to be removed; and the optimum timing of spraying with regard to humidity, wind conditions, temperature, and topography of the area to be sprayed (Huddle, 1969). During this time the Hourglass spray system —the archetype for the spray equipment used initially aboard the Ranch Hand C-123s—was developed. The Hourglass, or MC-1, spray system was capable of distributing herbicide at a rate of 1 to 1.5 gallons per acre; however, after evaluation and modification, the 1,000 gallon C-123/MC-1 spray system was capable of depositing 3 gallons per acre on swaths 240 feet wide when flying at an airspeed of 130 knots and an altitude of 150 feet. In 1966, the MC-1 was replaced in all C-123s by a modular spray system designed for internal carriage in cargo aircraft (Young et al., 1978).

In June 1959, an experiment led by Dr. James Brown at Camp Drum,

New York, demonstrated the long-term effectiveness of aerially dispensed herbicides in improving visibility for military operations (Buckingham, 1982). An improvised helicopter spray system delivered a 1:1 mixture of 2,4-D and 2,4,5-T over a 4-square-mile area at a quantity of one-half gallon per acre. Evaluation of the effectiveness of the defoliants on vegetation was made one year later and again in October 1962. In 1960, no signs of regrowth had occurred in the sprayed area. Upon reexamination in 1962, it was observed that maple trees, which had been predominant in the area, appeared to be dead. Sprouting had occurred in some other species of trees, and one species appeared to have recovered from the chemicals' effects. In general, trees throughout the area had been killed, and visibility had been improved nearly 100 percent (Warren, 1968). Additional field tests in the Florida Everglades and Puerto Rico demonstrated the chemicals' defoliant activity (MRI, 1967).

By 1960, the U.S. Army had tested numerous herbicides and aerial delivery techniques (MRI, 1967). With the anticipated intensified involvement of U.S. military advisory forces in Vietnam, the large-scale use of herbicides was pursued. In 1961, the U.S. Department of Defense conducted the first operational field tests in Vietnam of 2,4-D and 2,4,5-T, the major herbicides to be disseminated in Vietnam over the next 10 years. The primary purpose of the early missions was to test the soundness of the defoliation concept as well as to measure optimum chemical concentrations and methods of delivery (Collins, 1967; Warren, 1968). Results of these early defoliation tests were mixed, and military authorities urged continued testing and evaluation of the herbicides in Vietnam (Buckingham, 1982).

A test program was conducted in Thailand during 1964-1965 to evaluate the effectiveness of aerial applications of various formulations of 2,4-D, 2,4,5-T, and other chemicals in the defoliation of jungle vegetation representative of Southeast Asia on several 10-acre plots. Aerial spray treatments were applied at rates of 0.5 to 3.0 gallons per acre, and at two- to three month intervals, to determine minimal effective rates and proper season of application. Defoliation effectiveness was measured in terms of rate, volume, canopy penetration, vegetation response, and season of application. Results of the test program showed that (1) 2,4-D and 2,4,5-T were effective for long-term defoliation, with more complete defoliation and longer duration of effective defoliation at higher rates of application; (2) best results were achieved during the rainy or growing season; (3) defoliation responses were influenced more by rate than by volume of chemical applied; (4) woody species varied in the duration and degree of defoliation; and (5) complete defoliation of all species in mixed forest types was not achieved (Warren, 1968).

Use of Herbicides in Vietnam

Phenoxy herbicides are synthetic chemical analogues of hormones found in plants that regulate the rate and pattern of plant growth; these herbicides cause aberrant growth or death of certain plant species. The types of herbicide used in Vietnam were very effective at killing certain types of tropical vegetation, and the aerial spraying of herbicides allowed for easy application over a large-area. The herbicides were applied aerially at a rate of approximately 3 gallons per acre. According to military records of Operation Ranch Hand, from August 1965 to February 1971, a total of 17.6 million gallons of herbicide was sprayed over approximately 3.6 million acres in Vietnam (NAS, 1974).

The different types of herbicide used by U.S. forces in Vietnam were identified by a code name referring to the color of the band around the 55 gallon drum that contained the chemical. These included Agents Orange, White, Blue, Purple, Pink, and Green. From 1962 to 1965, small quantities of Agents Purple, Pink, and Green were used. From 1965 to 1970, Agents Orange, White, and Blue were employed, and from 1970 to 1971, only Agents White and Blue were used in the defoliation program (Young and Reggiani, 1988).

Agent Orange was the most extensively used herbicide in Vietnam; it consisted of a 50:50 mixture by weight of the *n*-butyl esters of two phenoxy acids: 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). A synthetic contaminant of 2,4,5-T is the compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), informally known as dioxin. TCDD is an unavoidable by-product of the manufacture of 2,4,5-T and a contaminant in Agent Orange (Gough, 1986). Levels of TCDD contamination in Agent Orange ranged from less than 0.05 to almost 50 parts per million, with a mean of about 2 parts per million (NAS, 1974). An estimated 368 pounds of dioxin was sprayed in Vietnam over a six year period (Gough, 1986).

The military use of 2,4,5-T, and thus Agent Orange, was suspended by the Department of Defense in April 1970 (Young and Reggiani, 1988). Following the suspension of 2,4,5-T, the White House announced on December 26, 1970, that it was initiating an orderly yet rapid phaseout of the entire herbicide operation. On February 12, 1971, U.S. Military Assistance Command, Vietnam announced that herbicides would no longer be used for crop destruction in Vietnam and the last Ranch Hand fixed-wing aircraft (C-123) was flown. Subsequent spraying of herbicides was limited to controlled use around U.S. fire bases by helicopter or ground troops (MACV, 1972). On October 31, 1971, nearly 10 years after the herbicide program began in Vietnam, the last U.S. helicopter herbicide operation was flown (NAS, 1974). The military use of herbicides is discussed in further detail in Chapter 3.

EARLY CONCERNS ABOUT THE USE OF HERBICIDES IN VIETNAM

Early Accounts of Dioxin (TCDD)

Dioxin (TCDD) arises during the hydrolysis of tetrachlorobenzene to form 2,4,5-trichlorophenol, the industrial precursor of 2,4,5-T. TCDD is a solid that is insoluble in water and slightly soluble in fats or hydrocarbons. TCDD decays slowly in the soil under normal environmental conditions, which indicates that "its potential hazards may be very persistent" (NAS, 1974). Further characteristics of dioxin can be found in Chapter 4 on toxicology.

In 1872, two German chemists prepared the first chlorinated dioxin, but its structure was not understood until much later. In 1957, Dr. W. Sandermann of the Institute of Wood Chemistry in Hamburg published results of his synthesis of TCDD. While working on the synthesis, his laboratory assistant was exposed to the substance being tested when some of it blew into his face. He soon developed skin lesions over his entire face and decided to seek treatment from Dr. Karl Schulz, a dermatologist who treated chemical workers and had observed chloracne in some of them (Gough, 1986). After examining Sandermann's laboratory assistant, Schulz identified the skin lesions on his face as chloracne. When the laboratory assistant explained that the compound he was synthesizing was TCDD, Schulz was the first to correlate the presence of chloracne with exposure to dioxin. To further confirm this assumption, Schulz applied a TCDD solution to the skin of his forearm and noted that chloracne appeared (Young and Reggiani, 1988).

In September 1971, an early account of research on the appearance of TCDD in trace quantities in samples of 2,4,5-T was presented at a session on the origin and fate of chlorodioxins at the American Chemical Society meeting. TCDD was defined to be the most toxic of all chlorodibenzodioxins studied at that time (Young and Reggiani, 1988). Further accounts of dioxin's toxicity were presented at a meeting on "Perspectives on Chlorinated Dibenzodioxins and Dibenzofurans" sponsored by the National Institute of Environmental Health Sciences in North Carolina in April 1973. The major findings indicated "… that there was a variation of sensitivity among species, the liver was the target organ, the toxic effects were delayed after absorption, and the mechanism of teratogenesis was still incompletely understood … patterns of absorption and of distribution among organs were beginning to emerge" (Young and Reggiani, 1988).

In 1974, the National Academy of Sciences' Committee on the Effects of Herbicides in Vietnam reported that "TCDD is extremely toxic to some laboratory animals. … TCDD has been found to be teratogenic in mice; results with other laboratory animals have not been conclusive. The lethal

dose in humans is not known, nor is that required to cause birth defects, if indeed there is such an activity. TCDD is strongly implicated as the main cause of chloracne, a disease that has affected employees in some plants manufacturing 2,4,5-T or its precursor, 2,4,5-trichlorophenol" (NAS, 1974).

Concerns Over the Long-Term Use of Herbicides

Public concern over the use of herbicides in Vietnam began in 1964, even before the toxicity of TCDD was first reported. At that time, the Federation of American Scientists urged the government not to use chemical and biological weapons unless they were used first by the enemy. The federation was concerned about the use of defoliants in Vietnam because the government was not discriminating between fighting forces and civilians while using the herbicides, and that constituted biological and chemical warfare (Young and Reggiani, 1988). In January 1966, 29 scientists banded together to protest the U.S. policy on the use of herbicides and demand their complete abolition. They requested that President Lyndon B. Johnson begin discussions with the allies on adherence to the ban on the use of herbicides in Vietnam. "Even if it can be shown that the chemicals are not toxic to man, such tactics are barbarous because they are indiscriminate; they represent an attack on the entire population of the region where the crops are destroyed, combatants and non-combatants alike. [This is] … a precedent for the use of similar but even more dangerous chemical agents against our allies and ourselves" (Dux and Young, 1980).

In December 1966, the Council of the American Association for the Advancement of Science (AAAS) sent a letter to the Secretary of Defense, Robert McNamara, calling for studies of the short- and long-term consequences of the massive use of herbicides in Vietnam (Young and Reggiani, 1988). In February 1967, a second petition signed by more than 5,000 scientists, including 17 Nobel laureates, was delivered to President Johnson requesting that he end the use of herbicides in Vietnam (Dux and Young, 1980). A Department of Defense (DOD) official, responding to criticisms regarding the questionable military use of herbicides, stated that "qualified scientists, both inside and outside the government, and in the governments of other nations, have judged that seriously adverse consequences will not occur. Unless we had confidence in these judgments, we would not continue to employ these materials." Several members of the AAAS council agreed that this statement was unjustified, noting that there was insufficient evidence to arrive at this conclusion (Wolfle, 1989).

Noting the strong opposition by some of the nation's leading scientists to the military use of herbicides, the Department of Defense commissioned a study by the Midwest Research Institute (MRI) in Kansas City, Missouri, to assess whether the use of the herbicides would have a long-term ecological

impact. The MRI assessment did not include field studies or trips to Vietnam, but involved a review of approximately 1,500 scientific papers. The study, *Assessment of Ecological Effects of Extensive or Repeated Use of Herbicides*, was completed in December 1967 (MRI, 1967). The report could not provide conclusive answers about the long-term effects of chronic exposure to herbicides on the ecological system or on the population, and recommended further studies of the long-term effects on the environment and the population in order to assess properly the consequences of repeated use of herbicides (MRI, 1967).

In 1965, the National Cancer Institute contracted with Bionetics Research Laboratory in Maryland to investigate the possible teratogenic effects of a number of pesticides and herbicides. The study, *Evaluation of Carcinogenic, Teratogenic, and Mutagenic Activities of Selected Pesticides and Industrial Chemicals*, noted that among the herbicides tested on mice and rats were 2,4-D and 2,4,5-T (Bionetics, 1968). This study provided the first indication of the teratogenicity and fetotoxicity of 2,4,5-T (Lilienfeld and Gallo, 1989). The researchers determined that 2,4,5-T was teratogenic, causing malformations and stillbirths in mice when administered in high doses, and that 2,4-D was potentially harmful. This report was released to the public in 1969. Bionetics later reanalyzed the 2,4,5-T used for its initial study and revealed that the cause of toxicity was the contaminant TCDD and that 2,4,5-T itself was not teratogenic (Young and Reggiani, 1988).

Another study, *Congenital Malformations, Hydatidiform Moles and Stillbirths in the Republic of Vietnam, 1960-1969*, was conducted by R.T. Cutting on behalf of the government of South Vietnam and the U.S. Military Assistance Command, Vietnam (Cutting et al., 1970). Cutting examined maternity records of 22 hospitals for two time periods: the buildup of herbicide use (1960-1965) and larger-scale military herbicide use (1966-1969). He found that there were no differences in the incidence of stillbirths, congenital malformations, and hydatidiform moles between the two periods (Cutting et al., 1970; U.S. Congress, House, 1978). It was later revealed that the study was biased because of unreliable data and hospital records (Young and Reggiani, 1988).

In early 1970, the AAAS set up a commission to assess the effects of large-scale use of herbicides on the environment and population of Vietnam. The members of the Herbicide Assessment Commission (HAC) were Matthew Meselson, Arthur Westing, John Constable, and Robert Cook. In June 1970, HAC held a conference at Woods Hole, Massachusetts, with individuals who had experience with the herbicide program in Vietnam. They determined what HAC members would investigate and observe while in Vietnam, and prepared questionnaires for use in interviews of Vietnamese residents. In August 1970, they traveled to Vietnam on an inspection field trip to

examine the extent to which the herbicides had destroyed the vegetation and local food crops in areas where they had been sprayed.

After returning from Vietnam, HAC members wrote a report on the defoliation of Vietnam in which they noted that the Department of Defense had stated that the herbicides were used "for crop destruction of small, isolated crop patches along infiltration routes …" (Wolfle, 1989) and limited to areas of low population. HAC, however, found that "crops had been sprayed in an area with an estimated population of 180 persons per square kilometer and that nearly all of the food being destroyed would have been used by mountain-dwelling Montagnard civilians instead of by enemy troops" (Wolfle, 1989). The commission maintained that the military use of herbicides had been considerably more destructive than previously imagined—half of the mangrove forests had been destroyed and there were indications of serious health effects (Wolfle, 1989). The HAC members documented reports of stillbirths and birth defects in Vietnamese, noting that these adverse reproductive effects were possibly associated with 2,4,5-T (Young and Reggiani, 1988) and its contaminant, TCDD. On December 26, 1970, the White House announced that it was initiating an orderly yet rapid phaseout of the herbicide operation. The AAAS council adopted a resolution commending the U.S. government for its intention to phaseout the use of herbicides in Vietnam (Wolfle, 1989).

At the end of 1970, Congress directed the Department of Defense to contract with the National Academy of Sciences (NAS) to study the ecological and physiological effects of the widespread military use of herbicides in Vietnam. The NAS recruited a 17-member committee and 30 consultants to carry out the study. Committee members and consultants spent approximately 1,500 man-days in Vietnam in order to develop an inventory of the areas sprayed by herbicides, review the effects on various vegetation types, study the persistence of herbicides in soil, examine the effects of herbicides on animal populations in estuaries of Vietnam, and attempt to identify the effects of herbicides on resident populations exposed to them (NAS, 1974).

The resulting report, *The Effects of Herbicides in South Vietnam* (NAS, 1974), concludes that (1) the committee was unable to gather any definitive indication of direct damage by herbicides to human health, although there were reports from Montagnards of respiratory distress in children; (2) although attempts to assess the social, economic, and psychological effects of the herbicide spraying were less than satisfactory, the effect of herbicide spraying on the health of the Vietnamese appeared to have been smaller than feared; (3) the evidence of spraying on food crops indicated that they were highly vulnerable to the herbicides; (4) the mangrove forests were found to have been extremely vulnerable to herbicide spraying; and (5) although it was difficult to assess the damage to the inland forests because

the committee had to rely on aerial photographs, the committee concluded that most of the damage occurred in overused open or thin forests and in young secondary forests.

Public concern about the military use of herbicides during the Vietnam conflict did not end when Operation Ranch Hand terminated with the last official herbicide spraying in 1971 or with the final departure of American troops in 1975. In April 1975, President Gerald Ford issued Executive Order 11850, in which the United States renounced the first use of herbicides in war except "under regulations applicable to their domestic use, for control of vegetation within U.S. bases and installations or around their immediate defensive perimeters." In a historical account of Operation Ranch Hand, it was noted, "As long as this policy stands, no operation like Ranch Hand could happen again" (Buckingham, 1982).

CONCERNS ABOUT EXPOSURE TO AGENT ORANGE

Vietnam Veterans Return Home

Historians have noted that during the 1970s, many Vietnam veterans returned to a society that did not welcome them (Schuck, 1987). The country had been greatly divided over the war, and a strong antiwar sentiment pervaded most of the final years of the Vietnam conflict (Karnow, 1991; Spector, 1993). There were antiwar demonstrations held throughout the country during these years, and when the veterans came home, many Americans did not want to acknowledge their patriotic effort (Bonoir et al., 1984; Salisbury, 1985). There was also a lack of unanimity among veterans about their service in the Vietnam conflict. Some veterans were bitter at having served in a war they felt could not be won; however, an equal number of veterans would have returned to Southeast Asia if they were called upon by their country (Wilcox, 1989).

The returning veterans were also presented with more difficult adjustments than veterans of other foreign wars. Because of improved emergency medical care, more disabled veterans returned home. Of those discharged for disabilities during World War II, 18 percent were amputees and 3.1 percent were paralyzed; the comparable figures for Vietnam were 28.3 percent and 25.2 percent, respectively (Schuck, 1987). The returning veterans also had a difficult period of adjustment due to the fact that most of them were discharged from service one at a time. Since their tour of duty was for only one year, many veterans did not forge close attachments with each other as in earlier wars. Following the war, some veterans began to develop health problems, and in time, more veterans reported serious illness and claimed that their children were born with birth defects (Gough, 1986).

The Beginning of the Controversy

During the early and mid-1970s, a growing number of veterans began to question the possible linkage between their conditions or diseases and their exposure to herbicides, mainly Agent Orange, in Vietnam. In 1977, Maude deVictor, a benefits counselor in the Chicago regional office of the Veterans Administration (VA), was contacted by the wife of Charles Owen, a Vietnam veteran who believed his terminal cancer was the result of exposure to Agent Orange. After learning that Charles Owen had died and that the VA had refused his widow's claim for benefits, deVictor began to research the health effects of exposure to Agent Orange (Wilcox, 1989). She contacted Alvin L. Young, Major, U.S. Air Force, an expert in plant physiology, and inquired about the types of herbicides used in Vietnam. DeVictor recorded the conversation in a memorandum to the file, which explained the use and toxicity of Agent Orange and Agent Blue (DeVictor, 1977). In response to this memorandum, a line-by-line commentary was prepared by Dr. Young, and a copy was recorded in a congressional hearing (U.S. Congress, House, 1980b).

DeVictor continued her inquiries into the possible connection between Agent Orange and certain health outcomes. She began gathering statistics on veterans' exposure to Agent Orange by questioning veterans who visited her office for benefits, widows of veterans, and wives of veterans about the health of their husband and children. When the VA learned that she was carrying out this research, she was asked to cease these additional inquiries and concentrate on her assigned duties, but she continued her research on Agent Orange. Soon after, someone contacted Bill Kurtis, a local television reporter, about deVictor's inquiries on veterans' exposure to Agent Orange (Linedecker et al., 1982). On March 23, 1978, WBBM, a CBS affiliate in Chicago, aired Kurtis' documentary *Agent Orange, the Deadly Fog*. Subsequently, local and national media began to report on Agent Orange and veterans' complaints with more frequency (Wilcox, 1989).

Early in 1978, Paul Reutershan, a former helicopter crew chief responsible for transporting supplies to the 20th Engineering Brigade, appeared on the "Today" show and shocked many of the show's viewers by announcing: "I died in Vietnam, but I didn't even know it." He told of how he flew almost daily through clouds of herbicides being discharged from C-123 cargo planes, and how he observed the dark swaths cut in the jungle by the spraying, and watched the mangrove forest turn brown and die (Wilcox, 1989). Even though he observed this destruction of the jungles and forests, he did not worry about his own health. He said that he was told by the Army that Agent Orange was "relatively nontoxic to humans and animals" (Wilcox, 1989). Upon returning home from Vietnam, Reutershan was diagnosed with cancer. On December 14, 1978, at the age of 28, Reutershan

died from the cancer that had invaded his colon, liver, and abdomen (Schuck, 1987).

Prior to his death, Paul Reutershan had read a news account about Maude deVictor's data correlating health problems in Vietnam veterans and exposure to Agent Orange. Convinced that he had identified the cause of his illness, he contacted Edward Gorman, a personal injury lawyer on Long Island, and requested that he file a suit in a New York State court naming Dow, Monsanto, and Diamond Shamrock (chemical companies that manufactured Agent Orange) as defendants. During this time, he also founded the Agent Orange Victims International (AOVI), and before his death, he named his colleague, Frank McCarthy, to carry on as AOVI director. Reutershan spent his remaining time alerting the public to his belief that his cancer was the direct result of his exposure to Agent Orange.

AGENT ORANGE PRODUCT LIABILITY LITIGATION

Class Action Suit

After the death of Paul Reutershan, Frank McCarthy met with the members of the AOVI and determined that Reutershan's lawsuit was an important part of its effort to alert the nation to ills caused by Agent Orange. Victor J. Yannacone, Jr., an expert in workmen's compensation claims, and in particular, toxic tort cases, was contacted and he became the prime mover of the Agent Orange litigation in its first four and a half years (Schuck, 1987). A class action lawsuit was filed on January 8, 1979, in the U.S. District Court for the Southern District of New York in Manhattan. The class consisted of Vietnam veterans, their spouses, their parents, and their children. The suit named five chemical manufacturers (others were added later) responsible for the production of the components of Agent Orange (Schuck, 1987). In May 1979, the case was consolidated into a multidistrict litigation (MDL 381) and designated *In re Agent Orange Product Liability Litigation* (Schuck, 1987).

The plaintiffs did not involve the federal government as a third-party defendant in the class action, in part, because of a rule of law—known as the Feres doctrine—that precludes recovery against the United States for injuries that arise out of or in the course of activity incident to military service (Jacobs and McNamara, 1986). Another reason was that the plaintiffs' felt that it would greatly protract the litigation by involving the federal government in the class action (Schuck, 1987). The chemical companies, however, sought to join the United States as a third-party defendant in the class action (Schuck, 1987).

After many preliminary proceedings, a trial date was set for May 7, 1984. Judge Weinstein, the trial judge, urged the parties to shape a settlement,

and on the eve of the trial, a settlement was reached. The settlement was approved in January 1985, after a series of hearings were held around the country to ensure that the settlement was fair and reasonable to the members of the class (Schuck, 1987). Under the settlement, the defendant chemical companies (Dow; Monsanto; Diamond Shamrock Corporation; Hercules, Inc.; Uniroyal Inc.; T-H Agricultural & Nutrition Company; and Thompson Chemicals Corporation) agreed to make available a fund of $180 million (Schuck, 1987). This fund was to be used to (1) finance a cash payment program for totally disabled veterans and survivors of deceased veterans; (2) establish a class assistance foundation to help meet the medical, social, and legal service needs of the members of the class; and (3) establish a trust fund for New Zealand and Australian class members (Jacobs and McNamara, 1986).

The product liability class action suit showed that many veterans were convinced that Agent Orange had hurt them. However, no causal relationship was ever established between the alleged health effects in Vietnam veterans and their exposure to Agent Orange because the case was settled out of court. Judge Weinstein noted that "causal connection may at some time in the future be proved. [However,] we can say that proof has not been produced in this court sufficient to go to the jury" (Agent Orange Product Liability Litigation, 1985).

CONCERNS ABOUT OTHER EXPOSURES TO 2,4,5-T AND TCDD

In the late 1890s, scientists began to develop the concept that herbicides could be produced to control the growth of certain types of plants; however, herbicides for general agricultural use were not developed for testing until 1942. In the early 1940s, the herbicidal properties of 2,4-D and 2,4,5-T were discovered. In 1945, Dow Chemical found that combining an equal mixture of 2,4-D and 2,4,5-T created a more efficient herbicide than when they were used separately (Lilienfeld and Gallo, 1989). The Department of Agriculture, under the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act registered 2,4,5-T as an herbicide in 1948. Farmers recognized its usefulness for killing broadleaf plants and for controlling weeds in pasturelands to enable desirable grasses to grow. Foresters also used the herbicide to control weeds, underbrush, and shrubs in forests. Because 2,4,5-T was inexpensive and easy to use, by the early 1970s it had become one of the most widely used herbicides in the United States (Gough, 1986).

Since the early production of chlorophenols—a group of chemicals that were also contaminated with TCDD—in the mid-1930s, accidents in chemical plants have occurred, releasing dioxin (TCDD) into the environment and exposing workers, but it was not until research on the toxicity of TCDD was

reported in 1971 that this issue received public attention (Fingerhut et al., 1987). The public became aware of the potential health effects of exposure to TCDD in tandem with the increased concern over possible health effects of exposure to herbicides sprayed in Vietnam. As studies were completed that demonstrated the toxicity of TCDD in animals, scientists began to realize that numerous individuals may have been exposed to TCDD. Researchers began to study populations (described below) that had potential health effects from exposure to TCDD, including residents living near soil contaminated with TCDD in Missouri; community residents who lived near sites sprayed with herbicides such as Alsea, Oregon; workers at the Monsanto plant in Nitro, West Virginia; residents living in and around Seveso, Italy, exposed during industrial accidents; pulp and paper mill workers who were exposed during the production process; and chemical plant workers who were occupationally exposed to TCDD during the production of 2,4,5-T and other products. For the studies introduced in the following sections, the methodological framework is described in Chapter 7, and the results are discussed in the health outcome chapters (8-11).

Occupational Exposure

Production Workers

Researchers have studied production workers in U.S. chemical plants for potential health effects of exposure to TCDD. Workers in plants manufacturing phenoxy herbicides can potentially be exposed heavily in four ways: (1) manufacture of the herbicides; (2) manufacture of intermediate chemicals used in the production of herbicides and other products; (3) packaging or transporting of the finished product; and (4) general maintenance and cleaning of the chemical plant (Lilienfeld and Gallo, 1989). Zack and Gaffey (1983) wrote that exposure to dioxins "can cause chloracne, a skin disorder characterized by comedones, cysts, and abscesses. Outbreaks of chloracne have been reported among workers associated with the production of 2,4,5-T and 2,4,5-T based products. Such incidents resulting from both accidental and routine occupational exposures have been reported from several countries."

The National Institute for Occupational Safety and Health (NIOSH) Industrywide Studies Section has conducted several epidemiologic studies of workers exposed occupationally to phenoxy herbicides (Lilienfeld and Gallo, 1989). In 1978, NIOSH "initiated an effort to identify the exposed workers at all U.S. chemical plants which produced TCDD-contaminated products. As a result, the NIOSH Dioxin Registry was established. It includes demographic and work history information for all workers assigned to the production of products contaminated with certain isomers of dioxin. …

Twelve [chemical] plants were included in the NIOSH Dioxin Registry" (Fingerhut et al., 1991b). In 1991, Fingerhut and colleagues published a study, based on data from the NIOSH Dioxin Registry, that was the first analysis of mortality in the cohort of U.S. workers exposed to TCDD between 1942 and 1984 (Fingerhut et al., 1991a,b). In ongoing analyses of the same population, Calvert and colleagues examined occupational exposure to dioxin and potential health outcomes including chronic bronchitis, chronic obstructive pulmonary disease, and ventilatory function (Calvert et al., 1991) as well as hepatic and gastrointestinal effects (Calvert et al., 1992). Sweeney and colleagues (in press) examined peripheral neuropathy in workers after occupational exposure to 2,3,7,8-TCDD.

Agricultural and Forestry Workers

Farmers and forestry workers have been exposed to a variety of chemicals including pesticides, insecticides, and herbicides. Several Swedish studies were published in the late 1970s, which reported associations between exposure to herbicides and certain cancers. "In Sweden, phenoxy herbicides have been used in forestry to combat hardwoods. There has been use of a combination of 2,4-D and 2,4,5-T but unlike Agent Orange the combination has been two parts of 2,4-D and one part of 2,4,5-T" (Hardell, 1990). In 1976, Hardell began a series of studies of workers exposed occupationally to phenoxy herbicides who subsequently developed soft tissue sarcomas (Hardell, 1990). In response to these Swedish studies, the National Cancer Institute (NCI) began a study in Kansas because "it is a wheat producing state and the amounts of herbicides that are used relative to the amounts of insecticides are greater for wheat than for some other crops such as corn. Both 2,4-D and 2,4,5-T were known to have been used in Kansas. [NCI] also chose Kansas because it has a population-based cancer registry. This means that [NCI was] able to base the study on incident diagnosed cases rather than using death certificate data …" (Hoar-Zahm and Blair, 1990; see also Hoar et al., 1986). NCI has published several studies on agricultural workers and workers who apply chemicals including pesticides and herbicides in Nebraska, Minnesota, Wisconsin, and Iowa, as well.

Pulp and Paper Mill Workers

More than 650,000 persons are employed in the manufacture of paper and allied products in the United States. Approximately half of these employees work directly in the production of pulp and paper stock, and are potentially exposed to a number of chemicals during the production process (Solet et al., 1989). "The [pulp and paper] production process is complex

and varied, but generally involves breaking down wood into liquid pulp by physical and chemical means. … The nature of chemical exposures varies with the industry, depending on the type of mill and the final product. Among potential exposures are both suspected and known human carcinogens, including asbestos, wood dust, formaldehyde, chlorinated phenols, and dioxin" (Solet et al., 1989). Occupational exposures may arise at any stage in the manufacturing process from preparation of the raw wood through the production of paper or pulp products (Robinson et al., 1986). Several studies of pulp and paper mill workers have been conducted. Robinson and colleagues (1986) surveyed pulp and paper mills in Washington, Oregon, and California in order to select five paper mills to study. The pulp and paper workers in the study cohort were followed from their last date of employment through March 31, 1977. Henneberger and colleagues (1989) initiated an investigation of the mortality of workers from different operations within a pulp and paper manufacturing company. In another study of pulp and paper workers, Solet and colleagues (1989) studied records of deceased members of the United Paperworkers International Union who had died between 1970 and 1984.

Major Event Associated with Occupational Exposure to Dioxin (TCDD)

The first major event associated with extraordinary occupational exposure to TCDD was the result of an accident at Monsanto's chemical plant in Nitro, West Virginia. After the accident, several researchers studied the exposed workers for related health effects.

Nitro, West Virginia On March 8, 1949, at the Monsanto Company's chemical plant in Nitro, West Virginia, an accident occurred in the autoclave in which trichlorophenol was being manufactured. The pressure inside the autoclave increased to a level that exceeded safety limits, and the safety valve gave way, allowing the pressurized contents (trichlorophenol containing TCDD) to vent out through the chimney and into the inside of the building (Gough, 1986). This accident was later recognized as a major event in occupational and environmental health, allowing scientists to study the health effects of TCDD on a group of workers exposed in a single incident (Zack and Gaffey, 1983).

There are differing stories about what the conditions were like inside the building after the accident, but many of the workers confirmed that they had to go into the building to clean residue off the walls. As they worked on their tasks of cleaning up the area, many of the workers began to complain of health problems. A number of workers who were the most severely affected were sent to the College of Medicine at the University of Cincinnati where they were examined by physicians (Gough, 1986).

After the accident, several researchers studied the exposed workers for related health effects. In a mortality study of workers employed at the plant, Zack and Gaffey (1983) followed the plant workers who had developed symptoms of chloracne after the accident, to examine the chronic health effects of exposure to TCDD. In 1984, Suskind and Hertzberg (1984) published their study on the human health effects of 2,4,5-T and TCDD, which was "conducted to determine the long-term health effects of workplace exposure to the process of manufacturing the herbicide (2,4,5-T) … including contaminants such as 2,3,7,8-TCDD." In the study of the health status of workers with past exposure to TCDD in the manufacture of 2,4,5-T, Moses and colleagues (1984), at the request of the United Steelworkers of America, conducted a health survey of current and retired workers of the plant: "The purpose of the survey was to determine if long-term health effects related to duration and/or intensity of past exposure to 2,3,7,8-TCDD could be demonstrated in the workers. Specifically, the study aimed to investigate effects documented from past studies."

Environmental Exposures

Domestic Use of Herbicides

Spraying of herbicides in the United States has been a practice of farmers, foresters, railroads, utility companies, and certain government agencies, for many years. Farmers used 2,4,5-T to kill broadleaf plants in pasturelands. Foresters, including the U.S. Forest Service and other federal agencies having jurisdiction over national lands, forests, and parks, have used herbicides to keep down brush and undergrowth and to eliminate unwanted hardwoods in pine forests. Other reasons for using 2,4,5-T were to limit the growth of weeds along railroad tracks, next to power lines, and along highways.

In April 1970, the U.S. Surgeon General reported that the use of 2,4,5-T could be hazardous to human health (Lilienfeld and Gallo, 1989). This prompted the U.S. Department of Agriculture to suspend the use of 2,4,5-T around homes, recreation areas, lakes, and ponds, and it canceled registration for the domestic use of 2,4,5-T, except for pastures and range lands (Gough, 1986; Lilienfeld and Gallo, 1989). The Environmental Protection Agency (EPA) finally banned the use of 2,4,5-T in the United States on February 28, 1979. The two major environmental events leading up to the domestic ban of 2,4,5-T were (1) the dioxin contamination of several sites in Missouri and (2) public concern about possible health effects of the spraying of herbicides in forests around Alsea, Oregon (Dux and Young, 1980).

Major Events Associated with Environmental Exposure to Dioxin (TCDD)

Several major events have been associated with extraordinary environmental exposure to TCDD. Included among these events are spraying of waste oil contaminated with dioxin in Times Beach, Missouri; herbicide spraying near Alsea, Oregon; and an accidental explosion at a chemical plant in Seveso, Italy.

Times Beach, Missouri During the early 1970s, several incidents of accidental spraying of dioxin-contaminated waste oil in Missouri left the state with the legacy of being the "Dioxin Capital of the United States" (Yanders, 1988). Through an unusual sequence of events, several areas in Missouri were highly contaminated with dioxin. The Northeast Pharmaceutical and Chemical Corporation (NEPACCO) in Verona, Missouri, was manufacturing hexachlorophene by using trichlorophenol that contained 3 to 5 parts per million of dioxin (Gough, 1986). The process of manufacturing hexachlorophene produced three different types of waste, each containing dioxin: (1) dioxin-containing water; (2) filter clay, a fine clay in which dioxin stuck to the clay particles; and (3) "still bottoms," a highly dioxin-concentrated thick oily residue from the bottom of process vessels. The toxic waste produced during this process had to be disposed of by NEPACCO. Most of the wastewater was fed into the water-treatment system of Hoffman-Taff (a company that produced Agent Orange for the Department of Defense and shared the production facility with NEPACCO), which leaked contaminated water into the Spring River (Gough, 1986). The filter clay was buried at the production site in Verona and at a local dump by NEPACCO. NEPACCO contracted with a local company that in turn subcontracted with another company to have the still bottoms removed from the process vessels at the manufacturing plant. The subcontractor, Russell M. Bliss, owned a company that removed waste oil from various locations and later sold the oil to clients (Yanders, 1988). In 1971, Bliss removed 18,500 gallons of still bottoms and was never told that the waste was contaminated with dioxin (Gough, 1986).

Waste oil is sometimes sprayed on dirt roads and other dirt surfaces to keep down dust. In May 1971, the Shenandoah Stables, northwest of St. Louis, contracted with Bliss to have its horse arena sprayed with waste oil. Almost immediately after the waste oil was sprayed, horses and other animals in the arena became sick and died. Eventually, 62 horses died from being exposed to the waste oil (Gough, 1986). The two young daughters of the owners of Shenandoah Stables also became very sick with flu-like illnesses immediately after being in the horse arena; however, the cause of the horses' deaths and the girls' illness could not be readily determined. The Centers for Disease Control (CDC) was called in to investigate the girls' illness, but did not identify the toxicant in the soil (Gough, 1986).

Shortly after the Shenandoah Stables had been sprayed, another horse arena, Timberline Stables, near Jefferson City, Missouri, contracted with Bliss to spray waste oil in its arena. Similar events occurred at Timberline—12 horses died and children became sick (Gough, 1986). CDC was called in to investigate these events and, in 1974, identified trichlorophenol and its contaminant dioxin in the soil from both horse arenas (Yanders, 1988). After discovering that the toxic substance in the waste oil was dioxin, CDC was able to trace the dioxin back to NEPACCO. CDC and EPA led an investigation of other areas that were contaminated with the dioxin-laced waste oil. Their investigation finally led them to the town of Times Beach, Missouri.

Times Beach is a small town on the banks of the Meramac River that has miles of unpaved roads. Between 1972 and 1976, Bliss sprayed more than 23 miles of these dirt roads with waste oil. In December 1982, after analyzing the soil from the sprayed roads in Times Beach, CDC and EPA scientists revealed that the results showed concentrations of dioxin as high as 300 parts per billion. The CDC recommended that Times Beach not be inhabited because of this dioxin contamination. On February 22, 1983, in St. Louis, Anne Burford, EPA Administrator, announced that the federal government and the state of Missouri would purchase the properties in Times Beach, and the residents began to abandon the town (Gough, 1986).

After the federal government began negotiations on purchasing the properties in Times Beach, the state of Missouri established a Task Force on Dioxin to review the situation and devise a plan of action to deal with the major dioxin contamination in Missouri (Yanders, 1988). One of the recommendations of the task force was to establish a dioxin research center in Times Beach for ongoing sampling and analysis of the contaminated soil (Yanders, 1988). The CDC established a registry of dioxin-exposed residents in Missouri in order to contact them with information about health risks and to examine them if necessary (Gough, 1986). Hundreds of former residents of Times Beach and 27 other dioxin-tainted sites in Missouri have recently settled lawsuits against four companies responsible for the toxic incidents. The town of Times Beach remains abandoned (New York Times, 1992a).

Several studies have been published that examine the health of Times Beach residents who have been environmentally exposed to dioxin. A pilot study was undertaken by Webb and colleagues (1987) to "ascertain what health effects might have resulted from TCDD exposure in one small area of Missouri. … The purpose of [the] pilot study was not to resolve the questions pertaining to the human effects of TCDD, but to suggest trends that can be investigated in future studies." In another study, Hoffman and colleagues (1986) examined residents of the Quail Run Mobile Home Park because TCDD measurements there were higher than at any other residential site in Missouri. In a study on the reproductive outcomes of mothers

with potential exposure to dioxin, Stockbauer and colleagues (1988) "conducted an epidemiologic investigation to determine if adverse human reproductive outcomes are associated with exposure to soil contaminated with dioxin. The [researchers] attempted to identify all births during the period of January 1, 1972 through December 31, 1982 that had potential exposure to dioxin, based on proximity of the maternal address to a location of known TCDD contamination."

Alsea, Oregon The Alsea region of Oregon includes the Siuslaw National Forest and privately owned timberland. Until 1979, the U.S. Forest Service and timber companies routinely used helicopters to spray 2,4,5-T in the forests around Alsea in the spring to control the underbrush.

In 1977, while attending a college course at the University of Oregon, Bonnie Hill, a teacher in Alsea, learned that dioxin caused spontaneous abortions in monkeys (Gough, 1986). Hill had experienced a miscarriage in the spring of 1975 and had heard of other women in Alsea who had also experienced miscarriages in the springtime. Hill decided to survey the women of Alsea, and among eight women that she interviewed, there were a total of 13 miscarriages that also occurred in the spring during the years 1972 through 1977. She felt that the pattern of the miscarriages correlated closely with the seasonal spraying.

Hill was able to obtain the spray locations around Alsea from the U.S. Forest Service and private timber companies. After matching the spray location records with a map of the area, she documented that the sprays were close to the homes of the women who had miscarried their pregnancies. Hill contacted the EPA, which sent two scientists from the University of Colorado to Alsea to interview the women who had suffered miscarriages (U.S. EPA, 1979; Wilcox, 1989). The purpose of the investigation was to assess the rates of spontaneous abortions (miscarriages) occurring in the region centered about the Alsea basin where 2,4,5-T had been used in forest management (U.S. EPA, 1979). The results from the data collected from these women—the Alsea I study—were reviewed by experts who concluded that it was impossible to judge an association between the miscarriages and 2,4,5-T based solely on data from nine women. Consequently, EPA had the scientists from the University of Colorado design a second study, known as Alsea II. This epidemiologic study compared the pregnancy experience of the women in Alsea with women in another part of Oregon that had never been sprayed. Alsea II was severely criticized by public authorities, chemical companies, and independent scientists, who said that the study was based on incomplete data of 2,4,5-T spraying and that there were inaccuracies in the data on the incidence of spontaneous abortions (McCulloch, 1984). However, on February 28, 1979, immediately after the release of Alsea II, the EPA issued an Emergency Suspension Notice of 2,4,5-T in forestry, rights-of-way, and pastureland.

The EPA began a series of hearings on the cancellation of 2,4,5-T in March 1980. Approximately 100 witnesses testified on issues including animal tests on dioxin, epidemiologic studies of Swedish workers in the timber industry, and the miscarriages in the women of Alsea. Subsequently, the chemical companies began to cancel their registration for 2,4,5-T; eventually, 2,4,5-T disappeared from the U.S. market (Gough, 1986).

The Seveso Accident The ICMESA (Industrie Chimiche Meda Societa Anonima) chemical manufacturing plant, near Seveso, Italy, produced 2,4,5-trichlorophenol contaminated with TCDD—the intermediate compound in the manufacturing of hexachlorophene—for its parent company, the Swiss Givaudan Company (Whiteside, 1977). In July 1976, an increase of pressure in the 2,4,5-trichlorophenol reactor caused an explosion of the reaction vessel, which produced a chemical cloud that was carried southward by the wind, spreading approximately a half pound of dioxin over several square kilometers inhabited by almost 40,000 people (Bisanti et al., 1980; NRC, 1982b).

Within a couple of days of the accident, small animals and birds became sick and died—most likely from eating vegetation contaminated with trichlorophenol. Almost two weeks after the accident, the Givaudan Company conceded that the chemical fallout was contaminated with dioxin (Whiteside, 1977). At this time, the Italian authorities began to develop safety precautions for citizens in the surrounding areas. One of the first steps they took was to map out the contaminated areas (see Figure 2-1). Zone A, directly south of the plant, designated the most highly contaminated area, was evacuated and sealed off by the authorities. Zone B, which had approximately 5,000 residents, was designated as a less contaminated area. These residents were allowed to stay (except that children under 15 years of age were removed from the zone during the day) (Whiteside, 1977), although strict measures were taken with regard to food and water supplies and the use of crops, milk, and farm animals. Zone R (zone of caution or "respect") was originally judged free from contamination, but subsequently, it was found to be slightly and unevenly contaminated; local residents in this area were allowed to stay, and only minor measures were taken with regard to food and water supplies (Bisanti et al., 1980; Young and Regianni, 1988).

To assess the health effects on the general population exposed to the dioxin that had been released into the environment, the Ministry of Health in Rome established the Technical Committee for the Seveso Accident to review all data collected between 1976 and 1981. In 1977, the National Academy of Sciences was invited by the Italian government to join in a collaborative effort to investigate the effects of the chemical contamination at Seveso (NRC, 1982b).

The Italian government cleaned up zone A by removing the highly contaminated soil in certain areas, depositing it in a plastic-lined landfill,

and monitoring the landfill for potential leakage. Clean soil was then laid over areas previously contaminated, and trees and grass were planted. In less contaminated areas, the soil was turned over frequently because sunlight degrades dioxin in the soil. The Seveso accident provided a great deal of information regarding high level exposure of men, women, and children to dioxin, and ways to manage this type of accidental environmental exposure (Gough, 1986).

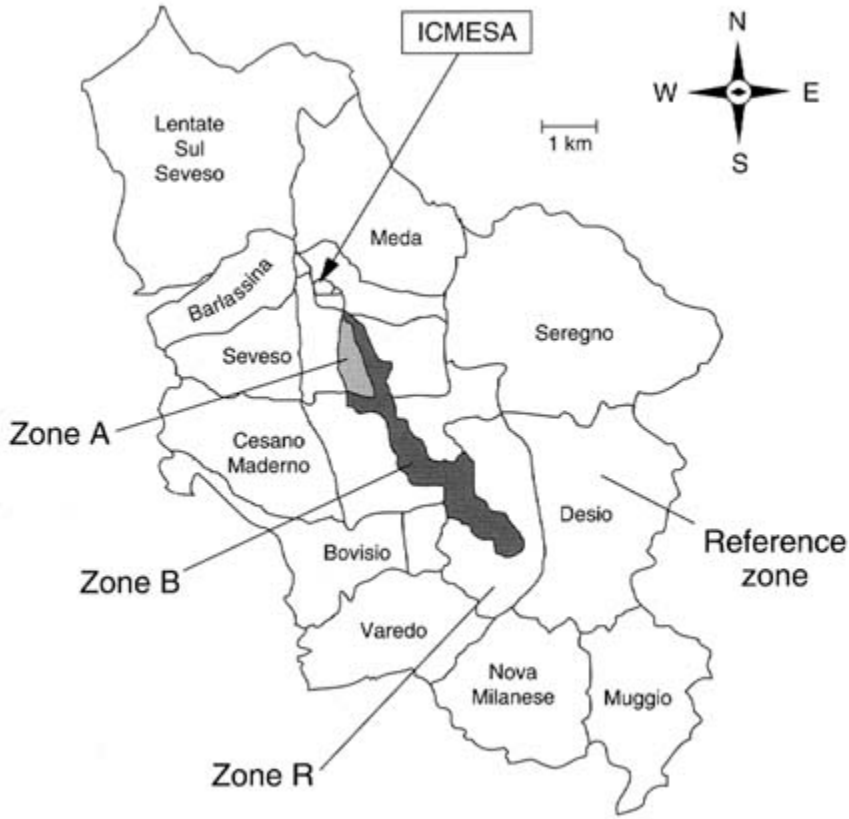

FIGURE 2-1 Area affected by the ICMESA plant accident in Seveso, Italy, in 1976. The three zones A, B, and R are indicated.
Adapted from Bertazzi et al., 1989b.

Several studies have been published that examine the health effects of the residents in the Seveso region. In one of the initial studies, Boeri and colleagues (1978) investigated the incidence of neurological disorders in the Seveso population exposed to TCDD and in a comparison group. Bisanti and colleagues (1980) chronicled the experiences from the accident to determine

the dioxin exposure of the local residents and potential health effects. In a study examining exposure to dioxin in children, Mocarelli and colleagues (1986) investigated whether the TCDD that was released to the atmosphere had any effect on the liver function and lipid metabolism of exposed children. In a 10 year mortality study of the population, Bertazzi and colleagues (1989) examined the mortality between 1976 and 1986 in the exposed population in comparison to mortality that would be expected in the general population. Pesatori and colleagues designed a cancer incidence study, 1977-1986, of the population living in the Seveso area from 1976 to 1986 (Pesatori et al., 1992).

THE FEDERAL GOVERNMENT'S RESPONSE TO PUBLIC CONCERNS

The federal government has been involved with international and domestic policy issues over the military use of herbicides and subsequent human health concerns about exposure to herbicides, particularly Agent Orange, since the onset of the defoliation program. In the late 1960s, several members of the international community became concerned over the U.S. use of herbicides in the Vietnam conflict, and on December 16, 1969, the United Nations General Assembly passed a resolution stating that "the Geneva Protocol embodies the generally recognized rules of international law prohibiting the use in international armed conflicts of all biological and chemical methods of warfare" (Dux and Young, 1980).

On December 16, 1974, the U.S. Senate consented to ratify the Geneva Protocol, which broadly sought an international commitment from all governments that they would never use chemical or biological weapons in war. A U.S. delegate had signed the protocol in 1925; however, the U.S. Senate did not give its consent to ratify the document at that time. President Richard Nixon signed it into force for the United States on February 10, 1975 (von Glahn, 1981). Chemical warfare includes additional aspects, such as the use of herbicides, not specifically covered by the Geneva Protocol. In April 1975, President Ford set forth future U.S. policy governing the use of herbicides in war in Executive Order 11850: "The United States renounces, as a matter of national policy, first use of herbicides in war except use, under regulations applicable to their domestic use, for control of vegetation within U.S. bases and installations or around their immediate defensive perimeters" (von Glahn, 1981; Buckingham, 1982).

White House

In response to the rising concern among veterans about possible health effects of dioxin exposure in Vietnam, President Jimmy Carter established

the Interagency Working Group on the Long-Term Health Effects of Phenoxyherbicides and Contaminants in December 1979. "This group's mission was to bring together knowledgeable government scientists to oversee the matters in the area of phenoxy herbicides and dioxin-related matters, to identify areas where scientific study was needed, and to report the results as soon as they became available to Congress and the public. The group reported to the Secretary of the [then] Department of Health, Education, and Welfare and to the White House Domestic Council" (AOTF, 1990). In August 1981, President Ronald Reagan renamed the working group the Agent Orange Working Group (AOWG), elevated it to be part of the Cabinet Council on Human Resources, and enlarged its scope of work. On April 11, 1985, the cabinet councils were reorganized from eight separate councils into two, the Council on Economic Policy and the Council on Domestic Policy, to which AOWG reported (AOWG, 1985, 1987).

Representatives of the Department of Health and Human Services; the Department of Defense; the State Department; the Veterans Administration; the Environmental Protection Agency; the Department of Agriculture; the Department of Labor's Occupational Safety and Health Administration; the White House Offices of Science and Technology Policy and Management and Budget; the Council of Economic Advisors; and the U.S. Congress Office of Technology Assessment (observer status) served as members of the AOWG (AOWG, 1985, 1987).

The AOWG evaluated the government's scientific research in Agent Orange and related issues (AOWG, 1985). A Science Panel was established to monitor research that the federal government was sponsoring in this area and, in addition, to provide scientific peer review of protocols and subsequent studies (U.S. Congress, Senate, 1988). These studies included the U.S. Air Force Ranch Hand Study; the CDC's Birth Defects Study, Vietnam Experience Study, Agent Orange Study, and Selected Cancers Study; the Department of Veterans Affairs' Proportional Mortality Study; and other related studies. On March 29, 1990, the Agent Orange Task Force (AOTF) was established under the Domestic Policy Council's Working Group on Health Policy to replace the AOWG (AOTF, 1990).

U.S. Congress

Congress has been following the issue of the military use of herbicides and potential health effects thought to be associated with exposure to the herbicides since 1970. Beginning in 1978, members of Congress began to raise questions about the health concerns of veterans potentially exposed to Agent Orange in Vietnam. There are three specific categories that health concerns of Vietnam veterans fall into: (1) access to health care for current problems that might be related to the exposure; (2) scientific answers to

questions about the health effects of exposure to Agent Orange; and (3) compensation for disabilities possibly related to exposure (U.S. Congress, Senate, 1989). Over the past 20 years, congressional committees have held hearings and introduced bills on this topic, and in an attempt to resolve this issue, Congress passed several laws dealing with the human health effects of exposure to Agent Orange used in Vietnam during the Vietnam era.

Hearings

The first congressional hearing related to herbicides used in Vietnam was held on April 7 and 15, 1970, by the Subcommittee on Energy, Natural Resources, and the Environment of the Senate Committee on Commerce (U.S. Congress, Senate, 1970). In the late 1970s, veterans began to ask serious questions regarding their potential exposure to Agent Orange in Vietnam (U.S. Congress, Senate, 1989). Lawmakers seeking answers to these difficult questions began to hold hearings on this issue. Since 1978, numerous hearings have been held by several committees and subcommittees to understand the complex issues concerning exposure assessment and to gather the most current scientific knowledge on the long-term health effects of exposure to herbicides and dioxin (TCDD) used in Vietnam during the Vietnam era. Lawmakers heard testimony from leading scientists, representatives of government agencies, members of veterans organizations, and individual veterans and their families. Listed in Table 2-1 are selected congressional hearings and reports on Agent Orange and dioxin.

Legislation on Agent Orange

Congress passed its first legislation that dealt with the issue of military use of herbicides in Vietnam (Public Law 91-441) on October 7, 1970. This law directed the Secretary of Defense to contract with the National Academy of Sciences to conduct a comprehensive study of the ecological and physiological dangers inherent in the use of herbicides, and of the defoliation program carried out in Vietnam.

A major focus of the Senate and House Committees on Veterans' Affairs has been to understand better the human health effects of exposure to Agent Orange used in Vietnam during the Vietnam era. Legislation on Agent Orange falls primarily into three categories: (1) health care—access to VA medical centers for veterans exposed to Agent Orange during service in Vietnam; (2) scientific research—the human health effects of exposure to Agent Orange in Vietnam, and how best to address the special needs of those veterans who may have been exposed to it; and (3) compensation—to address the issue of compensation for disabilities that might have resulted from exposure to Agent Orange in Vietnam (U.S. Congress, Senate, 1989).

TABLE 2-1 Selected Congressional Hearings and Reports on Agent Orange and Dioxin

Committee/Subcommittee	Hearing Description	Date of Hearing
U.S. Senate		
Committee on Commerce Subcommittee on Energy, Natural Resources, and the Environment	Effects of 2,4,5-T on man and the environment	April 7 and 15, 1970
Committee on Veterans' Affairs	Appendix on Agent Orange activities; Readjustment of Vietnam era veterans with emphasis on Agent Orange; Status of Agent Orange-related activities; Issues related to Agent Orange (adverse health effects from potential exposure to herbicides used in Vietnam); Scientific knowledge and studies regarding the long-term health effects of exposure to Agent Orange; Scientific knowledge relating to Agent Orange exposure and the concerns of veterans who may have been exposed in Vietnam; Legislative hearing on S. 1153 and Agent Orange issues	April 10, 1979; January 25-26, 1980, and; February 21, 1980; September 10, 1980; November 18, 1981; June 15 and 22, 1983; May 12, 1988; June 22, 1989
U.S. House of Representatives		
Committee on Interstate and Foreign Commerce	Involuntary exposure to Agent Orange and other toxic spraying	June 26 and 27, 1979
Subcommittee on Oversight and Investigations	Vietnam veterans' exposure to Agent Orange	September 25, 1980

Committee on Veterans' Affairs Subcommittee on Medical Facilities and Benefits	Herbicide use in Vietnam with the focus on Agent Orange; Testimony on Agent Orange; Scientific Community Report on Agent Orange	October 11, 1978; February 25, 1980, and; July 22, 1980; September 16, 1980
Subcommittee on Oversight and Investigations	Status of Agent Orange studies; Status of federal Agent Orange activities; Status of federally conducted Agent Orange studies	May 6, 1981; September 15, 1982; May 3, 1983
Committee on Veterans' Affairs Subcommittee on Compensation, Pension, and Insurance	H.R. 1961, Vietnam Veterans Agent Orange Relief Act; Veterans Agent Orange Exposure and Vietnam Service Benefits Act of 1989	April 26-27, 1983, and; July 12, 1983; May 2, 1990
Committee on Veterans' Affairs Subcommittee on Hospitals and Health Care	Report on a mission to Vietnam; Centers for Disease Control's birth defects study; Agent Orange Studies; Scientific research on the health of Vietnam veterans; Review of the status of Agent Orange studies	January 31, 1984; October 3, 1984; July 31, 1986; June 8, 1988; July 10, 1989
Committee on Veterans' Affairs	CDC's Selected Cancers Study and the scientific reviews of the study	April 4, 1990
Committee on Government Operations Human Resources and Intergovernmental Relations Subcommittee	Review of CDC's Agent Orange study; Links between Agent Orange, herbicides, and rare diseases; Report on the Agent Orange cover-up: a case of flawed science and political manipulation Health risks of dioxin	July 11, 1989; June 26, 1990; August 2, 1990; June 10, 1992

Legislation has also been enacted to appropriate funds for Agent Orange research, to provide clarification on payments received from the Agent Orange settlement fund, and to review and evaluate scientific literature regarding associations between diseases and exposure to dioxin and other chemical compounds in herbicides used in Vietnam.

Health Care Public Law 97-72, enacted on November 3, 1981, expanded eligibility for health care services to include veterans exposed to Agent Orange in Vietnam during the Vietnam era. The effect of this legislation was to provide health care for Vietnam veterans for conditions requiring treatment that resulted from exposure to Agent Orange. The veteran does not need to demonstrate any link with Agent Orange; rather, care is provided unless the condition is shown to be due to something other than exposure (e.g., congenital or developmental conditions or conditions resulting from postservice trauma; Conway, 1993). Public Law 99-166, enacted December 3, 1985, extended the program through September 30, 1989. Public Law 100-687, enacted November 18, 1988, extended the program through December 31, 1990; and Public Law 102-4, enacted February 6, 1991, extended the program through December 31, 1993.

Epidemiologic Studies Public Law 96-151, enacted on December 20, 1979, mandated the Veterans Administration to conduct an epidemiologic study of the possible health effects in Vietnam veterans of exposure to dioxin as found in the herbicides used in Vietnam. The legislation also required that the Office of Technology Assessment review and approve the protocol for the study. Public Law 97-72, enacted on November 3, 1981, expanded the scope of the epidemiologic study legislated in Public Law 96-151 to include an evaluation of the impact on the health of Vietnam veterans of other environmental factors that occurred in Vietnam; this is referred to as the "Vietnam Experience Study." To understand possible health effects experienced by women in Vietnam, Congress enacted Public Law 99-272 on April 7, 1986, directing the VA to conduct an epidemiologic study of the long-term health effects on women who served in Vietnam.

Compensation Congress enacted Public Law 98-542 on October 24, 1984, the Veterans' Dioxin and Radiation Exposure Compensation Standards Act, to address the issue of compensation for disabilities that might have resulted from exposure to Agent Orange in Vietnam. This law "provided for payment, during a two year interim period from October 1, 1984 to September 30, 1986, of disability and death benefits for Vietnam veterans with chloracne and porphyria cutanea tarda (an uncommon disorder of urinary porphyrin metabolism manifest in-patients by thinning and blistering of the skin) which became manifest within one year after service in Vietnam and the survivors of veterans with such conditions" (U.S. Congress, Senate, 1989). Public Law 98-542 also set forth a mechanism for the VA to issue

standards for determining claims for compensation based upon exposure to Agent Orange. Additionally, the law required VA to establish the Veterans Advisory Committee on Environmental Hazards. The purpose of the committee was to advise the Administrator on the content of VA regulations relating to claims for compensation based on exposure to Agent Orange. Also, the committee was to provide advice and recommendations on completed research on other administrative and legislative initiatives (Conway, 1993). Public Law 102-4, the Agent Orange Act of 1991, was enacted on February 6, 1991, to grant disability compensation payments for chloracne, non-Hodgkin's lymphoma, and soft tissue sarcoma (other than osteosarcoma, chondrosarcoma, Kaposi's sarcoma, or mesothelioma) associated with Agent Orange. This law also transferred the responsibility of reviewing the scientific literature concerning the association between herbicide exposure during Vietnam service and each health outcome suspected to be associated with such exposure from the DVA's Advisory Committee on Environmental Hazards to the National Academy of Sciences.

Appropriations In Public Law 98-181, enacted on November 30, 1983, Congress appropriated $57.4 million to CDC to conduct research on the health risks for Vietnam veterans exposed to Agent Orange. On November 18, 1988, Congress enacted Public Law 100-687 to appropriate funds to cover expenses of the U.S. Air Force Ranch Hand study. This law also required the VA to carry out an outreach program to keep Vietnam veterans (including veterans listed in the Agent Orange Registry) informed of new developments regarding Agent Orange-related information.

Agent Orange Settlement Fund Two public laws were enacted that dealt with payments received from the *In re Agent Orange Product Liability Litigation* settlement fund. Public Law 100-687 provided that payments to veterans or members of their families from the Agent Orange litigation settlement fund should not be considered as income for purposes of VA needs-based programs. Public Law 101-239, enacted on December 19, 1989, provided that the payments should not be considered as income in determining eligibility for the amount of benefits under any federal or federally assisted program.

National Academy of Sciences This report addresses the congressional mandate in Public Law 102-4, that the Department of Veterans Affairs contract with the National Academy of Sciences to review and evaluate the available scientific evidence regarding associations between diseases and exposure to dioxin and other chemical compounds in herbicides used in Vietnam during the Vietnam era. The National Academy of Sciences is also required to make recommendations concerning the need, if any, for additional scientific studies to resolve areas of continuing scientific uncertainty.

Office of Technology Assessment

The technical support office of the U.S. Congress is the Office of Technology Assessment (OTA). In 1979, Public Law 96-151 mandated that the OTA director review and approve plans for the VA's epidemiologic study on the possible long-term health effects resulting from exposure to dioxin-containing herbicides in Vietnam, as well as the study on women Vietnam veterans' health (U.S. Congress, Senate, 1989).

The question of the constitutionality of the congressional mandate that created the role for OTA to review VA protocols on Agent Orange studies was raised by the executive branch. The question was based on providing OTA, a congressional branch agency, veto authority over the execution of a study by an executive branch agency. The executive branch attorneys considered this a violation of the separation of powers doctrine and concluded it was unconstitutional, but legislative branch attorneys concluded that it was constitutional (Gough, 1988).

OTA assembled an Advisory Panel on Agent Orange to assist in reviewing the Agent Orange study protocols, including the CDC's Vietnam Experience Study, Agent Orange Study, and Selected Cancers Study (Gough, 1988), and the VA's Women Vietnam Veterans Health Study. OTA has also reviewed studies for VA committees, has testified at numerous congressional hearings, and was involved in the cancellation of CDC's Agent Orange Study.

General Accounting Office

The General Accounting Office (GAO) has prepared several reports on health effects of exposure to Agent Orange in Vietnam during the Vietnam era. GAO issued its first report on August 16, 1978, on the Department of Defense's use of herbicides in Vietnam and the Veterans Administration's handling of herbicide exposure disability claims submitted by Vietnam veterans (U.S. GAO, 1978). On April 6, 1979, a second report focused on the VA's response to veterans' concerns about exposure to herbicides used in Vietnam (U.S. GAO, 1979a). The report recommended that the Department of Defense, with the assistance and guidance of an appropriate interagency group, conduct a survey of long-term health effects on military personnel who served in Vietnam and possibly were exposed to herbicides.

The GAO, in its third report issued on November 16, 1979, focusing on U.S. ground troops in Vietnam, concluded that a large number of marines in the I Corps section of Vietnam from 1966 to 1969 were close to areas sprayed with Agent Orange both on the day of the spraying and within four weeks afterward (U.S. GAO, 1979b). Some Army units were also close to Agent Orange spraying (U.S. GAO, 1979b). Issued on October 25, 1982,

another GAO report reviewed the VA Agent Orange examination program, and the reliability and completeness of the Agent Orange Registry (U.S. GAO, 1982). The most recent GAO report, dated September 1990, examined the contracting practices of the CDC studies on the effects of Agent Orange on the health of Vietnam veterans (U.S. GAO, 1990). These studies include the Vietnam Experience Study, the Agent Orange Study, the Selected Cancers Study, and the Agent Orange Validation Study. This report reviewed (1) the amount of funds CDC received to do the studies, (2) how CDC used the funds it received, and (3) the contracts awarded for the studies to identify weaknesses that may have occurred in CDC's contracting and contract administration practices for the above-mentioned CDC studies (U.S. GAO, 1990).

Department of the Air Force

In 1979, the Air Force began an epidemiologic study of Ranch Hand personnel who participated in the aerial spraying of herbicides in Vietnam to determine whether they suffered long-term health effects from their exposure to Agent Orange and other herbicides. The 20 year Ranch Hand study is designed to determine whether adverse health effects exist and can be attributed to occupational exposure to Agent Orange. The health of Ranch Hand personnel is being compared to other Air Force servicemen who served in Vietnam but were not exposed to herbicides (U.S. Congress, Senate, 1989). The study consists of mortality and morbidity components, based on follow-up examination results. The following reports have been published to date:

Baseline Mortality Report (AFHS, 1983)

Baseline Morbidity Report (AFHS, 1984a)

First Follow-up Exam Results (AFHS, 1987)

Second Follow-up Exam Results (AFHS, 1990)

Serum Dioxin Level Follow-up Exam Results (AFHS, 1991b)

Mortality Updates 1984, 1985, 1986, 1989, 1991 (AFHS, 1984b, 1985, 1986, 1989, 1991a)

Reproductive Outcomes (AFHS, 1992)

A follow-up exam was conducted in 1992, and additional follow-up exams are scheduled for 1997 and 2002.

Department of Veterans Affairs

The Department of Veterans Affairs (DVA; formerly the Veterans Administration) has been involved in conducting and assessing research, and monitoring studies on the health effects of Agent Orange for almost 15

years. Its Advisory Committee on Health-Related Effects of Herbicides (whose members included government scientists, representatives of veterans organizations, and academic scientists) was in existence from June 1979 until September 1990. In October 1984, the Congress enacted Public Law 95-542, which established the Veterans Advisory Committee on Environmental Hazards. The committee had responsibility, in part, for reviewing the scientific literature relating to the health effects of exposure to Agent Orange. This function was transferred to the National Academy of Sciences following the enactment of Public Law 102-4 in February 1991.

Health Care

The DVA provides certain health care services to veterans of the Vietnam era (defined as August 5, 1964-May 7, 1975) possibly exposed to herbicides (contaminated with dioxin) used for military purposes in Vietnam. Prior to being admitted for health care services, the veteran must provide proof of service in Vietnam. Health care services are limited to hospital and nursing home care and outpatient care in DVA facilities, on a pre- or posthospitalization basis or to prevent a need for hospitalization (U.S. DVA, 1992). When a Vietnam veteran requests DVA medical care, he or she is evaluated by a physical examination and appropriate diagnostic studies, which may serve as the Agent Orange examination (U.S. DVA, 1992).

Research Efforts

The DVA's Environmental Epidemiology Service (EES) has conducted several research studies on Vietnam veterans. The Agent Orange Registry serves as a health surveillance data base; it contains 10 percent of the entire Vietnam veteran population (self-selected) and is routinely reviewed for changes in health outcome and mortality patterns. Several studies have been published on posttraumatic stress disorder among veterans in the Agent Orange Registry (True et al., 1988; Farberow et al., 1990; Bullman et al., 1991). The DVA has conducted studies on soft tissue sarcoma and military service in Vietnam (Kang et al., 1986; 1987). Several mortality studies have been conducted on Vietnam veterans using data from Army and Marine death records for the period 1965-1982 (Breslin et al., 1988) and for the period 1965-1984 (Watanabe et al., 1991), and on the Army I Corps veterans (Bullman et al., 1990). A study has been published on the morbidity and mortality experience of Army Chemical Corps Vietnam veterans (Thomas and Kang, 1990), based on rosters compiled from morning reports for all Chemical Corps units assigned to Vietnam between 1968 and 1987. Thomas and colleagues (1991) examined the mortality among women Vietnam

veterans for the period 1973-1987. For the above-mentioned studies, the methodological framework is described in Chapter 7, and the results are discussed in the health outcome chapters (8-11).

Compensation and Benefits

The Department of Veterans Affairs compensates veterans for certain diseases related to service in Vietnam and exposure to herbicides containing dioxin. Whenever the Secretary determines, on the basis of sound medical and scientific evidence, that a positive association exists between the exposure of humans to an herbicide agent, and the occurrence of a disease in humans, the Secretary prescribes regulations providing that a presumption of service connection is warranted for that disease.

The current DVA compensation policy provides that in making determinations, the Secretary shall take into account reports from the National Academy of Sciences and all other sound medical and scientific information and analysis. In evaluating any study for the purpose of making such determinations, the Secretary shall take into consideration whether the results are statistically significant, are capable of replication, and withstand peer review [38 USC 1116 (b)(2)]. An association between the occurrence of a disease in humans and exposure to an herbicide agent is considered to be positive if the credible evidence for the association is equal to or outweighs the credible evidence against the association [38 USC 1116 (b)(3)]. Proposed regulations on compensation or denial of compensation for these diseases are published in the *Federal Register*. The DVA solicits comments from the public before final regulations are issued.

For claims based on exposure to herbicides (containing dioxin), the DVA currently compensates for chloracne and soft tissue sarcomas (excluding osteosarcoma, chondrosarcoma, Kaposi's sarcoma, and mesothelioma) (38 USC 1116). Regulations currently proposed would compensate for peripheral neuropathy and deny compensation for lung cancer (57 FR, 2236-7, July 10, 1992). Any veteran who served in the Republic of Vietnam during the Vietnam era (defined as August 5, 1964-May 7, 1975) is presumed to have been exposed to an herbicide containing dioxin. In 1984, Public Law 98-542 authorized interim benefits for a two year period for porphyria cutanea tarda (PCT), which has since expired on its own accord. At its meeting on August 23, 1990, the Advisory Committee on Environmental Hazards did not find a significant statistical association between exposure to herbicides and PCT (U.S. DVA, 1990); the DVA does not currently compensate for PCT.

For claims based on service in Vietnam, the DVA currently compensates for non-Hodgkin's lymphoma (NHL) [38 USC 501(a)]. Service in Vietnam during the Vietnam era, together with the development of NHL

manifested subsequent to such service, is sufficient to establish service connection for this disease.

As of September 1992, DVA data indicate that 76 veterans were receiving service-connected compensation for chloracne, 339 were receiving compensation for STS, and 695 were receiving compensation for NHL. DVA does not have available data on claims in which compensation for these or other conditions was denied. Statistics maintained on pending claims for service-connected disability compensation are not broken down by the type of disability claimed (Hickman, 1993).

Outreach Activities

The DVA's Environmental Agents Service (EAS) is responsible for the development and implementation of its national medical policies and procedures regarding exposure of military veterans to possible environmental hazards, including Agent Orange.

The Agent Orange Registry (AOR), maintained by EAS, is a computerized index of Agent Orange medical examinations. The AOR was initiated by the DVA in mid-1978 to address the health concerns of Vietnam veterans and provide a data base for Vietnam veteran health surveillance. Any Vietnam veteran is eligible for inclusion in the AOR. Veterans reporting to the DVA are examined by a DVA physician, and any diagnoses made by the physician are recorded in the AOR. Between 1978 and 1982, approximately 85,000 veterans reported for an AOR exam and their diagnoses were recorded using one or more of 30 broad diagnostic categories. Beginning in 1982, International Classification of Diseases (ICD) codes were used to record any diagnosis. As of December 1991, there were 129,709 veterans on the computerized registry, whose diagnoses were recorded using ICD codes. In addition to diagnostic data, the AOR also has a variety of self-reported demographic and military characteristics (U.S. DVA, 1992).

Agent Orange Registry participants (all self-selected) are added to the mailing list to receive the *Agent Orange Review*, a newsletter that provides updated information about Agent Orange. The EAS also compiles fact sheets, *Agent Orange Briefs*, about Agent Orange and related concerns; copies of these briefs are available through the Agent Orange Coordinator at all DVA medical centers. In addition to the newsletter and the briefs, videotape programs explaining the issues surrounding Agent Orange are available at all DVA medical centers. Each medical center has a designated environmental physician and an Agent Orange Coordinator to manage its Agent Orange program (U.S. DVA, 1992). There are other information resources available to veterans, including the multivolume document *Review of Literature on Herbicides, Including Phenoxy Herbicides and Associated Dioxins* (U.S. DVA, 1981-1992).

Department of Health and Human Services

Centers for Disease Control

Beginning in the late 1970s, Vietnam veterans began to express their concern over the possibility that they had an increased risk of fathering babies with birth defects from their exposure to herbicides used during the Vietnam conflict. Anecdotal reports also put forth the claim that Vietnamese men who served in the conflict were at increased risks. Because of this growing concern, the CDC began a study in 1982 to determine whether Vietnam veterans were at risk of fathering babies with birth defects, utilizing a comprehensive registry of birth defects in Atlanta, Georgia (Erickson et al., 1984; U.S. Congress, Senate, 1989).

Public Law 96-151 (signed on December 20, 1979) directed the Veterans Administration to conduct an epidemiologic study to determine whether an association exists between exposure to herbicides used in Vietnam and long-term health effects. Public Law 97-72 expanded the scope of the study to include an evaluation of other environmental factors in Vietnam that may have affected the health of veterans (U.S. GAO, 1990). The VA contracted with the University of California, Los Angeles, to develop the study protocol for the epidemiologic study; however, the Office of Technology Assessment did not approve this study protocol. Due to the length of time that passed without producing an approved protocol, VA signed an interagency agreement, in January 1983, with the CDC to transfer the authority for the design, implementation, analysis, and scientific interpretation of the studies directed by Public Laws 96-151 and 97-72 (U.S. Congress, Senate, 1989; U.S. GAO, 1990). The CDC was identified as the organization most qualified to conduct the study (U.S. GAO, 1990). The CDC developed protocols for three components of this epidemiologic study: the Vietnam Experience Study (VES), Agent Orange Study (AOS), and Selected Cancers Study (SCS); OTA and AOWG reviewed and approved the protocols for these studies (U.S. Congress, Senate, 1989). The National Academy of Sciences' Institute of Medicine also reviewed the protocols for the VES and SCS and, in addition, reviewed and commented on the validation study results.

Vietnam Experience Study (VES) *Health Status of Vietnam Veterans* examined the possible long-term effects of military service in Vietnam. According to CDC, the VES was designed to address the concern that there may have been other factors in addition to herbicide exposure that could have adversely affected veterans who served in Vietnam, in contrast to those who served elsewhere (CDC, 1989b). In the VES, CDC used a random sample of military records to find a large cohort of U.S. Army enlisted men who had served a single tour in Vietnam and a comparison cohort of U.S. Army enlisted men who had served elsewhere. The VES had four

components, including (1) mortality assessment, (2) telephone interview, (3) medical and psychological examination, and (4) a reproductive outcome assessment (CDC, 1987, 1988a-c; U.S. GAO, 1990).

Agent Orange Study (AOS) The Agent Orange Study was designed to determine if there were differences in the health of veterans who were exposed to herbicides in Vietnam compared to Vietnam veterans who were not exposed to herbicides (U.S. Congress, Senate, 1989). "Achieving this goal was problematic because a critical component of such a study was that there existed an accurate assessment of exposure to Agent Orange. … Thus the November 1983 protocol for the Agent Orange Study proposed an approach to estimating the opportunity for exposure to Agent Orange" (Young et al., 1986). CDC proposed to sort veterans into low-, medium-, and high-exposure categories based on pre-set criteria on which OTA and AOWG had agreed. This method was thought to be acceptable because it was assumed there would be a large group of clearly heavily exposed veterans. It was assumed that veterans would be classified based on their company location each day. CDC found there was not enough location information to use companies, and it proposed using battalions. OTA rejected this as causing potentially overwhelming misclassification. Further work with the Environmental Support Group and CDC determined that companies could be used, after all. However, when the veterans were ranked on the composite exposure scores, almost none were placed in the high- or even the medium-exposure category. CDC then proposed analyzing veterans based on their actual scores (not in categories), using regression analysis. There was so little spread in the scores, however, with almost all veterans showing no exposure, that OTA called for a reassessment of the study (Gelband, 1993). In 1986, the AOWG Science Panel Subpanel on Exposure Assessment noted (Young et al., 1986):

> Additional pilot data reviewed at this time confirmed this finding, and the paucity of clearly exposed combat veterans makes it questionable whether a sufficient number can be assembled to conduct an epidemiological study of the type originally designed. It is clear from the available data that health studies designed to assess the effects of Agent Orange and its associated dioxin can be done on more appropriate populations than those identified through military records, e.g., industrial workers and commercial herbicide applicators. Recent advances in analytical chemistry may make it feasible to identify chemical (e.g., 2,3,7,8-TCDD) or biological (DNA adducts) markers that will permit a more reliable exposure assessment. This Subpanel recommends that any study of ground troops, which is dependent upon military records for the assessment of exposure to herbicides, not be conducted without an additional method to verify exposure.

CDC developed a protocol for a study to relate levels of dioxin in veterans to information from military records, which was approved by OTA

in October 1986 (U.S. Congress OTA, 1987). The study, *Comparison of Serum Levels of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin with Indirect Estimates of Agent Orange Exposure Among Vietnam Veterans* (CDC, 1989a), also referred to as the "validation study," was designed to validate several indirect estimates of opportunity for exposure to Agent Orange among U.S. Army Vietnam combat veterans (646 veterans). The estimates, based on military records, were compared with serum levels of 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD). CDC identified a sample of U.S. Army Vietnam veterans and asked them to have blood drawn to measure the level of TCDD in their serum. Veterans were selected from among those who served in 65 combat battalions in III Corps. For comparison, a sample of non-Vietnam U.S. Army veterans of the same era was also examined (CDC, 1989a).

OTA and AOWG reviewed the validation study and concluded that it had been properly conducted and was scientifically valid. According to the CDC, the validation study confirmed that little exposure had taken place, producing results entirely consistent with the records-based exposure scores (Gelband, 1993). In a letter to Senator Alan Cranston, Don M. Newman, chairman, Domestic Policy Council, Agent Orange Working Group, stated that the "Agent Orange Exposure Study … has proved scientifically impossible to do" (U.S. Congress, Senate, 1989).

Selected Cancers Study (SCS) *The Association of Selected Cancers with Service in the U.S. Military in Vietnam* examined the risk of selected cancers among veterans; in the study, the CDC investigated certain uncommon forms of cancer that had been linked in prior studies with occupational exposures to phenoxy herbicides or chlorophenols (CDC, 1990a). In the population-based study, CDC examined the risk of (1) non-Hodgkin's lymphoma, (2) soft tissue and other sarcomas, (3) Hodgkin's disease, (4) nasal cancer, (5) nasopharyngeal cancer, and (6) primary liver cancers among Vietnam veterans (CDC, 1990a-d).

Environmental Protection Agency

In 1991, the Environmental Protection Agency began a scientific reassessment of the risks of exposure to 2,3,7,8-TCDD and chemically similar compounds, collectively known as dioxin. The reassessment is part of EPA's goals for improving the research and scientific base of the agency, and incorporating improved research and science into its decisions. EPA is undertaking this project on dioxin in response to emerging scientific knowledge and information on the mechanisms of action of dioxin. After EPA's Science Advisory Board reviewed the 1985 and 1988 assessments of the human health risks associated with environmental exposures to dioxin, it suggested that EPA look carefully at the current science and use a biologically

based dose-response model for predicting various exposures to dioxin and related compounds. The report, due in 1993, will include chapters on mechanisms of toxic action; disposition and pharmacokinetics; toxicology: acute subchronic and chronic, reproductive/developmental effects, immunotoxic effects, and carcinogenicity; and epidemiology/human data (U.S. EPA, 1992a,b).

RESPONSE BY OTHERS TO PUBLIC CONCERNS

State Governments

During the 1980s, many state governments set up commissions to address Vietnam veterans' concerns about health effects related to exposure to Agent Orange and dioxin. By 1993, only a few of these commissions remained active.

One of the more active of these groups, the New Jersey Agent Orange Commission, conducted a study in cooperation with Rutgers University known as the Pointman Project. This study examined exposure levels of Vietnam veterans who were herbicide handlers. It compared blood and fat dioxin levels in pairs of exposed and nonexposed veterans (Kahn et al., 1988). In the follow-up study, Pointman II, individuals in Army and Marine infantry units and Navy riverboat units were matched with controls with regard to age, race, dates of service, and rank (Kahn et al., 1992a-c).

Several studies have been conducted on Massachusetts Vietnam veterans; one such study was a cancer surveillance of veterans from 1982 to 1988 (Clapp et al., 1991). This study examined the findings of a previous mortality study of Massachusetts veterans who died between 1972 and 1983, and utilized the Massachusetts Cancer Registry, which collects information on all cases of malignant disease diagnosed after January 1, 1982. Other states have conducted studies and published reports on Vietnam veterans; these include Hawaii, Iowa, Maine, New Mexico, New York, Pennsylvania, Wisconsin, West Virginia, and Texas.

Veterans' Advocates

The American Legion, Vietnam Veterans of America, and the National Veterans Legal Services Project were dissatisfied with the efforts of the DVA and its Advisory Committee on Environmental Hazards in their rule-making procedures to determine which adverse health effects, if any, are associated with exposure to dioxin. These veterans organizations assembled the Agent Orange Scientific Task Force—a group of independent scientists knowledgeable about scientific studies concerning the health effects of exposure to dioxin. The task force prepared a report that summarized its review of the scientific literature related to potential human health effects

of exposure to herbicides and associated contaminants (dioxins). The task force was also asked to determine which adverse health effects are associated with exposure to dioxin, using the DVA standard of determination (Clapp et al., 1990).

On October 6, 1989, Edward J. Derwinski (then Secretary of Veterans Affairs) appointed Admiral E.R. Zumwalt, Jr., as a special assistant to help him in determining whether it is at least as likely as not that there is a statistical association between exposure to Agent Orange and specific health outcomes. On May 5, 1990, Admiral Zumwalt presented his report to the Secretary of Veterans Affairs. This report reviews relevant data in accordance with DVA standards relating to the evaluation of health effects of dioxin exposure. In addition to reviewing the data, Admiral Zumwalt reviewed the work of the DVA's Advisory Committee on Environmental Hazards' Scientific Council, and the protocol and standards employed by government-sponsored studies, to assess the credibility of these studies according to generally accepted scientific practice (Zumwalt, 1990).

Australia and South Korea

Australia, New Zealand, and South Korea also sent troops to Vietnam to serve during the Vietnam era. In response to concerns of Australian veterans similar to those of American veterans about possible exposure to herbicides used in Vietnam, the Australian government conducted its own inquiries into the association between health effects and exposure to herbicides.

In October 1981, the Australian Senate Standing Committee on Science and the Environment undertook a study on pesticides and the health of Australian Vietnam veterans. This committee reviewed the use of pesticides, particularly phenoxy herbicides and chemicals containing dioxin, with reference to their ecological effects, and their effects on human and animal health, dealing primarily with the possible effects on Vietnam veterans of exposure to herbicides; it included all issues adversely affecting those who served in Vietnam. The Senate Standing Committee conducted 10 public hearings in several different cities in Australia as part of its inquiry (Australian Senate, 1982).

In May 1983, the Australian government established a commission to inquire about the use of chemical agents in Vietnam during the Vietnam era; the effects on Australian personnel of exposure to the chemical agents used; and the operation and administration of relevant Australian laws relating to claims by Australian personnel of chemical-caused disabilities (Evatt, 1985).

Approximately 4,700 South Korean veterans have filed claims with the South Korean Vietnam War Veterans Office, reporting that their medical problems appear to be related to Agent Orange. South Korea sent approximately

320,000 soldiers to Vietnam, the second largest force after the United States. South Korea has indicated a new willingness to address the Agent Orange issue by reviewing recommendations from its Veterans Office to cover health costs of alleged Agent Orange victims and possibly file legal action against U.S. chemical manufacturers (New York Times, 1992b).

National Research Council and Institute of Medicine

A number of components of the National Research Council (NRC) and the Institute of Medicine (IOM) have studied the effects of Agent Orange and other herbicide use in Vietnam. Several groups developed conclusions directly related to the issues that are currently being examined by the IOM.

The NRC first became involved in the evaluation and understanding of the health effects of exposure to Agent Orange in 1970. As mentioned above, Public Law 91-441 of 1970 directed the Department of Defense to contract with the NAS for a study of the ecological and physiological effects of the military use of herbicides in Vietnam. A committee, organized through the Assembly of Life Sciences (ALS) of the National Research Council, developed an inventory of the areas sprayed by herbicides, based on DOD log books on herbicide missions. These data were transferred to data tapes (the so-called HERBS tapes) and provide information on 6,542 spray missions that occurred from August 1965 to February 1971, using a total of 17.6 million gallons of herbicide, of which approximately 11.3 million gallons and 4,109 missions involved Agent Orange. The committee reviewed the effects on various types of vegetation, studied the persistence of herbicides in the soil, and attempted to identify effects of the herbicides on resident populations believed to have been exposed. Its investigation determined that mangrove forests were particularly vulnerable to herbicide spraying, as were standing food crops. The extent of damage to the inland forest was more difficult to estimate since, for security reasons, the committee had to rely on aerial photographs in estimating the extent of damage (NAS, 1974).

The committee was unable to determine the direct effects of herbicides on human health. Individual case reports of adverse health effects could not be substantiated, although one component of the report published eight years later (Kunstadter, 1982) reviewed hospital records on births and birth defects in Vietnam. This report remained inconclusive as to the relationship between maternal exposure to herbicides and the incidence of birth defects.

In 1979, the Air Force requested that NAS review a protocol for an investigation of the health effects of Agent Orange exposure on Ranch Hand personnel. A panel, organized by the ALS, suggested that the number of Air Force personnel exposed to Agent Orange was too small to have sufficient statistical power to detect meaningful health effects in the proposed time

frame, that the number of health end points to be followed was too large, and that the public perception of the study's credibility would suffer if it were conducted by the Air Force (NRC, 1980). The Ranch Hand study was eventually conducted by the Air Force, over a longer time period than originally intended.

In 1977, the Italian government invited the National Academy of Sciences-National Research Council to join in a collaborative effort to investigate the effects of area-wide chemical contamination at Seveso, Italy. The NAS-NRC recommended the development of a continued relationship between U.S. and Italian scientists for the purposes of exchanging scientific and technical information, conducting complementary research, and organizing conferences to examine the impacts on health and the environment. In 1980, the ALS, as part of this collaborative effort, established the Committee on Response Strategies to Unusual Chemical Hazards. This committee published the proceedings of its international workshop on plans for clinical and epidemiologic follow-up after area-wide chemical contamination, which was held in Washington, D.C., in March 1980 (NRC, 1982b).

In 1982, the NRC Commission on Life Sciences (CLS) reviewed and commented on a proposed epidemiologic study of the health effects of exposure to Agent Orange and Vietnam service in general. This report made a number of recommendations about a study protocol proposed by the Veterans Administration, including the need for separate studies of exposure to Agent Orange and of Vietnam service in general, rather than one combined study; the need for a formal review of the methods used to classify subjects according to degree of exposure to Agent Orange; and the need for quality control and other validity studies (NRC, 1982a). The protocol that the CLS reviewed was never carried out by the VA, but some of the committee's recommendations were consistent with studies that were eventually carried out by CDC.

In a series of letter reports from 1986 through 1990 (IOM, 1986a,b, 1987a-e, 1988a-e, 1989a,b, 1990a,b), the IOM advised the CDC on its conduct of studies on the health effects of Vietnam service (Advisory Committee on the CDC Study of the Health of Vietnam Veterans). These studies, mandated by Public Laws 96-151 and 97-72, were to determine the long-term health effects of veterans' exposure to herbicides (the Agent Orange Study), the possible long-term effects of military service in Vietnam (the Vietnam Experience Study), and the risk of selected cancers among veterans (the Selected Cancers Study). Although the Agent Orange Study was canceled, the IOM committees reviewed study protocols and preliminary results for the VES and SCS (IOM, 1990a,b), and their recommendations were incorporated into the analyses and final results published by CDC. The IOM advisory committee did review and comment on the validation study results that contributed to the decision not to complete the Agent

Orange Study (IOM, 1987c) but it was not asked, and did not make, a recommendation about the cancellation of that study.

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3

The U.S. Military and the Herbicide Program in Vietnam

From 1962 to 1971, the U.S. Air Force sprayed nearly 19 million gallons of herbicides in Vietnam, of which at least 11 million gallons was Agent Orange, in a military project called Operation Ranch Hand. An additional quantity (1.6 million gallons has been documented) of herbicides was applied to base perimeters, roadways, and communication lines by helicopter and surface sprayings from riverboats, trucks, or backpacks. Herbicide operations in Vietnam had two primary military objectives: (1) defoliation of trees and plants to improve observation, and (2) destruction of enemy crops.

Estimates of the number of U.S. military personnel who served in Vietnam during this period of herbicide use vary from 2.6 to 3.8 million. The total number of U.S. servicemen and women exposed to herbicides is also not known, although some individuals, such as those of the Air Force Operation Ranch Hand and the Army Chemical Corps, were more likely to have been exposed by the nature of their job assignments.

This chapter describes the characteristics of the Vietnam veteran population at potential risk of herbicide exposure during service in Vietnam, and reviews the U.S. military herbicide program of the 1960s and early 1970s. It summarizes what is known about the use of herbicides in Vietnam, their chemical formulations, and the quantities applied, and serves as background information for Chapter 6, which summarizes and evaluates the various methods for estimating exposure to herbicides in epidemiologic studies of veterans as well as other environmentally and occupationally exposed populations.

MILITARY AND DEMOGRAPHIC CHARACTERISTICS OF VIETNAM VETERANS

As one historian notes in his account of the Vietnam conflict, "there was no 'typical' U.S. soldier in Vietnam … the three million Americans who served there went through many varied experiences—partly because the quality of the war varied in different areas of the country, and partly because the nature changed over time" (Karnow, 1991). Individual experiences also varied according to job assignment, military unit of service, rank, and branch of service. Artillery units, for example, tended to be less mobile than cavalry because of the heavy equipment involved. An individual assigned to base headquarters with an Army personnel position experienced a different tour of duty than an infantry commander, a field engineer, or an officer stationed aboard a Navy vessel off the coast of I Corps. Personnel assigned to units in the Mekong Delta might slog week after week across paddy fields, while others patrolling the perimeters of major U.S. installations at Danang, Bien Hoa, and Camranh were often targets for sniper attacks (Karnow, 1991). Individuals and units also varied in their consumption of locally grown foods and water from local supplies, as well as in their personal hygiene practices. Ground forces—the Army and Marines—were likely to experience more of the day-to-day fighting than Navy or Air Force personnel (Card, 1983). Sociological assessments of the American soldier in Vietnam suggest that no one factor is more important in understanding the experiences of the individual veteran than the degree of exposure to combat (Moskos, 1975; Fischer et al., 1980; Martin, 1986; Shafer, 1990).

In order to properly evaluate existing epidemiologic studies of Vietnam veterans and to consider the possibility of new studies, the size and characteristics of the exposed population must be known. Remarkably, the number of U.S. military personnel who served in Vietnam during the Vietnam conflict is not known precisely. Estimates depend on definitions regarding time and place of service, and the source of the data on which the estimates are based. Although detailed records of demographic information were not compiled during the Vietnam era, some federal estimates are available. In addition, data from several national surveys of the Vietnam veteran population supplement the government estimates.

Estimates of the Number of Military Personnel Serving in Vietnam

According to official records, U.S. military advisory assistance to Vietnam began as early as 1950, during the First French-Indochina War; 128 personnel "spaces" were allotted for the U.S. advisory group (MACV, 1972). After the division of Vietnam along the seventeenth parallel in 1954, U.S.

advisors were assigned the responsibility of training the South Vietnamese army. By the end of 1960, nearly 900 U.S. military advisors were stationed in the country—the vanguard of an estimated 2.6 to 3.8 million U.S. military personnel to serve in Vietnam over the next 13 to 15 years. Two advisors were killed in 1960 in a raid at Bien Hoa military base—the first American casualties of the Vietnam conflict (MACV, 1972).

As American concerns about Communist control of South Vietnam heightened, U.S. involvement increased. Toward the end of 1962, 11,000 U.S. military advisors and personnel were serving in South Vietnam, and the U.S. Military Assistance Command, Vietnam (MACV) was organized. During the next two and a half years, the number of personnel would increase to nearly 60,000 and America began bombing North Vietnam—first as "retaliation for North Vietnamese aggression" and ultimately, in February 1965, as a sustained activity (Shafer, 1990). Shortly afterward, President Lyndon Johnson ordered the deployment of U.S. military troops to South Vietnam (see Table 3-1).

The Vietnam era was characterized by heavy conscription that began to gain momentum in 1965 (Card, 1983). Between 1964 and 1968, as American involvement in Vietnam escalated, U.S. troop strengths doubled, then nearly tripled, peaking at 543,482 in April 1969. The number of military personnel declined in the following years, in keeping with President Nixon's policy of "Vietnamization," falling to 475,000 at the end of 1969 and to 334,600 at the end of 1970. By the end of 1972, fewer than 25,000 American troops remained in Vietnam. The final U.S. withdrawal of American combat troops in Vietnam was completed in March 1973. The remaining Americans, including the ambassador to Vietnam, were evacuated from the U.S. embassy in Saigon in April 1975 (Karnow, 1991).

Although the military maintained accurate records of the number who died, reliable records-based information on the number and characteristics of men and women who served in the Vietnam conflict was more difficult to maintain during the wartime period. Because there is no master list of the millions of veterans who served during the Vietnam era, studies of Vietnam veterans must create their own sampling frames from which samples of veterans can be selected and from which national estimates can be generated. These estimates of the national Vietnam veteran population are necessary for epidemiologic studies of veterans that attempt to quantify the risk of various health effects for the entire veteran population-based on the results observed in representative samples. The extent to which the study sample is representative of the entire Vietnam veteran population in these epidemiologic studies is of utmost importance in designing programs to address the health needs of veterans (see Chapter 5 for further discussion of epidemiology and the evaluation of such studies).

The identification of an appropriate veteran group for study often involves

a time-consuming and labor-intensive search of military records for names or Social Security numbers. The General Services Administration, under an agreement with the Department of Defense, maintains the military personnel records of veterans, including those from the Vietnam era at the National Personnel Records Center (NPRC) in St. Louis, Missouri. These records include military service information, on microfiche, for all American veterans. Once an individual has been identified by name or Social Security number, he or she can be matched to their military personnel file at NPRC. A computerized index lists the physical location of the individual's personnel file; the file can then be retrieved to verify the service information. It is currently impossible to readily determine from these records the true number of U.S. military personnel who served in Vietnam during the Vietnam era because this information has not been entered systematically into a computerized database.

TABLE 3-1 Summary of U.S. Military Strength in Vietnam and Quantities of Herbicides Sprayed: 1960-1973

		Quantity of Herbicide Sprayed (million gallons)[b]			
Year	No. of Troops[a]	Orange	White	Blue	Total
1960	900				
1961	3,200				
1962	11,300	NA	NA	NA	
1963	16,300	NA	NA	NA	
1964	23,300	NA	NA	NA	1.27[c]
1965	184,300	0.37	0	0	0.37
1966	385,300	1.64	0.53	0.02	2.19
1967	485,600	3.17	1.33	0.38	4.88
1968	536,100	2.22	2.13	0.28	4.63
1969[d]	475,200	3.25	1.02	0.26	4.53
1970	334,600	0.57	0.22	0.18	0.97
1971	156,800	0	0.01	0	0.01
1972	24,200	0	0	0	0
1973	8 250	0	0	0	0
TOTAL		11.22	5.24	1.12	18.85

NOTE: NA = no data available.

[a] Data represent year-end troop strengths. These counts include those who served more than one tour of duty in Vietnam.

[b] As recorded on the HERBS tape.

[c] Cumulative total from 1962 through 1964.

[d] Peak U.S. troop strength of 543,482 occurred in April 1969.

SOURCES: U.S. Department of Defense, 1978:Table P28.01S; National Academy of Sciences, 1974:Table 1

Because of the difficulties in obtaining representative samples (and due

to the nature of the question being examined), many studies do not attempt to generalize their findings to the entire veteran population, but rather focus on a discrete subpopulation of veterans, for example, U.S. Army ground combat troops who served in III Corps during 1967-1968, Marines who served in I Corps, or Air Force Operation Ranch Hand personnel. The results of such studies are limited to the group under study and do not necessarily apply to the entire Vietnam veteran population.

Comparison of the studies that do provide national estimates representative of the Vietnam veteran population is complicated, however, by differences in terminology and methodology. For example, the time and place of Vietnam service are not defined consistently across studies. Some studies define the Vietnam era as the period of service between August 1964 and June 1975. This period of service was defined as the "Vietnam era" by presidential proclamation on May 7, 1975 (Fischer, 1980). Others use January 1, 1965, to mark the beginning of the Vietnam era, and March 1973, the time of final withdrawal of combat troops, to designate its end. Yet other veteran population estimates refer to service in the "Vietnam theater" rather than Vietnam per se. The Vietnam theater includes Vietnam, Laos, Cambodia, and the adjacent waters of the South China Sea; service in Thailand may be also be included.

Estimates of the number of military servicemen and women who served during the Vietnam era are provided by several federal agencies, including the Department of Defense (DOD), the Department of Veterans Affairs (DVA), and the Department of Labor. A number of postwar surveys have also attempted to determine retrospectively, from samples of the veteran population, the total number of U.S. military personnel who served in Vietnam during the Vietnam era. A comparison of the differences in definition and methodology for deriving these estimates is provided below.

Federal Estimates

According to DOD calculations (Defense Almanac, 1992), 8.7 million served in the military during the Vietnam era (defined here as the period August 4, 1964, through January 27, 1973). The DOD estimates that 2.6 million, or approximately one-third, of these Vietnam era veterans served in Vietnam. Nearly 40 percent of Vietnam era veterans, or 3.4 million, served in the Vietnam theater (U.S. DOD, 1976). These estimates are based on military records tabulated by MACV from summaries prepared by individual units that recorded end-of-the-month troop numbers. Although the completeness of these records varied by unit, they are the best official documents available for estimating the number of U.S. servicemen in South Vietnam.

According to estimates of the Department of Veterans Affairs (DVA),

approximately 8.3 million veterans of the Vietnam era (August 4, 1964, to May 7, 1975) were represented among the adult civilian U.S. population (U.S. VA, 1985). Approximately one-third, or some 2.7 million, of the Vietnam era veterans served in the Vietnam theater (defined as service in Laos, Cambodia, Vietnam, or the surrounding waters). The DVA adjusts its veteran population estimates based on the U.S. decennial census; estimates of Vietnam era service are based upon receipt of the Vietnam Service Medal, as identified on individual military discharge forms. Qualification for a service medal is limited to military units that supported operations within Vietnam and to those individuals that served in the Vietnam theater between July 1965 and April 1974 (Fischer et al., 1980). Therefore, the DVA estimate of the number who served in the Vietnam theater is restricted, based on use of the Vietnam Service Medal as an indicator of service.

Various demographic data on veteran populations, in addition to employment and disability statistics, are reported by the Bureau of Labor Statistics (BLS). These data, tabulated from the Current Population Survey (U.S. Department of Labor, 1992a,b), suggest that of the 7.9 million male veterans of the Vietnam era (August 1964 to May 1975), nearly one-half, or 3.7 million, reported having served in the Vietnam theater of operations (Vietnam, Laos, or Cambodia, or in the waters or air surrounding these countries). Estimates of the number of veterans who served in Vietnam per se are not available from these data.

Other Survey Estimates

Various other postwar surveys of Vietnam era veterans also provide estimates of the number who served. A 1979 Louis Harris survey of the U.S. adult, noninstitutionalized population who served on active duty in the military during the Vietnam era (defined as the period between August 1964 and June 1975) reported that approximately 3.8 million (42.5 percent of Vietnam era veterans) served in Vietnam (Fischer et al., 1980). An estimated 4.3 million, or 47.8 percent, of Vietnam era veterans reported service in the Vietnam theater during the Vietnam era.

The National Survey of the Vietnam Generation was conducted as part of the National Vietnam Veterans Readjustment Study (Kulka et al., 1988). The reference population for this survey was 8.3 million veterans who served during the Vietnam era (August 1964 to May 1975). According to these data, an estimated 3.1 million men and 7,000 women, or 37 percent of Vietnam era veterans, served in the Vietnam theater (defined as Vietnam, Laos, Cambodia, or the surrounding waters); nearly 2.6 million were stationed directly in Vietnam.

These various estimates of the Vietnam veteran population are summarized in Table 3-2. From these data, it is estimated that one-third to one-half

of Vietnam era veterans, or 2.7 million to 4.3 million persons, served in the Vietnam theater of operations, depending on the definition of the period and/or location of military service. Comparable estimates of those who served in Vietnam range from 2.6 million to 3.8 million.

TABLE 3-2 Estimates (in millions) of the Vietnam Veteran Population

Reference	Definition of Service	*N*
U.S. DOD, 1976	Vietnam, 1/1/65-3/31/73	2.6
Fischer et al., 1980	Vietnam, 8/64-6/75	3.8
U.S. VA, 1985	Vietnam theater, 8/4/64-5/7/75	2.7
Kulka et al., 1988	Vietnam theater, 8/64-5/75	3.1
U.S. DOD, 1976	Vietnam theater, 1/1/65-3/31/73	3.4
U.S. Dept. of Labor, 1990	Vietnam theater, 8/64-5/75 (males)	3.7
Fischer et al., 1980	Vietnam theater, 8/64-6/75	4.3
U.S. Dept. of Labor, 1990	Vietnam era, 8/64-5/75 (males)	7.9
U.S. VA, 1985	Vietnam era, 8/4/64-5/7/75	8.3
U.S. DOD, 1976	Vietnam era, 8/4/64-1/27/73	8.7

SOURCES: U.S. Department of Defense, 1976; Fischer et al., 1980; U.S. Veterans Administration, 1985; Kulka et al., 1988; U.S. Department of Labor, Bureau of Labor Statistics, 1990.

Military and Demographic Characteristics

Selected military and demographic statistics on U.S. personnel who served in Vietnam are available from these official records and postwar surveys. According to BLS estimates of the veteran population in 1989-1990, veterans who were between 40 and 44 years of age comprise the single largest age cohort of male Vietnam era veterans; 38 percent of male Vietnam era veterans and 43 percent of male Vietnam theater veterans were born between 1946 and 1950 (see Table 3-3). These statistics on the current age distribution of Vietnam veterans corroborate findings from several recent veteran surveys (Fischer et al., 1980; Kulka et al., 1988; CDC, 1989; U.S. DVA, 1990).

Some veteran surveys obtain information retrospectively on the veteran's background characteristics upon entering military service, whereas others describe the veteran's characteristics at the time of the survey (which could be 10 to 25 years after military discharge). Differences in sampling procedures limit comparisons of the surveyed populations. As discussed previously, some studies provide data only on Vietnam theater veterans, including those who served in Laos and Cambodia or other areas of Southeast Asia, depending on how theater is defined. Some studies do not include a comparison group of nonveterans or do not provide contrasts among various

subgroups (i.e., race, branch or region of service), whereas others select only discrete groups for study (e.g., veterans of a particular age cohort or unit of service during a specific period of time). Furthermore, most surveys sample only males; less information on the characteristics of women who served in Vietnam is available (Schwartz, 1987). With these deficiencies in mind, estimates from several surveys involving large samples of veterans are presented below.

TABLE 3-3 Age Distribution of Vietnam Era and Vietnam Theater Veterans 1989-1990 (numbers in thousands)

Age Group (years)	Vietnam Era, *N* (%)	Vietnam Theater, *N* (%)
All ages	8,071[a]	3,886[a]
≤ 34	133 (1.6)	32 (0.1)
35-39	1,109 (13.8)	369 (9.4)
40-44	3,031 (37.6)	1,676 (43.1)
45-49	2,301 (28.5)	1,090 (28.0)
50-54	675 (8.4)	280 (7.2)
55-64	511 (6.3)	322 (8.3)
≥ 65	178 (2.2)	83 (2.1)

[a] Totals reflect the addition of an estimated 33,000 women who served in the Vietnam theater and 137,000 who served during the Vietnam era; these data are not included in the age distributions since they were not available by 5 year age groups.
SOURCE: U.S. Department of Labor, Bureau of Labor Statistics, 1990.

Approximately 50 percent of Vietnam era veterans served in the Army, 20 percent in the Navy, 20 percent in the Air Force, and the remaining 10 percent in the Marines or Coast Guard (Kulka et al., 1988). These data are in close agreement with DOD (Defense Almanac, 1992) estimates of the proportion serving by branch of service. Other than IV Corps, where 7 percent served, military personnel in Vietnam were relatively evenly distributed throughout the four military regions of the country. Approximately 80 to 85 percent of male Vietnam veterans were white, 10 to 12 percent black, and the remaining Hispanic or other (Fischer et al., 1980; Kulka et al., 1988; U.S. Department of Labor, 1990). Of those surveys that provided comparison groups, there were no differences in the racial composition of Vietnam era veterans compared to those who did not serve in Vietnam during the Vietnam era (Fischer et al., 1980; Card, 1983). Although race was not associated with military service, whites who did serve, and especially those who enlisted willingly, came from poorer families than whites who did not serve. For blacks, there were no significant socioeconomic differences between those who did and did not serve. However, blacks who

served, and especially those who served more than two years, had completed more years of education than those who did not serve (Card, 1983).

Twenty percent of soldiers sent to Vietnam were assigned to combat units (Shafer, 1990), although surveys of veterans indicate much higher percentages who reported having experienced combat. A survey published by the Veterans Administration indicated that 70 percent of those sampled reported exposure to combat, which meant that they had come under some kind of attack (U.S. Department of Labor, 1990; Karnow, 1991). The CDC Vietnam Experience Study found that 57 percent of Army veterans had served in combat type units (i.e., infantry, artillery, armor, cavalry, and engineer; CDC, 1989).

The average age of those who experienced combat in Vietnam was 19 years, compared to 27 years in World War II (Shafer, 1990). Draftees were more likely to see heavy combat in Vietnam, compared to those who enlisted and served in other parts of the theater (Fischer et al., 1980; Kulka et al., 1988). No differences among racial or ethnic groups were found for either service in the Vietnam theater or exposure to combat. However, those who served in Vietnam with less than a high school education at the time of entry into military service were three times as likely to see heavy combat as those with college educations, and those who were less than 20 years of age at the time they went to Vietnam were twice as likely to be exposed to heavy combat as those aged 35 years or older (Fischer et al., 1980). A 1981 survey of a 1960 male high school cohort of military age during the Vietnam era found that veterans with lower academic aptitude, as measured by a series of cognitive tests scores received in the ninth grade, reported more combat experiences, such as seeing Americans wounded, firing a weapon at the enemy, or receiving fire from the enemy, than those with higher test scores (Card, 1983).

Just as there was no "typical" American soldier or typical military experience in Vietnam, there was no one combat experience (Shafer, 1990; Karnow, 1991). The combat experiences of individual soldiers varied according to assignment, geographical region of duty, and the period during which they served. In addition, most soldiers who were sent to Vietnam after the first American troops arrived in 1965 were sent as individual replacements, rather than as units (Karnow, 1991). The military found it more efficient administratively to replace losses piecemeal than to replace units and rebuild them. Unfortunately, this operational strategy minimized prospects for unit cohesion and contributed to low troop morale (Shafer, 1990).

It should be noted that these estimates describe the surviving veteran population: more than 58,000 U.S. men and women were killed and over 300,000 were wounded, of which more than one-half were wounded seriously enough to require hospitalization (U.S. VA, 1985). The Combat Area

Casualties Current File, maintained by the Department of Defense, contains records on U.S. personnel who died as a result of hostilities (killed in action, died from wounds, died while missing, or died while captured) or other causes (died from illness or nonhostile injury or other nonhostile causes) while serving in Cambodia, China, Laos, Vietnam, or Thailand during the conflict in Southeast Asia. As of November 1992, the file contained information on 58,166 deaths. Information potentially available in each casualty record includes branch of service, military grade and occupation code, birth date, cause and date of death, and length of service. Forty-four percent of the 58,166 U.S. military deaths occurred in those under age 21; three-fourths were aged 23 and younger. Among the recorded deaths, enlisted personnel suffered six times as many casualties as officers (86 percent and 14 percent, respectively), and pay grades E-3 and E-4 suffered the heaviest losses. The majority of deaths occurred among U.S. Army forces (66 percent), 25 percent occurred among the Marine Corps, 4.5 percent among the Navy, and 4.5 percent among the Air Force (U.S. DOD, 1986). As a group, Marines sustained the heaviest proportion of losses. Although blacks made up 11 percent of the American population and 12.6 percent of American forces in Vietnam, they accounted for 20 percent of Army combat deaths from 1961 to 1966 (Shafer, 1990). Black casualties ultimately accounted for 15.1 percent of total Army casualties and 13.7 percent of total U.S. casualties.

Studies of Women Veterans

There are no centrally maintained records or files of all women who were stationed in Vietnam during the Vietnam era (Thomas et al., 1991). The Veterans Administration (1985) reported that 263,000 women served in the military between 1964 and 1975 (the Bureau of Labor Statistics estimates that about 210,000 women served during the Vietnam era (U.S. Department of Labor, 1990); an estimated 5,000 to 7,000 women served in Vietnam (Thomas et al., 1991). Eight women were killed in Vietnam; all were nurses.

Very little information on the demographic characteristics of women veterans exists (Schwartz, 1987). The National Vietnam Veterans Readjustment Study (Kulka et al., 1988) sampled 736 Vietnam era female veterans, 432 of whom were Vietnam theater veterans. Over 70 percent of women who served in the Vietnam theater were born during the period 1940-1949. An estimated 97 percent were white. Nearly 80 percent served in the Army, 90 percent were officers, and 87 percent were military nurses (Kulka et al., 1988).

Thomas and colleagues (1991) conducted a study of 4,582 female Vietnam veterans who served between July 1965 and March 1973. The women

were identified from the review of morning reports of Army hospital and administrative support units stationed in Vietnam, and from Air Force personnel files, Navy muster roles, and Marine Corps listings of all women assigned to Vietnam. More than 90 percent (93.9 percent) of the women in the study were white. They also tended, on average, to be older than male veterans; about one-third were younger than age 25 at the time they entered Vietnam service. Most female veterans (75 percent) served in the Army, followed by the Air Force (16 percent), Navy (8 percent), and Marines (less than 1 percent). Eighty percent of the female veterans included in this study were nurses. These estimates are similar to those determined by Kulka and colleagues (1988) in their review of women serving in the military during the Vietnam era.

THE MILITARY USE OF HERBICIDES IN VIETNAM

In 1960, U.S. assistance to the Diem government in South Vietnam was limited to military advisors, economic aid, and some logistic support (Karnow, 1991). American military advisory forces in South Vietnam numbered fewer than 900 (MACV, 1972). Some leaders within the U.S. government and military warned that the time to act against a Communist takeover of South Vietnam had come and that further U.S. intervention was inevitable. Defoliation operations were among several supplemental actions proposed that could be conducted while decisions regarding the commitment of combat troops were pending (Buckingham, 1982). The use of herbicides in South Vietnam was recommended for several reasons: to remove foliage along thoroughfares used as cover for enemy ambushes, to defoliate vegetative areas surrounding enemy bases and communication routes, to improve visibility in heavily canopied jungle, and to destroy enemy subsistence crops (Collins, 1967; Huddle, 1969; U.S. Army, 1972). Although the first combat troops did not arrive in Vietnam until April 1965, preparations for the testing and conduct of a major aerial defoliation program proceeded in cooperation with the South Vietnamese government. In December 1961, President Kennedy authorized the use of herbicides, and on January 12, 1962, the first U.S. Air Force herbicide missions of Operation Ranch Hand were flown over South Vietnam (Warren, 1968; MACV, 1972).

Much of the currently available information on the military use of herbicides in South Vietnam during the period 1962 to 1971—the chemical formulations used, the quantities applied, the operational procedures for aerial spray missions, and the aircraft used—was compiled in the 1970s and early 1980s from military records kept during the conflict, DOD technical reports, and procurement records. In 1974, the National Academy of Sciences' Committee to Review the Ecological Consequences of Herbicides in Vietnam evaluated the available DOD records of herbicide spray missions

conducted from 1965 to 1971. During the Vietnam era, thousands of pieces of information on fixed-wing herbicide spray missions from the MACV log books was compiled and recorded on the HERBS data tapes. The HERBS tapes are considered to contain the most complete, accurate, and authoritative compilation of data available on *aerial fixed-wing* herbicide operations conducted in Vietnam (Dashiell, 1973).

Herbicides were also applied by other methods. An unknown, but smaller, quantity of herbicides was applied around base perimeters and lines of communication to improve visibility and reduce the likelihood of enemy ambush. Records of these smaller-scale uses of herbicides were not systematically logged and do not appear on the HERBS tapes. A review of various Army records and military reports identified the use of an additional 1.6 million gallons of herbicides, and information on these sprays was recorded on the Services HERBS tape. Together these tapes of herbicide sprays account for approximately 20 million gallons of herbicides used in Vietnam from 1962 to 1971.

Operation Ranch Hand

The defoliation program in Vietnam began on December 4, 1961, when President Kennedy authorized the Secretary of Defense to test the military effectiveness of the defoliation of several lines of communication (MACV, 1968). Operation Ranch Hand, the tactical military project for the aerial spraying of herbicides in South Vietnam, was the first (and only) large-scale experience with chemical defoliants in U.S. military operations. According to MACV records, the first U.S. Air Force Ranch Hand missions over Vietnam were flown on January 12, 1962 (MACV, 1972); however, it was not until August 1962 that President Kennedy approved the program on a larger scale. The first major operation, to clear enemy infiltration routes, was carried out over the mangrove forests in the Ca Mau peninsula in the southernmost region of the Mekong Delta in September 1962 (Dux and Young, 1980).

Operation Ranch Hand had two primary objectives: (1) defoliation of trees and plants to improve visibility for military operations, and (2) destruction of essential enemy food supplies. Targets for defoliation by Ranch Hand included base camps and fire support bases (specifically constructed sites for storage of artillery in support of combat operations), lines of communication, enemy infiltration routes, and enemy base camps. Clearance of these areas improved aerial observation, opened roads to free travel, and hindered enemy ambush.

All large-area defoliation missions were flown exclusively by Ranch Hand crews (Collins, 1967). According to DOD records, the aerial application of herbicides was accomplished by spraying from C-123 fixed-wing

aircraft and helicopters (UH-1 and H-34). During 1967-1968, requirements for herbicide missions increased to the point that the number of available C-123 aircraft was not sufficient to complete all approved targets within the desired time frame (MACV, 1968). In order to permit a more timely response to defoliation requirements, helicopter operations were recommended for smaller targets, such as in support of local base defense, maintenance of deforested areas, and the uncovering of known small ambush sites along lines of communication (MACV, 1968). As Ranch Hand operations declined in 1970-1971, the number of helicopter herbicide operations increased and gradually became the only aerial means of herbicide delivery.

With the buildup of American troops in 1965, Operation Ranch Hand also intensified: the number of C-123 aircraft assigned to the operation increased from 3 to 12 (36 aircraft were assigned to the program from 1967 until it was phased out in 1971); permanent personnel were assigned to the team (Dux and Young, 1980); and the number of missions increased nearly sixteenfold from 107 in 1962 to more than 1,600 in 1967 (Huddle, 1969; NAS, 1974). Typical missions early in the conflict included 3 to 4 aircraft, increasing to as many as 19 in the later years. The operation of a single aircraft was termed a sortie. In the period from 1966 through 1968, more than one sortie per day was often common. During the first six months of 1968, the 24 C-123 aircraft assigned to Ranch Hand averaged nearly 39 sorties per day (Young et al., 1978). All missions within a target area formed a project (Young and Reggiani, 1988). Ranch Hand missions were also frequent targets of ground fire due to the low altitude and slow speed of the aircraft, and flights required fighter cover for protection (Collins, 1967; Warren, 1968; Spey, 1993). As early as 1963, fighter cover was used in conjunction with defoliation missions to provide mission protection. In 1966, it was reported that nearly one-third (29 percent) of all C-123 defoliation sorties received "hits" from ground fire. The ratio of hits per sortie decreased in later years with improved fighter tactics (Warren, 1968). The helicopter delivery system was also particularly vulnerable to ground fire because of the slow delivery speed (Collins, 1967).

The control of the use of herbicides was a joint effort by the government of South Vietnam and the United States. Authorization in Saigon and at the Corps level was mandatory for all Ranch Hand crop destruction and defoliation operations by fixed-wing aircraft (MACV, 1969b; NAS, 1974). The authorization procedure required approval prior to spraying by the Government of Vietnam (local and national); the U.S. Ambassador; and the commander of the U.S. Military Assistance Command, Vietnam. Procedures governing these herbicide operations required that all herbicide spraying be "limited to areas of low population" and that "defoliation operations not normally be undertaken when it is apparent that damage will occur to crops … unless the military advantage is very clear" (MACV, 1969b).

Following each Ranch Hand mission a report was to be filed that included information on the number of aircraft scheduled and the number productive, the type and number of gallons of herbicide sprayed, the type of mission (crop destruction or defoliation), and the location of the spray run.

The normal altitude of the C-123 for spray application was 150 feet, flying at a speed of 130 to 150 knots, and producing a swath width of 240 m per aircraft (MRI, 1967; NAS, 1974). Under these ideal conditions, a 1,000 gallon tank permitted a 3- to 4-min spray time at a total distance of about 8.7 statute miles, or about 340 acres treated per aircraft, with a deposition rate of 3 gallons per acre (Young et al., 1978). The HIDAL (Helicopter, Insecticide Dispersal Apparatus, Liquid) was initially developed as a potential insecticide delivery system. The capacity of the UH-1 spray tank was 200 gallons, but because of weight limitations, only 100 gallons was carried. The helicopter spray system was capable of depositing 1.5 gallons per acre when flown at 55 knots and cut a swath of 75 m.

In addition to aircraft altitude and speed, distribution of the spray was also affected by climate, wind, terrain, and turbulence from the aircraft. Although missions generally were flown in the early morning when the wind was calm, to minimize spray drift, the NAS (1974) study showed that crop damage resulting from drift on defoliation missions was greater than that caused by crop destruction missions—indicating that widespread crop damage resulted from drift. Maximum defoliation results were achieved during the growing season, when the vegetation was in full-leaf and actively growing (Young et al., 1978). The rainfall pattern (in relation to the fastest growing season), the vegetation composition, and the number of canopies were important in determining the proper herbicidal agent and the number of applications required to uncover completely the ground underneath (U.S. Army, 1972). Tschirley (1967) estimated that in a moist, tropical, triple-canopy forest, approximately 80 percent of the spray droplets were intercepted by the uppermost canopy, 14 percent fell to the inner level, and only 6 percent reached ground-level vegetation (Tschirley, 1967). Air turbulence from the aircraft also helped to distribute spray droplets throughout the foliage and was an important factor in the dispersal of the spray (MRI, 1967).

Ranch Hand aircraft were also responsible for the spraying of insecticides to control malaria-carrying mosquitoes, and began spraying in South Vietnam in 1966. According to Air Force records, Ranch Hand "silver bugs" flew approximately 20 insecticide sorties per month in 1967. One sortie could cover about 15,000 acres. They were commonly spotted over allied camps, spraying malathion and DDT (dichlorodiphenyltrichloroethane). The insecticide delivery planes were not camouflaged like the other Ranch Hand C-123 aircraft. The spraying involved longer missions; therefore, fuel conservation was a concern. On the other hand, navigation of the insecticide

sprays along exact coordinates was not as vital as for the herbicide missions (Collins, 1967; Buckingham, 1982: Young and Reggiani, 1988). It was reported that 44 percent of the land area of Southeast Asia (primarily South Vietnam) was sprayed with malathion (Westing, 1984).

Herbicide Formulations

Four major compounds were used in the Ranch Hand herbicide formulations—2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), picloram, and cacodylic acid. These compounds have been used worldwide for the control of weeds and unwanted vegetation, although the application of 2,4,5-T is no longer permitted in the United States following a series of Environmental Protection Agency directives in the 1970s. A considerable amount of information is available on the physical, chemical, and toxicological properties of these compounds, although it is greater for 2,4,5-T and 2,4-D than for picloram and cacodylic acid (see Chapter 4).

The term herbicide includes chemicals that regulate normal plant growth. Some organic chemicals are considered growth regulators and are effective in minute amounts at stimulating or inhibiting plant growth. Some herbicides, such as 2,4-D, stimulate plant growth when applied at extremely low dosages, but inhibit growth or are lethal at higher concentrations.

Herbicides may be classified on the basis of their effects on plants and their site of application (foliage or soil). Selective herbicides may kill some plants and cause little or no damage to other plant species. By contrast, nonselective herbicides exhibit a broad spectrum of herbicidal effects on most plant species (Huddle, 1969; U.S. Army, 1970). Foliage-applied herbicides include contact herbicides or desiccants, which kill primarily by contact with plant tissues, and systemic herbicides, which are absorbed and translocated within the plant from the point of entry. Soil-applied herbicides kill germinating seeds and established plants by uptake of the chemical from the soil.

Which of these four major chemicals (2,4-D, 2,4,5-T, picloram, or cacodylic acid) was chosen for a specific application depended on the desired effects. 2,4-D and 2,4,5-T are chlorinated phenoxy acids, and each is effective against a wide array of broadleaf plant species (Irish et al., 1969). They persist in soil only a few weeks (Buckingham, 1982). Picloram, like 2,4-D and 2,4,5-T, regulates plant growth. Compared to 2,4-D, picloram is more mobile, and therefore better able to penetrate the plant's roots and be transported throughout the plant's tissues. Unlike the phenoxy herbicides, picloram is extremely persistent in soils. The fourth compound, cacodylic acid, contains an organic form of arsenic. Cacodylic acid is a desiccant,

causing a plant's tissues to lose their moisture and eventually killing the plant. It is a contact herbicide that is rapidly rendered ineffective in soil.

Military Herbicides

The major herbicides employed in Operation Ranch Hand were code named according to the color of an identification band painted on the 55 gallon storage drum. Agents Purple and Blue were the first defoliants introduced into Vietnam in 1962. Agent Purple was a 50:30:20 mixture of the *n*-butyl ester of 2,4-D, and the *n*-butyl and isobutyl esters of 2,4,5-T (see Table 3-4). Purple was first tested in the Camp Drum, New York, defoliation test in 1959 and found to be most effective on broadleaf plants. Young and colleagues (1978) reported that 145,000 gallons of Agent Purple was procured and applied in Vietnam during 1962-1964. Because of its volatility, Agent Purple was replaced by Agent Orange in 1965.

Blue was the code designation for a liquid formulation of cacodylic acid and its sodium salt. The term Blue was first applied to cacodylic acid in a powder form that was mixed in the field with water. It was later replaced by the liquid formulation Phytar 560-G. Cacodylic acid is a highly soluble organic arsenic compound that is readily broken down in soil. According to military herbicide records, more than 1.1 million gallons of Agent Blue was dispensed in the DOD herbicide program. Approximately one-half of all Agent Blue was used for crop destruction missions; it was the agent of choice for destruction of rice crops. Blue was employed in situations requiring rapid defoliation, causing noticeable browning or discoloration in one day, with maximum desiccation and leaf fall occurring within

TABLE 3-4 Major Herbicides Used in Operation Ranch Hand: 1962-1971

Herbicide Code Name	Formulation	Purpose	No. of Gallons Sprayed	Period of Use
Purple	2,4-D; 2,4,5-T	General defoliation	145,000	1962-1964
Blue (Phytar 560-G)	Cacodylic acid	Rapid defoliation, grassy plant control, rice destruction	1,124,307	1962-1971
Pink	2,4,5-T	Defoliation	122,792	1962-1964
Green	2,4,5-T	Crop destruction	8,208	1962-1964
Orange, Orange II	2,4-D; 2,4,5-T	General defoliation	11,261,429	1965-1970
White (Tordon 101)	2,4-D; picloram	Forest defoliation, long-term control	5,246,502	1965-1971

SOURCES: MRI, 1967; NAS, 1974; Young et al., 1988.

two to four weeks (Darrow et al., 1969). The remainder was used in defoliation or sprayed around base perimeters, being delivered by helicopters or ground vehicles with sprayers attached to them (Young et al., 1978).

Agents Pink and Green were used in small quantities; however, official records of herbicide sprays during the early years of the program (1962-1964), when these two herbicides were used, are incomplete. Young and colleagues (1978) reported the use of 122,792 gallons of Agent Pink, a 60:40 mixture of the *n*-butyl ester and isobutyl ester of 2,4,5-T. Pink had been previously tested in the 1953-1964 defoliation program in Thailand (U.S. Army, 1965). Agent Green was a single-component formulation of the *n*-butyl ester of 2,4,5-T. Slightly more than 8,000 gallons of Agent Green was sprayed, primarily in defoliation missions (Young et al., 1978).

In January 1965, two additional herbicides, code named Orange and White, were introduced into the herbicide program. Agent Orange, a 1:1 mixture of 2,4-D and the *n*-butyl ester of 2,4,5-T, accounted for approximately 61 percent of the recorded herbicide use. Orange was the general-purpose herbicide for defoliation and crop destruction, with leaf fall in three to six weeks and control persisting for seven to twelve months. According to military estimates of herbicide use, 90 percent of Agent Orange was used in Ranch Hand forest defoliation missions; 8 percent was used in Ranch Hand crop destruction missions; and 2 percent was sprayed from the ground around base perimeters and cache sites, waterways, and communication lines (NAS, 1974). Mangrove forests were especially sensitive to the effects of Agent Orange—a single application killed them (NAS, 1974). Annual crops were killed rapidly by one application of Agent Orange; root and tuber crops, and perennial and woody tropical crops such as jackfruit, papaya, and mango, were also susceptible to Agent Orange (Young et al., 1978).

Orange II was introduced later in the program. It differed from the original Agent Orange in that the *n*-butyl ester of 2,4,5-T was replaced by the isooctyl ester; however their herbicidal effects were similar. According to procurement records, less than 10 percent of the total Agent Orange used was Orange II (Craig, 1975).

White was the code name for Tordon 101, a liquid mixture of 2,4-D and picloram. Approximately 5.25 million gallons of Agent White was dispensed during Ranch Hand operations. More than 95 percent of Agent White was applied in defoliation missions (NAS, 1974; Young and Reggiani, 1988). Because of the persistence of Agent White in soil, it was not recommended for use on crops, but was most often used in areas where longer persistence rather than immediate defoliation was desired, such as inland forests. White was effective principally on broadleaf herbaceous and woody plants. The herbicide's action on woody plants was usually slow, however, and full defoliation did not normally occur for several months (Young and Reggiani, 1988).

In addition to these four major compounds, Dinoxol, Trinoxol, and diquat were applied on native grasses and bamboo (Brown, 1962). Soil-applied herbicides were also reportedly used around base camp perimeters, mine fields, ammunition storage areas, and other specialized sites requiring control of grasses and woody vegetation (Darrow et al., 1969). Additional accounts include the use of fungicides, insecticides, wetting agents, wood preservatives, insect repellents, and other herbicides (Gonzales, 1992). The number of military personnel potentially exposed to these chemicals is not available.

An undetermined amount of herbicides and insecticides was procured and distributed by Australian forces in Vietnam during 1966-1971. The use of these chemicals was confined largely to defoliation around base camps, improving security, and controlling mosquito-borne diseases. It appears that the chemicals were largely dispersed by use of ground delivery techniques, although low-volume aerial applications of insecticides, usually by helicopter, have been reported. The chemicals tested and used included 2,4-D, chlordane, DDT, diazinon, lindane, malathion, and picloram (Australian Senate Standing Committee, 1982).

Level of Toxic Contaminants

2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is a contaminant of 2,4,5-T, but not of 2,4-D, and is a very toxic material. The levels of TCDD found in any given lot of 2,4,5-T depend on the manufacturing process (Young et al., 1976), and different manufacturers produced 2,4,5-T with various concentrations of TCDD. The primary source of 2,4,5-T in the herbicides used in Vietnam was Agent Orange. It is the unknown concentration of TCDD in Agent Orange that is of particular concern.

Of all the herbicides used in South Vietnam, only Agent Orange was formulated differently from the materials for commercial application that were readily available in the United States (Young et al., 1978). TCDD concentrations in individual shipments were not recorded, and levels of TCDD varied in sampled inventories of herbicides containing 2,4,5-T. Analysis of the TCDD concentration in stocks of Agent Orange remaining after the conflict, which had either been returned from South Vietnam or had been procured but not shipped, ranged from less than 0.05 to almost 50 parts per million (ppm), averaging 1.98 and 2.99 ppm in two sets of samples (NAS, 1974; Young et al., 1978). Comparable manufacturing standards for domestic use of 2,4,5-T in 1974 required that TCDD levels be less than 0.05 ppm (NAS, 1974). Therefore, depending on which stocks were sampled, the level of dioxin contamination in Agent Orange could have been up to 1,000 times higher than the level of dioxin found in phenoxy herbicides domestically available at the time.

Agents Green, Pink, and Purple, also contained 2,4,5-T and were used from 1962 through mid-1965. These 2,4,5-T formulations used early in the program (prior to 1965) contained 16 times the mean dioxin content of formulations used during 1965-1970 (Young et al., 1978). Analysis of archive samples of Agent Purple reported levels of TCDD as high as 45 ppm (Young, 1992). The mean concentration of TCDD in Agent Purple was estimated to be 32.8 ppm and in Agents Pink and Green, 65.6 ppm (Young et al., 1978).

Termination of the Program

After a relatively slow buildup in military herbicide operations from 1962 to 1965, herbicide use increased rapidly during 1966 and 1967, was relatively stable in 1968 and 1969, and then dropped sharply in 1970. According to information on Ranch Hand spray missions, 80 percent of herbicides sprayed in the herbicide program (1962 to 1971) were applied during the period from 1966 to 1969.

As the military use of herbicides in Vietnam intensified, various questions were raised concerning the legality, morality, and possible long-term consequences of the program. By the end of the decade, the controversy had become a contributing element in the growing opposition to American involvement in Vietnam. The crop destruction program was of particular concern. Official military reviews of the herbicide program frequently cited the appearance of adverse political and psychological effects from the military operations on the civilian population in North Vietnamese-controlled areas (Warren, 1968).

In October 1969, the Department of Defense restricted the use of Agent Orange to areas remote from populations. This action was prompted by a National Institutes of Health report that 2,4,5-T could cause malformations and stillbirths in mice. At about the same time it was recognized that 2,4,5-T was contaminated with TCDD. In December 1969, the American Association for the Advancement of Science (AAAS) declared that recent research showing that 2,4-D and 2,4,5-T could cause birth deformities in experimental animals supported the conclusion that 2,4,5-T posed a probable health threat to humans. The AAAS also maintained that the levels of application of 2,4-D and 2,4,5-T in Vietnam exceeded levels in civilian usage and called on the Department of Defense to cease use of these chemicals (Buckingham, 1982). In April 1970, the Secretaries of Agriculture; Health, Education, and Welfare; and the Interior jointly announced the suspension of certain uses of 2,4,5-T (Young et al., 1978). The Department of Defense temporarily suspended all use of 2,4,5-T (and therefore Agent Orange) in all military operations pending "further evaluation of its chemical constituents" (U.S. GAO, 1978). Since Agent Orange was no longer available for military use, all defoliation missions were shifted to Agent White,

but White was not as desirable for military purposes because defoliation required four months with White compared to three to four weeks with Orange (Buckingham, 1982). In May 1970, Ranch Hand flew its last defoliation (but not crop destruction) mission in Vietnam.

According to Department of Defense records, on February 12, 1971, MACV further announced that herbicides would no longer be used for crop destruction in Vietnam, and the last fixed-wing herbicide-dispensing aircraft was flown. Subsequent herbicide operations (Agents Blue and White) were limited to certain strictly controlled use around allied fire bases by helicopter or on the ground (MACV, 1972). The last U.S.-authorized helicopter herbicide operation was flown on October 31, 1971 (NAS, 1974).

Disposal of Surplus Herbicides

In 1977, the U.S. Air Force (USAF) disposed of 2.22 million gallons of Agent Orange by incineration at-sea (Young et al., 1978). This operation, known as PACER HO, was accomplished under strict criteria of the U.S. Environmental Protection Agency (EPA) ocean dumping procedures. In order to obtain an ocean dumping permit, the EPA required the USAF to conduct extensive research on the environmental impact and occupational safety of the land transfer and loading operations and of shipboard incineration operations (Young et al., 1978).

When the Department of Defense suspended the use of 2,4,5-T in April 1970, the U.S. Air Force "had an inventory of 1.37 million gallons of [Agent] Orange in South Vietnam and 0.85 million gallons at the Naval Construct Battalion Center in Gulfport, Mississippi" (Young et al., 1978). In September 1971, the Department of Defense directed that all surplus Agent Orange in South Vietnam be removed and that the entire 2.2 million gallons be disposed of by an environmentally acceptable method. The 1.37 million gallons in South Vietnam was moved to Johnston Island, in the Pacific Ocean, for storage (Buckingham, 1982). The USAF reviewed various methods for the destruction and recovery of surplus Agent Orange. Techniques reviewed for destruction included soil biodegradation, high-temperature incineration, deep well injection, burial in underground nuclear test cavities, sludge burial, and microbial reduction (Tremblay and Virost, 1977). Techniques reviewed for recovery of a useful product included return to manufacturers, fractionation, and chlorinolysis (Tremblay and Virost, 1977). The USAF decided that destruction by high-temperature incineration was the method that warranted further research.

Although the DOD directed the Air Force to dispose of all remaining drums of Agent Orange in 1971, it was not until 1974 that a plan was offered for disposal of the 2.2 million surplus gallons of herbicide. On April 7, 1977, the EPA issued a research permit to the USAF and Ocean

Combustion Service allowing the transport of Agent Orange from Gulfport, Mississippi, to a site in the North Pacific Ocean for the purpose of at-sea incineration. The USAF contracted with the German-built, Dutch-owned incineration ship, the *Vulcanus*, in accordance with the provisions of the Marine Protection, Research and Sanctuaries Act of 1972 (Tremblay and Virost, 1977). The entire operation required three ship loadings (one from Gulfport and two from Johnston Island) for the total surplus of Agent Orange to be incinerated.

Ground Spraying of Herbicides

Although the number of U.S. military personnel exposed to herbicides is impossible to determine precisely, the majority of those assigned to Operation Ranch Hand can be presumed to have been exposed to Agent Orange and other herbicides. During the entire operation, approximately 1,250 military personnel served in Ranch Hand units. Although the Air Force maintained complete records of its Operation Ranch Hand fixed-wing herbicide missions, documentation of spraying conducted on the ground by boat, truck, or backpack and authorized at the unit level was less systematic. Authorization for herbicide missions by helicopter or surface spraying from riverboats, trucks, and hand-operated backpacks was delegated to the Republic of Vietnam and U.S. authorities at the Corps level; these operations required only the approval of the unit commanders or senior advisors. "Free-spraying" areas, including the Demilitarized Zone (DMZ) at the seventeenth parallel and the first 100 meters outside base camps, were also exempt from Ranch Hand regulations (NAS, 1974). This delegation of authority for spraying to the Corps level reduced the lag time that existed from proposal to completion of small defoliation projects, such as around depots, airfields, and outposts (Collins, 1967). However, because these helicopter and ground sprays were less rigidly controlled than fixed-wing aerial sprayings, the recording of such sprays was not as systematic as those of Operation Ranch Hand.

The U.S. Army Chemical Corps, using hand equipment and H-34-type helicopters, conducted smaller spray operations, such as defoliation around Special Forces camps; clearance of perimeters surrounding airfields, depots, and other bases; and small-scale crop destruction (Warren, 1968; Thomas and Kang, 1990). Twenty-two Army Chemical Corps units were assigned to South Vietnam between 1966 and 1971. Approximately 950 veterans who served in the Army Chemical Corps in Vietnam between 1966 and 1971 have been identified from unit morning reports. Men serving in these units were trained in the preparation and application of chemicals, as well as in the cleaning and maintenance of the spray equipment (Thomas and Kang, 1990).

Units and individuals other than the members of the Air Force Ranch Hand and Army Chemical Corps were also likely to have handled or sprayed herbicides around bases or lines of communication. For example, Navy riverine patrols were reported to have used herbicides for clearance of inland waterways. Engineering personnel required the use of herbicides for removal of underbrush and dense growth in constructing fire support bases. It is estimated that 10 to 12 percent of the total volume of herbicides was dispensed from the ground by spraying from backpacks, boats, trucks, and buffalo turbines (NAS, 1974). The buffalo turbine was a trailer-mounted spray system used for roadside spraying and perimeter applications, which essentially "shot" the herbicide with a velocity up to 240 km/hour and a volume of 280 m^3/min (Young and Reggiani, 1988). Hand spray units consisted of a backpack type of dispenser with a capacity of 3 gallons (Collins, 1967).

Although some information is documented in military records, it is impossible to determine accurately from military records alone the extent of spraying conducted on the ground or the number of personnel involved in these operations with potential herbicide exposure. An unknown number of non-Ranch Hand personnel likely received various degrees of exposure to herbicides. Young and Reggiani (1988) report that the actual number "may be in the thousands since at least 100 helicopter spray equipment units were used in South Vietnam, and most military bases had vehicle-mounted and backpack spray units available for use in routine vegetation control programs." The dregs of the 55 gallon drums were pumped into smaller drums and sent to military camps for local defoliation of crops and control of perimeter foliage (Dux and Young, 1980).

According to official documents, the "small-scale use of herbicides, for example around friendly base perimeters, were at the discretion of area commanders. Such uses seemed so obvious and so uncontroversial at the time that little thought was given to any detailed or permanent record of the uses or results" (U.S. Army, 1972). The Department of Defense took few precautions to prevent troops' exposure to herbicides since they were considered to be a low health hazard. Precautions prescribed were consistent with those applied in the domestic use of herbicides existing before the Vietnam conflict (U.S. GAO, 1979b). The Army added that exposure of ground troops was very unlikely since DOD personnel did not enter a Ranch Hand-sprayed area until approximately four to six weeks after the mission, when defoliation was complete and the herbicide was biodegraded or photodegraded (U.S. Army, 1972). The restriction placed on troops' entering a previously sprayed area was primarily for operational reasons, to prevent troops from being injured by the fighter aircraft that often accompanied the herbicide spraying aircraft (U.S. GAO, 1979b).

The 1979 study by the U.S. General Accounting Office (U.S. GAO,

1979b) examined the military defoliation operation in the Con Thieu province of I Corps between January 1966 and December 1969. During this period, more than 2 million gallons of herbicides were sprayed in I Corps. By using average troop strength and turnover figures, an estimated 218,000 Marine infantry personnel were determined to have been assigned to I Corps between 1966 and 1969. By randomly selecting 276 of 976 Marine monthly battalion reports, the GAO tracked troop movement and compared troop locations with herbicide mission data. Nearly 26,000 U.S. Marines and Navy medical personnel were identified who entered within a radius of 2.5 km of the defoliated target areas within one day of spraying; 4,300 troops were identified as being within 0.5 km of the flight path; 11,700 were within 2.5 km within four weeks. In the Khe Sanh-Thon Son Lam area, an estimated 4,300-8,000 troops were within 0.5 km of the sprayed area within one day of spraying; within 28 days, 33,600-45,300 troops were determined to have been within 2.5 km of the defoliation target. Army records were found to lack sufficient information, so that estimates of the number of Army personnel close to sprayed areas could not be calculated. The GAO report concluded that "the chances that ground troops were exposed to herbicide Orange are higher than the DOD previously acknowledged … the group of personnel most likely to have been exposed could include ground troops as well as herbicide handlers and aircraft crew members" (U.S. GAO, 1979b).

The HERBS and Services HERBS Tapes

A log of the aerial herbicide applications was maintained by the Chemical Operations Division, United States MACV, from data received from the various bases that supported herbicide missions. These records became the source documents for HERBS, the computerized system for processing, storing, and retrieving monthly herbicide mission activity data. HERBS was designed and implemented for the Chemical Operations Division by the Data Management Agency, MACV, in May 1970 (MACV, 1970).

Data received from the field were processed on a mission-by-mission basis. For each aerial herbicide mission between July 1965 and February 1971, information was recorded on the type of herbicide; the number of gallons sprayed; the number of scheduled, actually flying, and productive aircraft; the mission flight path coordinates; the province in which the mission flew; the purpose of the mission (defoliation or crop destruction); and the number of aborts. Flight paths were identified by the UTM (Universal Transverse Mercator) coordinates, a standard grid system for identifying geographic points.

In 1974, the DOD furnished a version of the HERBS tapes to a NAS committee acting under a congressional mandate to study the ecological

effects of herbicide use in South Vietnam (NAS, 1974). The tapes provided by DOD included the date, mission number, location (UTM coordinates), type, and quantity of herbicide; the area covered; the purpose of mission for fixed-wing operations during the period August 1965 (when the logbook was started) through February 1971; and helicopter crop destruction missions flown from 1968 to 1971 (NAS, 1974). Additional printouts of the number of aircraft per mission and of herbicide missions conducted during March through October 1971, the stated termination of the U.S.-controlled herbicide operations, were also provided. The committee conducted an inventory of the herbicide operations and constructed maps of the herbicide missions based on data recorded on the HERBS tapes.

The HERBS tapes contain information on 6,539 herbicide missions and 17.6 million gallons of herbicides sprayed during operations for the period August 1965 through February 1971. Nearly 11.3 million gallons and 4,109 missions involved Agent Orange, 5.2 million gallons and 1,786 missions Agent White, and 1.1 million gallons and 640 missions Agent Blue. Nearly 70 percent of all missions (15.5 million gallons) were for defoliation, 13 percent were crop destruction missions (1.6 million gallons), and the remaining 17 percent were flown over or around base perimeters, cache sites, communication routes, and waterways (0.5 million gallons).

Certain deficiencies in the HERBS data were noted by the NAS committee. These deficiencies were associated with errors in transcription of the records to IBM punch cards, and incomplete or illegible listings; erroneous recording of flight paths, particularly for crop destruction missions when sprays were frequently shut off between targets, or when flying over mountainous terrains or along winding riverbeds; lack of information on herbicide missions before and after the 1965-1971 period; no records of chemical "dumps"; and no records of herbicide missions authorized by the Corps level (these would include defoliation missions by helicopter and sprays made by land or water-borne equipment). Because of the lack of information on these uses of herbicides, the NAS committee estimated that an additional 2.4 million gallons of herbicides may not be accounted for by the HERBS tapes (NAS, 1974).

The NAS committee compared the inventory of herbicide missions with procurement records of the DOD. The total number of gallons of herbicides sprayed as recorded on the HERBS tapes was nearly identical to the amount procured, 17.63 million gallons and 17.58 million gallons, respectively.

In 1986, the U.S. Army Joint Services Environmental Support Group recorded an additional 1.6 million gallons of herbicides sprayed from a review of U.S. Army Chemical records and various documentation on helicopter, backpack, and other types of ground sprays (see Table 3-5). Some of this usage was either improperly recorded, incompletely documented, or omitted from the HERBS tapes (U.S. Army and Joint Services ESG, 1986;

Young and Reggiani, 1988). The resulting computer tape was named the Services HERBS tape since it was thought that the records of other services would show ground spray operations; only one Navy mission, however, could be documented from available records (U.S. Army and Joint Services ESG, 1986). The Services HERBS tape contains data on helicopter spray missions prior to 1968, backpack, and other ground sprayings by Army personnel. When combined with the HERBS tapes on herbicide operations from August 1965 through February 1971, these two tapes account for 19.2 million gallons of herbicides sprayed in Vietnam.

TABLE 3-5 Quantity of Herbicides Recorded on the Services HERBS Tape (gallons)

Year	Agent Orange	Agent White	Agent Blue	Other/ Unknown	TOTAL
1962				3,700	3,700
1963				4,885	4,885
1964				14,560	14,560
1965	34,025			244,725	278,750
1966	242,800	45,900		182,161	470,861
1967	167,085	33,835	25,401	23,795	250,116
1968	77,259	80,245	36,846	72,977	267,327
1969	79,922	29,745	17,917	71,460	199,044
1970	27,805	10,655	26,623	28,495	93,578
1971			11,063	2,400	13,463
TOTAL	628,896	200,380	117,850	649,158	1,596,284

SOURCE: U.S. Army and Joint Services Environmental Support Group, 1986.

Geographical Distribution of Herbicide Sprays

South Vietnam was divided into four combat tactical zones, from I Corps lying south of the DMZ to IV Corps in the Mekong Delta region (Figure 3-1). Although spraying occurred in most provinces of Vietnam, certain areas of the country were subject to more intensive spraying. The herbicide mission maps (Figure 3-2) indicate that defoliation missions were not uniformly distributed but were concentrated in certain geographical areas—along transportation routes, in occupied areas around Saigon, and on infiltration routes along the Laotian and Cambodian borders and the DMZ where enemy attacks were likely (U.S. Army, 1972). Primary target areas for crop destruction missions were in I Corps and along the upland and mountain valleys of II Corps (NAS, 1974). The military purposes of these missions were to deny food to the enemy, to redirect enemy manpower to

FIGURE 3-1 South Vietnam during the Vietnam conflict.

crop production, and to weaken enemy strength in these areas (Warren, 1968).

According to a 1972 Department of the Army report, *Herbicides and Military Operations*, the dense forest along many of the key marine and land transportation routes served as effective cover for enemy ambush (U.S. Army, 1972). In particular, the Rung Sat Special Zone, an area of dense mangrove forests, afforded enemy concealment along the main shipping route to Saigon. Defoliation of the area began in the mid-1960s, and by the late 1960s, most of the mangrove forests adjacent to the shipping routes were defoliated. The defoliation operation was so complete that it eliminated enemy attacks on shipping in the Rung Sat area (U.S. Army, 1972).

Infiltration of the enemy and their supplies into Vietnam was also a major problem for military operations. The predominant points of entry were in densely forested areas, where U.S. patrols were subject to enemy ambush, and the forest cover concealed the enemy and its supplies. The Ca Mau peninsula was a temporary staging area for infiltration into the Mekong Delta and for attacks on local shipping and Navy patrol craft along the peninsula's streams and canals. Defoliation operations in 1967 and early 1968 aided military operations by improving observation of formerly heavily forested jungle areas (U.S. Army, 1972).

The enemy infiltration terminated in base camps within South Vietnam; several were located near the Cambodian border and others were located near Saigon. These enemy camps were the source of raids on and harassment of friendly forces, terrorist attacks on local inhabitants, and attempted infiltration into cities. War Zone C (an area in III Corps on the Cambodian border), and War Zone D and Bear Cat (both near Saigon), were three such enemy base camp areas noted by the Army that were sprayed repeatedly to reach all levels of the canopy forest and restrict regrowth.

Perimeter spraying by hand or helicopter at base camps and other installations was required to control the growth of tall grasses and brush. In areas where bamboo or tall grass surrounded a base, it was necessary to respray every two or three months to keep the vegetation low; however, the Army notes that in most locations, the topography, hazardous conditions, mine fields, and limited work force and equipment precluded the use of hand sprays for clearing base perimeters (U.S. Army, 1972).

Crop destruction targets were primarily located in I Corps and the western region of II Corps. Rice was the main target for destruction, and Agent Blue was found to be most effective. Although the immediate effect of the herbicides was to destroy the rice crop, the Army reported that new crops could be planted during the next growing season due to the lack of residuals in the soil that restricted subsequent plant growth.

Assuming a flight swath width of 80 m, the NAS estimated that from 1965 to 1971, 3.6 million acres, or nearly 10 percent of the land area of

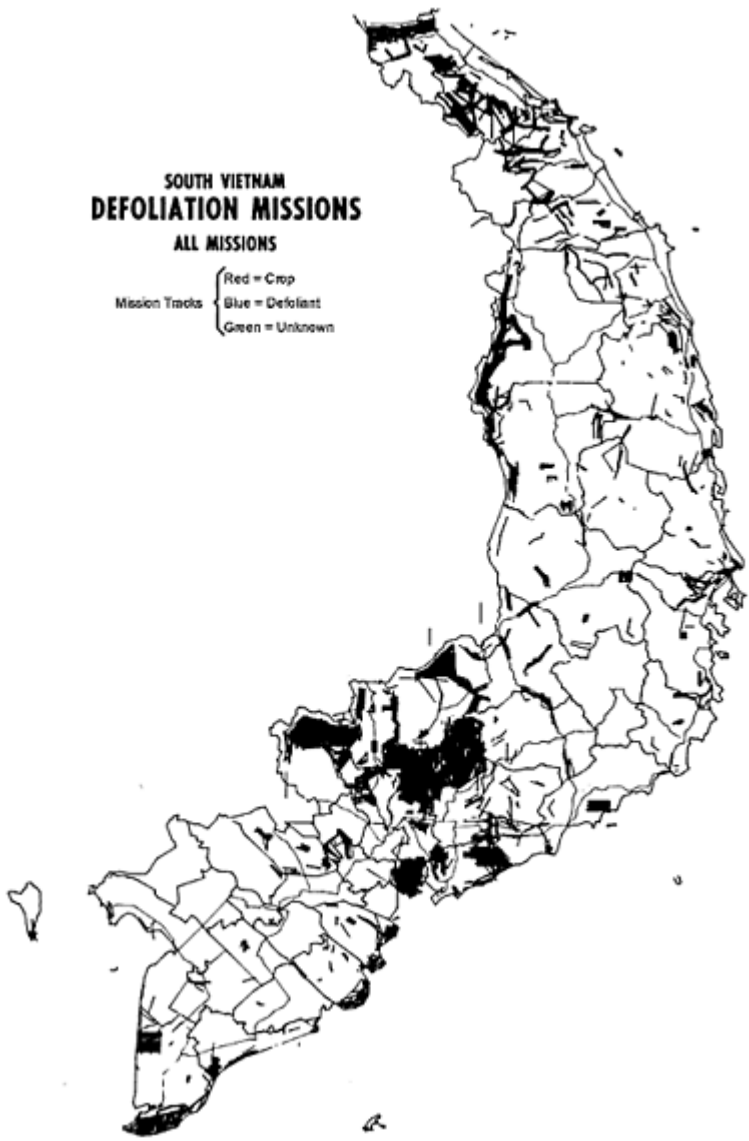

FIGURE 3-2a Herbicide defoliation missions in Vietnam as recorded on HERBS tape.
SOURCE: NAS, 1974. NOTE: Lines indicate mission tracks.

FIGURE 3-2b Herbicide defoliation missions in Vietnam during 1966. SOURCE: NAS, 1974.
NOTE: Lines indicate mission tracks.

FIGURE 3-2c Herbicide defoliation missions in Vietnam during 1967. SOURCE: NAS, 1974. NOTE: Lines indicate mission tracks. Shading indicates populated area.

FIGURE 3-2d Herbicide defoliation missions in Vietnam during 1968. SOURCE: NAS, 1974. NOTE: Lines indicate mission tracks.

FIGURE 3-2e Herbicide defoliation missions in Vietnam during 1969. SOURCE: NAS, 1974. NOTE: Lines indicate mission tracks.

South Vietnam, had been sprayed at least once with herbicides (NAS, 1974). About 1.2 million acres, or roughly 34 percent of the sprayed area, was sprayed more than one time. These calculations are based on figures for the spraying missions by the C-123s and do not take into account unrecorded helicopter or ground sprays, or the effects of wind drift, aircraft speed, and rates of delivery. III Corps was the most heavily sprayed area of Vietnam, receiving about 53 percent of all herbicide sprays from 1965 to 1971 (Table 3-6). Thirty percent of III Corps was sprayed at least once (Westing, 1984). War Zones C and D, and the Iron Triangle in III Corps, can also be identified as heavily sprayed areas in maps of herbicide defoliation missions. The Rung Sat Special Zone in III Corps near Saigon, where the Saigon and Dong Nai Rivers linked together, was the most heavily sprayed region in Vietnam, as well as a site of frequent U.S. Navy operations. In 42 missions, the C-123s sprayed thousands of gallons of herbicides on the mangrove swamps to flush out Vietcong from hidden strongholds, from which they attacked supply ships and instituted offensives in the Delta region and surrounding provinces (Dux and Young, 1980). The area was sprayed consistently until 1970; the NAS (1974) estimated that 57 percent of the Rung Sat Special zone had been sprayed.

TABLE 3-6 Herbicide Use by Military Region, 1965-1971 (million gallons)

Military Region	Agent Orange	Agent White	Agent Blue	TOTAL (%)
I	2.25	0.36	0.30	2.91 (16.5)
II	2.52	0.73	0.47	3.72 (21.0)
III	5.31	3.72	0.29	9.32 (52.7)
IV	1.23	0.44	0.06	1.73 (9.8)
TOTAL	11.31	5.25	1.13	17.68
(%)	63.9	29.7	6.4	100.0

SOURCE: Tschirley, 1992.

Another heavily sprayed area, the Ca Mau Peninsula at the southern tip of South Vietnam, was almost entirely covered with dense mangrove forests up to 1968. However after extensive spraying of the peninsula in 1967 and 1968, the NAS (1974) concluded that nearly half of the mangrove trees had been destroyed. Mangrove forests were more heavily affected by herbicide spraying than any other vegetation type in South Vietnam. One spray usually killed all mangrove trees (NAS, 1974).

Another limitation of the HERBS tapes, and the maps generated from it, is that the plotted lines represent the center of each mission. The assumed swath width for a sortie was 80 m. Typical missions consisted of

three aircraft, and some as many as 12 to 16; these differences in effective spray area are not reflected by the maps. A detailed study of the frequency of sprays in the Rung Sat Special Zone conducted by the NAS (1974) is shown in Figure 3-3.

For each herbicide mission, the date, number of gallons sprayed, and type of herbicide are indicated. These maps show how many times any

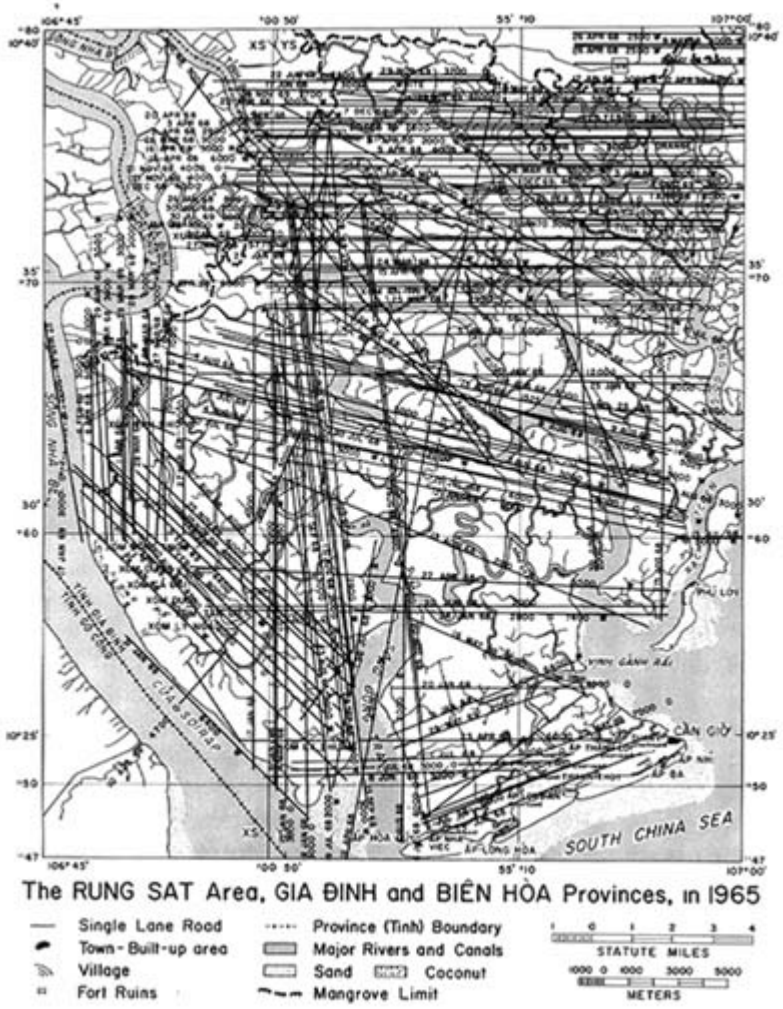

FIGURE 3-3 Herbicide spray missions (1966-1967) in the Rung Sat Special Zone. Data from HERBS tape include date of mission, number of gallons, and type of herbicide agent. SOURCE: NAS, 1974.

hectare had been sprayed due to repetition or overlapping of herbicide applications and, more accurately, depict the extent of spraying conducted in the Rung Sat Special Zone during 1966 and 1967. Areas designated as defoliation targets were much more likely to be sprayed repeatedly than targets of crop destruction missions. Less than 10 percent of the targets for crop destruction missions were sprayed more than once, and the intervals were usually 6 to 12 months; one-third of the areas classified as defoliation were resprayed, and approximately 70 percent received the second spray within 6 months of the initial spray (NAS, 1974).

Military documents report the use of herbicides over areas of Laos, particularly near the Vietnam border and along the Ho Chi Minh Trail. The purpose of the operation in Laos was to expose foot trails, roads, and other lines of communication that led into Vietnam. Herbicide operations began in December 1965; within a six month period, more than 200,000 gallons of herbicide had been sprayed over approximately 1,500 km of roads and trails in Laos (Collins, 1967).

SUMMARY

Some 3 million U.S. military personnel served in or near Vietnam, but the precise number cannot be readily determined from existing military records since individual service records have not been computerized. Surveys of veterans vary in their estimates because of differences in terminology and sample selection procedures. Existing military records do document assignments of military personnel to units and the location of most units at most times. Individual military experiences of Americans who served in Vietnam varied as the nature of the war in different areas of the country changed over time. Individual experiences also varied by branch of service, military occupation, rank, and type of military unit.

Between 1962 and 1971, U.S. military forces sprayed nearly 19 million gallons of herbicides over approximately 3.6 million acres in South Vietnam. The preparation known as Agent Orange accounted for approximately 11.2 million gallons of the total amount sprayed. Seven different herbicide formulations were used in varying quantities for a variety of purposes in different parts of the country. Approximately 65 percent of all herbicides sprayed were contaminated by TCDD, in varying concentrations. Herbicides were used to strip the thick jungle canopy that helped conceal opposition forces, to destroy crops that enemy forces might depend upon, and to clear tall grass and bushes from around the perimeters of U.S. base camps and outlying fire support bases.

Aerial spraying of herbicides by Operation Ranch Hand accounted for approximately 86 percent of all spraying, and existing computerized records indicate which herbicides were used and where. These records indicate that

the spraying was not uniform, but concentrated in certain geographical and tactical areas. Units and individuals other than the members of the Air Force Ranch Hand, such as the Army Chemical Corps, were also likely to have handled or sprayed herbicides around bases or lines of communication. Considerable quantities of herbicides were also sprayed from boats and ground vehicles, as well as by soldiers wearing back-mounted equipment. Although the recording of such sprays was not as systematic as those of Ranch Hand, some records do exist on the "Services HERBS" computer tape.

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4

Toxicology

In this chapter, the results of experiments in which animals were exposed to the substances of concern and observed for particular effects are reviewed, to provide a basis for evaluating the biologic plausibility of the epidemiologic evidence associating exposures and effects described in Chapters 8-11. Assessing the biologic plausibility of the outcomes reported in epidemiologic studies would strengthen any evidence for an association between exposures and effects.

Although there is evidence that multiple chemicals were used for various purposes in Vietnam, the use of four herbicides has been documented in military records; therefore, toxicologic assessment was limited to the compounds 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, and cacodylic acid (Figure 4-1). In addition, the toxicologic properties of a 2,4,5-T contaminant that has caused a great deal of controversy, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are described. The emphasis of the chapter is on the effects of TCDD, because there is considerably more information available on TCDD than on the herbicides.

The chapter begins with an overview that describes toxicology data on TCDD and the four herbicides in nontechnical terms. The overview is followed by complete toxicity profiles of each of the five substances considered. In reading these profiles, several characteristics of animal studies should be borne in mind. First, animals are exposed to various levels of a compound through multiple routes of exposure. In addition, animals may be exposed once to a very high dose of a compound or multiple times to lower doses. Thus, an effect observed in animals may not necessarily occur

in humans because of differences in dose, route, and timing of exposure. Second, for the most part, animals are exposed to a single agent and are generally healthy when exposure occurs. Although most of the people exposed to TCDD who are of interest in this report were healthy, they were certainly not exposed solely to TCDD. Third, the toxicity of a given compound varies widely depending on the health status (as determined by nutrition, age, infection, etc.) of the animal examined. When data are available, the contribution of nutrition, age, and other possible factors to the toxicity of the compounds is discussed. Fourth, there is a wide variability in the toxicity of TCDD depending on the species of animal tested. These differences are exemplified in the dose of TCDD required to kill 50 percent of the animals exposed (LD_{50}) (Table 4-1).

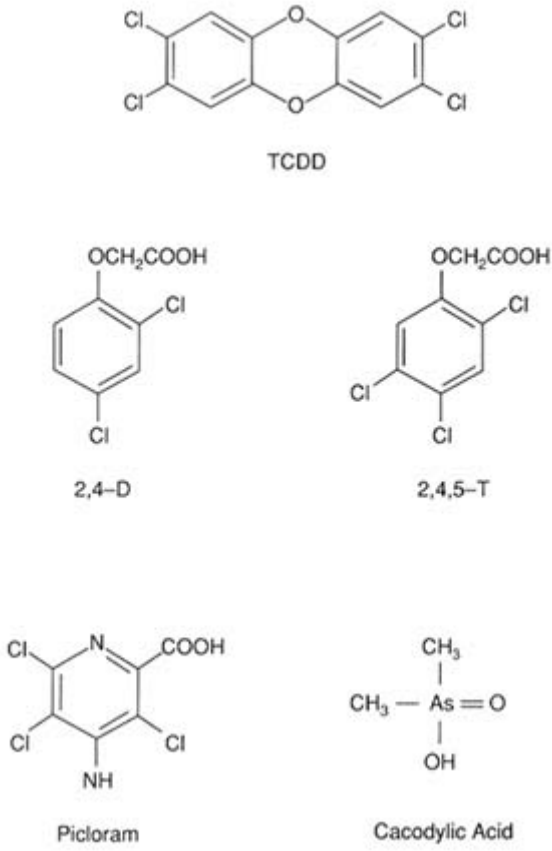

FIGURE 4-1 Chemical structures of the herbicides 2,4-D, 2,4,5-T, picloram, and cacodylic acid, and of the contaminant TCDD.

In the guinea pig (the most sensitive species to acute lethality by TCDD), the LD_{50} is 0.6-2.1 μg/kg. On the other hand, the LD_{50} of TCDD for the hamster (the least sensitive species examined) is 1,157-5,051 μg/kg. It is currently unknown where on this spectrum humans lie; however, studies are under way to determine the sensitivity of humans to a number of effects of TCDD. Lastly, individuals within a species may vary widely in their sensitivity to the effects of a chemical. For example, two strains of mice, C57Bl/6 (sensitive) and DBA/2 (resistant), are very different in their sensitivity to the acute toxicity of TCDD. Studies involving congenic mice (mice that are identical at all genetic sites except one) suggest that for many of the toxicologic

TABLE 4-1 Acute Lethality of TCDD to Various Species and Substrains

Species/Strain/Sex	Route	LD_{50} (μg/kg)	References
Guinea pig/Hartley (male)	Oral	0.6-2.1	McConnell et al., 1978a; Schwetz et al., 1973
Mink/not reported (male)	Oral	4.2	Hochstein et al., 1988
Chicken/not reported	Oral	<25	Greig et al., 1973
Monkey/rhesus (female)	Oral	~70	McConnell et al., 1978b
Rat/L-E (male)	Intraperitoneal	~10	Tuomisto and Pohjanvirta, 1987
Rat/Sherman, Spartan	Oral		Schwetz et al., 1973
male		22	
female		13-43	
Rat/Sprague-Dawley	Intraperitoneal		Beatty et al., 1978
male		60	
female		25	
weaning male		25	
Rat/Fischer Harian (male)	Oral	340	Walden and Schiller, 1985
Rat/H/W/ (male)	Intraperitoneal	>3,000	Pohjanvirta and Tuomisto, 1987; Pohjanvirta et al., 1988a
Mouse/B6 (male)	Oral	182	Chapman and Schiller, 1985
D2A/2J (male)		2,570	
B6D2F1 (male)		296	
Mouse/B6	Intraperitoneal	132	Neal et al., 1982
Mouse/D2		620	
Mouse/B6D2F1		300	
Rabbit/New Zealand white	Oral	115	Schwetz et al., 1973
(male and female)	Dermal	275	
Rabbit/New Zealand white (male and female)	Intraperitoneal	~50	Brewster et al., 1988
Hamster/golden Syrian (male and female)	Oral	1,157-5,051	Henck et al., 1981
Hamster/golden Syrian (male and female)	Intraperitoneal	>3,000	Olson et al., 1980b

SOURCE: U.S. EPA, 1992.

effects described below, the differences in the sensitivity of these two strains are due to differences in the affinity of an intracellular protein—referred to as the Ah receptor—for TCDD. Although all of these considerations have implications for the interpretation of the data described below, it should be kept in mind that the primary purpose of this review is to contribute to a consideration of the biologic plausibility of the associations observed in epidemiologic studies that are relevant to herbicide exposure in Vietnam, not to resolve the continuing scientific and regulatory concerns about TCDD.

OVERVIEW

Information from tests in laboratory animals and other nonhuman systems is useful because it can be combined with information obtained from humans exposed to the herbicides (described in Chapters 6 and 7) to determine the biologic plausibility for health effects observed in humans (described in Chapters 8-11). Establishing the biologic plausibility of effects due to herbicide exposure in the laboratory strengthens the evidence for any effects of the herbicides that are suspected to occur in humans.

The herbicides that were used in the greatest quantities in Vietnam were 2,4-D, 2,4,5-T, picloram, and cacodylic acid. Agent Orange was a one-to-one mixture of 2,4-D and 2,4,5-T. A contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (commonly called TCDD or dioxin), was found at varying levels in different batches of Agents Orange, Pink, Purple, and Green.

Chemistry

TCDD forms as a by-product during the manufacture of 2,4,5-T. TCDD molecules contain carbon, hydrogen, oxygen, and chlorine. TCDD dissolves easily in fats and oils but not in water, and is persistent in the environment. The primary source of TCDD in the environment is combustion and industrial processes, but the primary source of human exposure is through food.

2,4-D and 2,4,5-T are called chlorophenoxy acids and are also made up of carbon, hydrogen, oxygen, and chlorine. They both dissolve in water and are very similar in structure to a natural plant hormone called auxin. As a result of this similarity, 2,4-D and 2,4,5-T can mimic the action of auxin in some plants, and this activity is thought to be the reason these chemicals are herbicidal.

Cacodylic acid contains carbon, hydrogen, oxygen, and arsenic and was called Agent Blue. Picloram contains carbon, hydrogen, oxygen, chlorine,

and nitrogen, and was combined with 2,4-D to become Agent White. Both compounds dissolve in water.

Exposure and Metabolism

When exposure to a chemical occurs, its effects on the body depend on a number of factors: it can be absorbed into the body, it can be distributed to different organs in the body, it can be metabolized by enzymes that change its chemical structure, and it can be eliminated from the body. A chemical's effects ultimately depend on the rate and extent to which all of these activities occur.

When TCDD is ingested by animals (e.g., through contaminated food), more than 50 percent is absorbed into the body through the gastrointestinal tract. Most of the TCDD breathed in the air is thought to be absorbed through the lungs, but this route of exposure is not well-studied. In contrast, TCDD is not absorbed well through the skin. The same pattern of absorption holds true for 2,4-D and 2,4,5-T, and probably for picloram and cacodylic acid, although much less information is available for them.

After a chemical is absorbed into the body, it can be transported to different organs through the blood or lymph system. TCDD is transported by both systems of circulation, and is distributed primarily to the liver and to body fat. Following single doses of TCDD to rats, a dose-related increase occurred in the proportion of the dose that distributed to the liver as compared to the fat. This observation may be due to increased binding of TCDD to liver cells as the doses increased, as well as to the loss of body fat that occurs in rats as doses of TCDD increase. The amount of time that TCDD remains in the liver or fat is different for different species: in rats, TCDD remains in fat longer than in the liver; in mice, it stays in both for about the same time; and in monkeys, it stays in fat for a very long time. Mice and rats eliminate TCDD from the body in both urine and feces, whereas all other species studied eliminate TCDD primarily through feces.

2,4-D and 2,4,5-T are distributed widely in the body and are eliminated quickly, mostly in the urine. The distribution patterns of picloram and cacodylic acid are not known, although they are eliminated rapidly from the body, mostly in urine. Some of the cacodylic acid that is absorbed is bound to red blood cells, however, and is eliminated when the red blood cells to which it is bound die naturally. Although cacodylic acid binds readily to rat red blood cells, it does not bind readily to human red blood cells.

TCDD is removed slowly from the body; as discussed later in Chapter 6, it takes more than 10 years for half of the body burden of TCDD to be removed. TCDD is metabolized by enzymes in the liver to form derivatives that can dissolve in water and thus be more easily eliminated from the body than TCDD itself, which does not dissolve in water. Water-soluble derivatives

of TCDD are thought to be much less toxic to animals than TCDD itself, although at present, no significant correlations have been made between the distribution, metabolism, and elimination of TCDD and its toxicity in different species.

2,4-D, 2,4,5-T, and cacodylic acid are not metabolized to any significant extent in the body. It is not known whether picloram is metabolized.

Carcinogenicity: TCDD

The ability of TCDD to cause cancer in animals has been studied using rats, mice, and hamsters exposed to TCDD for between one and two years. In these studies, TCDD was fed to animals, applied to their skin, injected under their skin, or injected into their abdominal cavities. Table 4-2 summarizes the results of the different studies that have been performed in animals to evaluate the ability of TCDD to cause cancer.

As the table shows, increased tumor rates have been reported to occur at several different sites in the body in different studies, although the liver was consistently a site of tumor formation in different studies and different species. In studies in which liver cancer occurred, other toxic changes in the liver also occurred. Other organs in which increased cancer rates were observed in animals exposed to TCDD include the thyroid and adrenal glands, the skin, and the lung. Organs in which decreased cancer rates were seen in animals exposed to TCDD include the uterus, pancreas, and the pituitary, mammary, and adrenal glands.

In addition to increasing cancer rates in animals by itself, TCDD can increase tumor formation by other chemicals. For example, when a single dose of a known carcinogen is applied to the skin of mice and that dose is followed by multiple doses of TCDD over a period of several months, more skin tumors are seen than would be expected from the single dose of carcinogen alone. Similar results are obtained in rat livers when a single dose of a liver carcinogen is followed by multiple doses of TCDD.

In rats, liver tumor formation associated with TCDD exposure is dependent on the presence of ovaries; in other words, only female rats that have not had their ovaries removed can develop liver tumors when they are exposed to TCDD. This observation indicates that complex hormonal interactions are likely to be involved in TCDD-induced carcinogenesis.

Mechanism of Action

TCDD has a wide range of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. TCDD may thus play a number of different roles that could affect tumor formation. Understanding how TCDD affects tumor formation in

TABLE 4-2 Summary of Carcinogenicity Bioassays of TCDD

Reference	Species/Strain/Sex	Protocol	Results
Van Miller et al., 1977	Sprague-Dawley rats, male, 10/group	0.001-1,000 ppb (0.0003-500 µg/kg/wk) in feed for 78 weeks; observed for 17 weeks	High mortality, poor reporting; total tumors increased in all but lowest dose group; possible increase in lung tumors and liver tumors; no tumors in controls
Kociba et al., 1978	Sprague-Dawley rats, male and female, 86/control group, 50/treated groups	21-2,200 ppt (0.001-0.1 µg/kg/day) in feed for 2 years	Males: increased tumors of tongue, nose/palate; females: increased tumors of lung, liver, nose/palate
Toth et al., 1979	Swiss mice, male, 100/control group, 45/treated groups	0.007-7.0 µg/kg/wk by gavage for 1 year; observed for lifespans	Liver tumors in 0.7 group; none in 0.007 group; higher dose died
NTP, 1982a	Osborne-Mendel rats, male and female, 75/control group, 50/treated groups	0.0014-0.071 µg/kg/day by gavage for 2 years	Males: increased tumors of thyroid and skin; females: increased tumors of skin, liver, and adrenal gland
NTP, 1982a	B6C3F_1 mice, male and female, 75/control group, 50/treated groups	Males: 0.0014-0.071 µg/kg/day; females: 0.0057-0.29 µg/kg/day; by gavage for 2 years	Males: increased tumors of lung and liver; females: increased lymphoma and tumors of liver, thyroid gland, skin
NTP, 1982b	Swiss-Webster mice, male and female, 45/control group, 30/treated groups	0.001-0.005 mg/dermal application, 3 times weekly for 2 years	Males: no effect; females: increased skin fibrosarcomas
Della Porta et al., 1987	B6C3F_1 mice, male and female, 42-50/group	2.5-5.0 µg/kg/week by gavage for 52 weeks; observed until 78 weeks	Both sexes: increased hepatocellular carcinoma
	B6C3F_1 and B6CF$_1$ mice, male and female, 89-106/group	1-30 µg/kg/week by intraperitoneal injection for 5 weeks; observed until 78 weeks	All: increased lymphoma; B6C3F_1 males: increased hepatocellular adenomas and carcinomas
Rao et al., 1988	Syrian golden hamsters, male	100 µg/kg by intraperitoneal injection; 2-6 treatments over a 4-week period; observed until 12-13 months	Increased squamous cell carcinoma of facial skin
		50-100 µg/kg by subcutaneous injection; 2-6 treatments over a 4-week period; observed until 12-13 months	Increased squamous cell carcinoma of facial skin

SOURCE: Adapted from Huff, 1992.

laboratory animals may help us understand whether TCDD would affect tumor formation in humans. For example, when a chemical's ability to induce tumors in animals is tested, it is administered at doses much higher than those to which humans are normally exposed in the environment. High doses of chemicals can cause toxic effects in animals that may increase their sensitivity to carcinogenesis; in other words, cancer can occur at high doses because of effects that would not occur at low doses (Cohen and Ellwein, 1990). In this case, it would not be appropriate to conclude that a chemical that caused cancer in laboratory animals would do so in humans. Understanding how a chemical causes cancer is thus a very important consideration when using information obtained in the laboratory to evaluate effects in humans.

A normal cell can be transformed into a cancer cell when the information that is coded into the DNA of the cell is changed in critical places. Such changes are called mutations and may result from the direct interaction of a chemical with DNA. TCDD is not considered toxic to DNA; that is, tests of its ability to alter the structure of DNA have been negative.

Another way that a normal cell can be transformed into a cancer cell is when changes occur in the regulation of the manner in which the information encoded in DNA is expressed, and incorrect information is received by the cell. Regulation of DNA is performed by proteins called receptors, which interact both with other molecules and with specific sites on DNA. There is a receptor in liver cells (and probably other cells as well), called the Ah receptor, that can interact with TCDD and then with sites on DNA. Binding of TCDD and the Ah receptor to each other and then to DNA results in a number of biologic effects such as increasing the activity of certain enzymes and affecting the levels of hormones and of molecules that control tissue growth. For example, TCDD treatment can increase the rate at which liver cells multiply; both this effect and TCDD-induced liver tumor formation are dependent on the presence of ovaries. It is thus possible that TCDD, together with the Ah receptor, could alter the information obtained from DNA in such a way that a normal liver cell is transformed into a cancerous liver cell, although direct proof of this possibility has not been obtained.

Carcinogenicity: Herbicides

Several studies of the carcinogenicity of 2,4-D, 2,4,5-T, picloram, and cacodylic acid have been performed in laboratory animals. In general they have produced negative results, although some were not performed using rigorous criteria for the study of cancer in animals, and some produced equivocal results that could be interpreted as either positive or negative. The studies and their results are summarized in Table 4-3.

2,4-D was administered to rats, mice, and dogs in their food, by injecting it under their skin, or by placing it directly into their stomachs. All the results were negative, except for one study that found an increased rate of brain tumors in male rats, but not female rats, receiving the highest dose. These tumors also occurred in the control group and might have occurred spontaneously and not as a result of 2,4-D exposure, however. In another study, the occurrence of cancer of the lymph system (malignant lymphoma) among dogs kept as pets was found to occur more frequently when owners used 2,4-D on their lawns than when they did not (although this test had limitations). These dogs were exposed to other chemicals in addition to 2,4-D, however. Another test using dogs exposed to 2,4-D in the laboratory produced negative results, so it is not clear whether 2,4-D was responsible for the lymphomas in dogs.

2,4,5-T has been tested in rats and mice in their food, in their drinking water, by injecting it under their skin, or by placing it directly into their stomachs. Cacodylic acid has been tested in a very limited study in mice both in their food and by placing it directly into their stomachs. Picloram has been tested in rats and mice in their food. Results of all of these studies were uniformly negative, with the exception of one study using picloram in which liver tumors appeared but were attributed to the presence of hexachlorobenzene as a contaminant.

Mechanism of Action

In the absence of any compelling evidence that the herbicides used in Vietnam are carcinogens in animals, it is difficult to draw conclusions regarding their mechanisms of action as such. The mechanisms of action of the herbicides have not been studied to the same extent as TCDD. Neither 2,4-D nor 2,4,5-T is considered toxic to DNA; that is, they do not interact directly with or change the structure of DNA. Tests on cacodylic acid indicate that it is toxic to DNA only at very high doses, and tests with picloram are extremely limited, but suggest that it is not toxic. None of these compounds is metabolized to reactive intermediates. They do not accumulate in the body. Thus there is as yet no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides used in Vietnam.

Immunotoxicity: TCDD

The immune system is a complex network of cells and molecules that play an important role in the maintenance of health and resistance to infection. Suppressing the activity of the immune system could lead to an increase in the incidence and severity of infectious disease and an increase in

TABLE 4-3 Summary of Carcinogenicity Bioassays of Herbicides Used in Vietnam

Reference	Species/Strain/Sex	Protocol	Results
Bionetics, 1968a; Innes et al., 1969	Strain (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1 mice, male and female, 18/group	46.4 mg 2,4-D/kg by gavage at 7 days of age, the same amount unadjusted for body weight daily until 28 days of age, then 149 mg/kg diet until 78 weeks of age	No effect
	Strain (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1 mice, male and female, 18/group	21.5 mg 2,4,5-T/kg by gavage at 7 days of age, the same amount unadjusted for body weight daily until 28 days of age, then 60 mg/kg diet until 78 weeks of age	No effect
	Strain (C57BL/6×AKR)F_1, male and female, 18/group	100 mg 2,4-D/kg by gavage at 7 days of age, the same amount unadjusted for body weight daily until 28 days of age, then 323 mg/kg diet until 78 weeks of age	No effect
	Strain (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1, male and female, 18/group	Single dose of 215 mg 2,4-D or 2,4,5-T/kg by gavage or subcutaneously on day 28 of age	No effect
Hansen et al., 1971	Osborne-Mendel rats, male and female, 25/group	0, 5, 25, 125, 625, or 1250 ppm 2,4-D in the diet for 2 years	No effect
Hazleton, 1986	Fischer 344 rats, male and female, 60/group	0, 1, 5, 15, or 45 mg 2,4-D/kg in the diet for 2 years	Females: no effect; males: increased astrocytomas at high dose only
	B6C3F_1 mice, male and female, 60/group	0, 1, 15, or 45 mg 2,4-D/kg in the diet for 2 years	No effect

Hayes et al., 1991	Dogs kept as pets	Case-control study, information from questionnaires and telephone interviews, no exposure data	Household with dogs developing malignant lymphoma used 2,4-D more frequently than those that did not; odds ratio = 1.3
Hansen et al., 1971	Beagle dogs, male and female, 3/group	0, 10, 50, 100, or 500 ppm 2,4-D in the diet for 2 years	No effect
Muranyi-Kovacs et al., 1976	XVII/G mice, 20 male and 19 female; C3Hf mice, 22 male and 25 female	100 mg 2,4,5-T/l drinking water for 2 months, followed by 80 mg/kg diet for their life spans	No effect
Kociba et al., 1979	Sprague-Dawley rats, male and female, 60/group	0, 3, 10, or 30 mg 2,4,5-T/kg/d in the diet for 2 years	No effect
Innes et al., 1969	Unspecified strain mice, male and female	46.4 mg cacodylic acid/kg on day 7 of age, same amount unadjusted for body weight daily until day 28 of age, then 121 ppm (about 18 mg/kg/d) in the diet for 18 months	No effect
Stott et al., 1990	Fischer rats, male and female, 50/group	0, 20, 60, or 200 mg picloram/kg/d in the diet for 2 years	No effect
NCI, 1978	Osborne-Mendel rats, male and female	0, 10,000, or 20,000 ppm picloram (0, 500, or 1,000 mg/kg/d) in the diet for 39 weeks, then 0, 5,000, or 10,000 ppm for 41 weeks; observed for additional 33 weeks	Increase in liver tumors attributed to contamination of picloram by hexachlorobenzene
	$B6C3F_1$ mice, male and female	0, 2,500, or 5,000 ppm picloram (0, 357, or 714 mg/kg/d) in the diet for 79 weeks; recovered for additional 10 weeks	No effect

some types of cancer. Increasing the activity of the immune system could result in the development of allergies and of autoimmune diseases. TCDD has been shown to have a number of effects on the immune systems of laboratory animals.

Studies in mice, rats, guinea pigs, and monkeys indicate that TCDD suppresses the function of certain components of the immune system in a dose-related manner; that is, as the dose of TCDD increases, its ability to suppress immune function increases. TCDD suppresses the function of cells of the immune system such as lymphocytes (cell-mediated immune response), as well as the generation of antibodies by B cells (humoral immune response). Increased susceptibility to infectious disease has been reported following TCDD administration. In addition, TCDD increased the number of tumors that formed when mice were injected with tumor cells.

The effects of TCDD on the immune system appear to vary among species, although most studies used different treatments and are not completely comparable. Studies indicate, however, that some species are more sensitive to the effects of TCDD on the immune system than others. It is not known whether humans would be more or less sensitive than laboratory animals.

Mechanism of Action

Studies of the mechanism of TCDD-mediated effects on the immune system are conflicting. Most studies indicate that the presence of the Ah receptor is required for TCDD-induced immunotoxicity, but other studies indicate that it is not. It is possible that the Ah receptor could play a role in some types of immunotoxicity and not in others. Additional studies indicate that an animal's hormonal status may contribute to its sensitivity to immunotoxicity. There is not enough information available on the mechanisms of TCDD-mediated immunotoxicity in laboratory animals to be able to predict whether it would be immunotoxic in humans, but the fact that TCDD induces such a wide variety of effects in animals suggests that it is likely to have some effect in humans as well.

Immunotoxicity: Herbicides

The potential immunotoxicity of the herbicides used in Vietnam has been studied to a very limited extent. Effects on the immune system of mice have been reported for 2,4-D administered at doses that were high enough to produce clinical toxicity, but these effects did not occur at low doses. The potential for picloram to act as a contact sensitizer (produces an allergic response on the skin) was tested, but other aspects of immunotoxicology

were not examined. The immunotoxicity of 2,4,5-T and cacodylic acid has not been evaluated in laboratory animals.

Reproductive and Developmental Toxicity: TCDD

TCDD has been reported to have a number of effects on the reproductive and developmental functions of laboratory animals. Reproductive toxicity is defined as the occurrence of adverse effects on the male or female reproductive system, whereas developmental toxicity is defined as the occurrence of adverse effects on the developing animal. Developmental toxicity can occur any time during the lifetime of the animal as a result of either parent's exposure to a toxic agent prior to conception, during the development of the fetus, or after birth until the time of puberty.

For example, administration of TCDD to male rats, mice, guinea pigs, marmosets, monkeys, and chickens can elicit reproductive toxicity by affecting testicular function, decreasing fertility, and decreasing the rate of sperm production. TCDD has also been found to decrease the levels of hormones such as testosterone in rats. These effects generally occur only at doses that are high enough to produce clinical toxicity, however, and are much less common at low doses. The reproductive systems of adult male laboratory animals are considered to be relatively insensitive to TCDD because high doses are required to elicit effects. Potential developmental toxicity following exposure of male animals to TCDD has not been studied.

Studies in female animals are limited but demonstrate reduced fertility, decreased ability to remain pregnant throughout gestation, decreased litter size, increased fetal death, impaired ovary function, decreased levels of hormones such as estradiol and progesterone, and increased rates of fetal abnormalities. Most of these effects may have occurred as a result of TCDD's general toxicity to the pregnant animal, however, and not as a result of a TCDD-specific mechanism that acted directly on the reproductive system.

Mechanism of Action

Little information is available on the cellular and molecular mechanisms of action that mediate TCDD's reproductive and developmental effects in laboratory animals. Evidence from mice indicates that the Ah receptor may play a role: mice with Ah receptors that have a relatively high affinity for TCDD respond to lower doses than mice with a relatively low affinity. Other as yet unidentified factors also play a role, however, and it is possible that these effects occur only secondarily to TCDD-induced general toxicity. Extrapolating these results to humans is not straightforward because

of the many factors that determine susceptibility to reproductive and developmental effects among species.

Reproductive and Developmental Toxicity: Herbicides

Several studies have evaluated the reproductive and developmental toxicity of herbicides in laboratory animals. Results indicate that 2,4-D does not affect male or female fertility and does not produce fetal abnormalities, but it did reduce the rate of growth of offspring and increase their rate of mortality when pregnant rats or mice were exposed. Very high doses were required to elicit these effects, however. The reproductive toxicity of 2,4,5-T has not been evaluated, although it was toxic to fetuses when administered to pregnant rats, mice, and hamsters. Studies of the reproductive toxicity of cacodylic acid are too limited to draw conclusions. Studies of its developmental toxicity indicate that it is toxic to rat, mouse, and hamster fetuses at high doses that are also toxic to the pregnant mother. Very limited data indicate that picloram is not a reproductive toxicant, although it may produce fetal abnormalities in rabbits at doses that are also toxic to the pregnant animal.

Studies of the reproductive toxicity of the herbicides are thus too limited to draw conclusions about their effects on male or female fertility. Studies of the developmental toxicity of the herbicides suggest that they can be toxic to developing animals, but high doses are required.

Other Toxicity: TCDD

TCDD has been reported to elicit several other kinds of toxicity in laboratory animals besides those described above. For example, the liver is a target organ for TCDD-induced toxicity in sensitive species. Sensitivity to TCDD-induced liver toxicity is dependent on the presence of Ah receptors with a high affinity for TCDD. Effects of TCDD on the liver include increasing the rate at which liver cells multiply, increasing the rate of liver cell death, increasing fat levels in liver cells, decreasing bile flow, and increasing the levels of protein and of substances that are precursors to heme synthesis. TCDD also increases the levels of certain enzymes in the liver, but this effect is not considered toxic. Mice and rats are susceptible to TCDD-induced liver toxicity, but guinea pigs and hamsters are not. It is possible that liver toxicity is associated with susceptibility to liver cancer.

Other toxic effects of TCDD that have been reported in laboratory animals include reduced blood glucose levels and starvation, increased rates at which cells in the gastrointestinal tract multiply, and changes in skin cells.

Other Toxicity: Herbicides

The herbicides used in Vietnam have also been reported to elicit adverse effects in a number of organs in laboratory animals. The liver is a target organ for toxicity induced by 2,4-D, 2,4,5-T, and picloram, with changes reportedly similar to those induced by TCDD. Some kidney toxicity has been seen in animals exposed to 2,4-D and to cacodylic acid. Exposure to 2,4-D has also been associated with effects on blood, such as reduced levels of heme and of red blood cells.

TOXICITY PROFILE OF TETRACHLORODIBENZO-P-DIOXIN

Introduction

"Dioxin" is a general term used to describe a subset of halogenated aromatic hydrocarbons, as listed in Table 4-4. The chemical structure of some of these compounds is shown in Figure 4-2. These chemicals are usually considered together because (1) their chemical structures are similar; and (2) they produce similar patterns of toxicity (although they differ in potency; Poland and Knutson, 1982).

As will be discussed further below, the greatest biologic potency is associated with halogenation at three or more lateral positions that gave the molecule a relatively planar configuration (Safe, 1986). Although there are

TABLE 4-4 Hierarchical Tree of Selected Halogenated Aromatic Hydrocarbons: Relationship of TCDD to Other Compounds

- Nonchlorinated
- Chlorinated

- Polychlorinated dibenzofurans (PCDFs; furan)
- Polychlorinated biphenyls (PCBs; biphenyl)
- Polychlorinated dibenzodioxins (PCDDs; dioxin)[a]

- Dioxins with other than four chlorines[b]
- Tetrachlorinated dibenzodioxin[c]

- Dioxins with four chlorines other than at the 2,3,7,8-positions
- 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin[d]

[a] Theoretically, 75 possible PCDDs differing only in the number of chlorine atoms and their location on the dioxin nucleus.
[b] OCDD, for example, would refer to a molecule with eight chlorines (O=octa-) on the ring structure.
[c] TCDD may exist as 22 different isomers, but the agent generally referred to as "TCDD" is the 2,3,7,8-isomer.
[d] Numbering system refers to the position of the chlorines on the aromatic rings.
SOURCE: Fishbein, 1987.

75 possible chlorine-substituted dibenzo-*p*-dioxin isomers, the data described below in the sections on toxicology and health effects concern the measured exposure to one dioxin isomer, 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD). In addition to TCDD, commercial formulations of chlorophenoxy herbicides contain a series of other polychlorinated dibenzodioxins (PCDDs) and dibenzofurans. Although studies have been conducted on many of these structurally similar molecules, such as polyhalogenated dibenzofurans and polyhalogenated biphenyls, these studies are not covered in this report because of the extensive literature base.

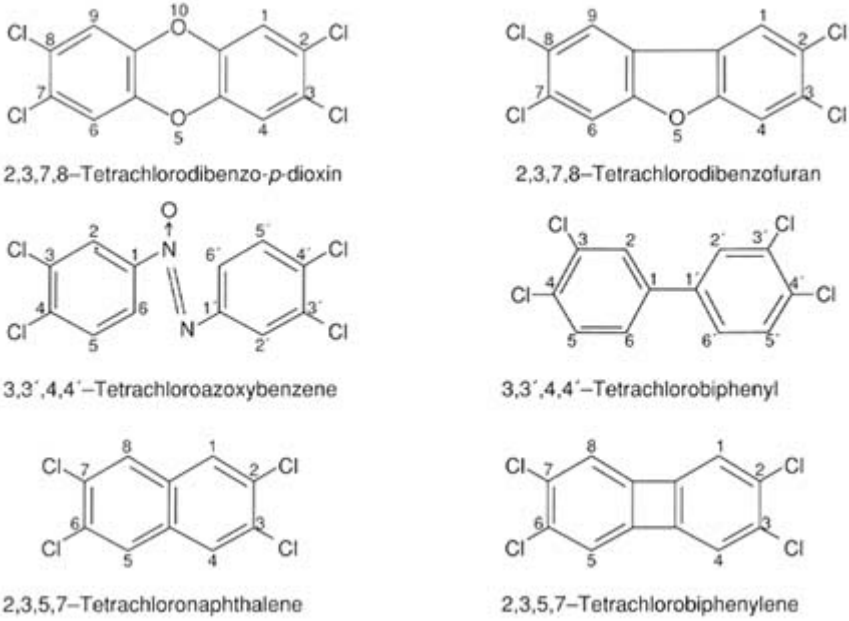

FIGURE 4-2 Chemical structures of some of the halogenated aromatic hydrocarbon compounds.

Some of the halogenated aromatic hydrocarbons are manufactured as commercial products, but others, like TCDD, occur as contaminants in commercial products. TCDD is formed as a contaminant in the synthesis of 2,4,5-trichlorophenol, which is used to manufacture 2,4,5-T (one of the components of several of the herbicides used in defoliation and crop destruction during the Vietnam war) and hexachlorophene. The degree of TCDD contamination is dependent on the temperature and pressure of the reaction conditions (Lilienfeld and Gallo, 1989). Young and colleagues (1978) reported on the levels of TCDD found in more than 450 samples of Agent Orange and one sample of Agent Purple (Table 4-5).

TABLE 4-5 Concentrations of TCDD in Samples of Agents Orange and Purple

	Number of Samples		Concentration of TCDD (μg/g)	
Source of Samples	Orange	Purple	Range	Mean
Johnston Atoll inventory, 1972[a]	200	(4)[b]	0.05-47	1.91
Johnston Atoll inventory, 1974	10		0.07-5.3	1.68
NCBC, Gulfport inventory, 1972[c]	42		0.05-13.3	1.77
NCBC, Gulfport inventory, 1975	238		0.02-15	2.11
Eglin AFB archived sample[d]		1	—	45.00
Eglin AFB inventory, 1972	2		—	0.04

[a] Surplus Agent Orange was shipped from South Vietnam to Johnston Atoll (near Hawaii) for storage in April 1972.
[b] Four of 200 samples may have been Agent Purple.
[c] The Naval Construction Battalion Center (NCBC), Gulfport, Mississippi, served as a storage site for surplus Agent Orange from 1969 to 1977.
[d] Agent Purple was used extensively in the evaluation of aerial spray equipment on Test Area C-52, Eglin Air Force Base (AFB) Reservation, Florida, 1962-1964.
SOURCE: Young et al., 1978.

Using various analytical methods, other investigators determined that TCDD was the dominant compound of its group in Agent Orange (IARC, 1986). Due to their chemical stability and lipophilicity, the chemicals are persistent in the environment and are magnified in the food chain. The primary source of dioxins for human exposure is the food supply (Travis et al., 1989). The main ultimate sources of dioxins are industrial processes and combustion. As stated, the syntheses of some organic chemicals are known to yield dioxins (U.S. EPA, 1980). The use of products contaminated with dioxins and waste disposal from these production processes are two major sources of dioxin exposure (U.S. EPA, 1985). Since 1980, the practices that led to the dispersal of dioxins have been greatly reduced.

TCDD is a molecule that forms colorless needles with a melting point of 295-306°C. It is insoluble in water, but is soluble in many organic solvents (e.g., acetone, alcohol, and benzene) and oils.

Exposure and Pharmacokinetics

The fate of experimentally administered TCDD has been studied in a variety of animal species (reviewed: Neal et al., 1982; Gasiewicz et al., 1983; Olson et al., 1983; Birnbaum, 1985). Drug disposition studies such as these provide important information in developing models that can predict the biodistribution and elimination of TCDD following human exposure.

Ultimately, the disposition of TCDD, like all agents, is influenced by many factors—including the rate of drug absorption, distribution, metabolism, and elimination, and its sequestration and storage in various tissues—all of which have the potential for having an impact on the magnitude of toxicity produced.

Bioavailability Following Various Routes of Exposure

The amount of bioavailable TCDD (i.e., that which is capable of reaching tissue sites sensitive to TCDD-mediated alterations) is dependent on the route of chemical entry. In animal studies, the oral exposure route is most significant because it is believed to be the primary route for human exposure. Although the actual percentage of the total orally administered TCDD dose that undergoes gastrointestinal absorption following oral administration is found to vary among mammalian species, in virtually all cases absorption from either oil vehicles or dietary supplementation is greater than 50 percent. In Sprague-Dawley rats, 84 percent was the mean absorption of a single oral dose of ^{14}C-TCDD (1.0 µg/kg) in an acetone corn oil vehicle (1:25 ratio by volume) (Rose et al., 1976). A similar percentage of the total dose was absorbed when rats were repeatedly administered low doses of TCDD (0.1-1.0 µg/kg) via the oral route, 5 days per week for 7 weeks (Rose et al., 1976). Following multiple oral administrations of high TCDD doses (50 µg/kg) in the rat, absorption was slightly lower, approximately 70 percent of the total dosage administered. Similarly, in hamsters, 75 percent of a single oral dose of [^{3}H]-TCDD administered in olive oil (650 µg/kg) was absorbed, and in the guinea pig, approximately 50 percent of a single oral dose of TCDD in acetone/corn oil was absorbed. Thoracic duct-cannulated rats showed that intestinal absorption of [^{14}C]-TCDD led to the transfer of the radioactive label to chylomicrons, which presumably transported the absorbed TCDD via the lymphatics into the circulation (Lakshman et al., 1986).

In Fischer 344 rats, dermal application of 60 µl of a TCDD solution (0.00015-1.0 µmol/kg in acetone) to a shaved 1.8-cm^2 area of the animal's back, which was subsequently covered with a perforated stainless steel cap (to prevent confounding effects due to animal grooming), revealed several trends (Brewster et al., 1989). First, the percentage of the total TCDD dose absorbed decreased as the dosage increased. Second, the absolute absorbed amount of TCDD increased nonlinearly with dose. Lastly, the majority of the applied dose remained at the site of application, associated primarily with the stratum corneum, the uppermost layer of the epidermis, and did not penetrate through to the dermis. Absorption kinetic studies over 120 hours in the Fischer 344 rat model following application of 200 pmol of TCDD (64 µg/l acetone) indicated that the rate of dermal absorption was very slow,

with an absorption rate constant of approximately 0.005 pmol/hour (Banks and Birnbaum, 1991). After dermal application of 26 ng of TCDD in 50 µl methanol, approximately 14 percent of the total dose was found associated with the liver at 24 hours, compared to 40 percent (in 50 percent ethanol vehicle) after administration of an equal dose of TCDD orally (Poiger and Schlatter, 1980). Studies using soil-bound TCDD showed that a marked decrease occurred in the percentage of TCDD absorbed (approximately 1 percent of total applied dose), compared to when methanol was used as the vehicle, as determined by the total amount of hepatic TCDD (Shu et al., 1988a,b). In an attempt to simulate dermal exposure from contaminated soil, TCDD was applied in a soil-water paste to rats for 24 hours. Only 2 percent of the applied dosage was detected in hepatic tissues, suggesting very poor absorption (Poiger and Schlatter, 1980). Studies using a number of other vehicles suggest that the percentage of TCDD absorbed dermally is dependent on formulation. Taken together, these findings indicate poor dermal absorption of TCDD.

Little information is available pertaining to pulmonary absorption of TCDD; however, it is believed to be very high. Intratracheal instillation of 1 nmol/kg TCDD in Emulphor into male Fischer 344 rats resulted in approximately 92 percent absorption (Diliberto et al., 1991).

Distribution

Once absorbed, the distribution of xenobiotics occurs through body fluids, primarily the lymphatics and blood, where agents either can be transported in the aqueous phase or are free to associate with various lipids and proteins that can serve as endogenous carriers. Following gastrointestinal uptake, TCDD enters the lymphatics where approximately 96 percent is found to be associated with the chylomicron fraction in thoracic duct-cannulated rats (Lakshman et al., 1986). TCDD is transported in this manner into the circulation. Disappearance of TCDD from plasma followed first-order kinetics, with the first 67 percent of absorbed TCDD leaving the blood compartment rapidly (half-life = 0.81 min). The majority of absorbed TCDD was found to be distributed to the liver and adipose tissue.

The amount of an agent distributed to any given tissue is dependent on a number of factors, including the amount of blood flow to that tissue and overall tissue size. The primary sites of initial TCDD distribution from the blood, in terms of percentage of total administered dose, are the liver, adipose tissue, skin, and muscle during the first hour following administration. However, within days the majority of TCDD redistributes to the liver and adipose tissue, the primary sites of TCDD deposition. This general profile of distribution for TCDD has been observed in a variety of animal species including mice, rats, nonhuman primates, guinea pigs, and hamsters (Rose

et al., 1976; Kociba et al., 1978; Gasiewicz and Neal, 1979; Olson et al., 1980a; Gasiewicz et al., 1983; Birnbaum, 1986; Pohjanvirta et al., 1990). Whole-body autoradiography has also revealed that in both mice and rats, in addition to the liver and adipose tissue, there was a distinct localization of ^{14}C-TCDD in the nasal olfactory mucosa (Appelgren et al., 1983; Gillner et al., 1987). The nasal olfactory mucosa was probably not identified in previous biodistribution studies because it is such an unlikely site for TCDD deposition, and therefore was most likely not previously examined.

There is evidence to suggest that the profile of TCDD tissue deposition may also be governed by the temporal kinetics of TCDD administration and the magnitude of the administered dose. Some studies suggest that TCDD tissue distribution is dose-dependent. Biodistribution studies following a single intraperitoneal administration of TCDD in rats revealed a marked increase in the concentration of TCDD in liver at doses greater than 10 ng/kg, with a concomitant decrease in adipose tissue-associated TCDD (Abraham et al., 1988). Similarly, following administration of single doses of TCDD, a dose-related increase was observed in the proportion of TCDD distributed to the liver as compared to adipose tissue (Poiger et al., 1989). Although the mechanism for this phenomenon is unclear, it may be partially related to the fact that rats also exhibit a concomitant and dose-dependent loss of adipose tissue. Other evidence suggests that an increase in hepatic TCDD retention is mediated by a liver-associated binding species. Several laboratories have demonstrated that this binding species is TCDD inducible (Poland et al., 1989a; Curtis et al., 1990; Leung et al., 1990). Pretreatment of rats with 5 or 15 μg/kg of TCDD increased the accumulation of TCDD in hepatic tissue in a dose-dependent manner, when followed by subsequent oral administration of TCDD. Similarly, increased hepatic uptake of TCDD-related compounds was observed after pretreatment with TCDD (Poland et al., 1989b; Leung et al., 1990). Findings by several independent laboratories suggest that the hepatic binding species is cytochrome P4501A2 (Voorman and Aust, 1987, 1989; Poland et al., 1989a,b). As would be expected for cytochrome P4501A2 involvement, Poland and coworkers (1989a) found that the TCDD-binding species was associated primarily with the microsomal fraction of the liver and was heat and trypsin sensitive, inactivated by mercurials, and liver specific. The prospect that cytochrome P4501A2 can act as a TCDD-binding protein is also consistent with the fact that the only other site at which this P450 isozyme is TCDD inducible other than the liver is the nasal olfactory mucosa, a tissue that exhibits high TCDD bioaccumulation (Tuteja et al., 1985; Gillner et al., 1987). Contrary to the premise that cytochrome P4501A2 represents the TCDD hepatic binding species was the observation by Poland and colleagues (1989b) that dietary administration of the cytochrome P4501A2 inducer, isosafrole, did not increase hepatic uptake of TCDD.

In contrast to studies describing dose-dependent tissue distribution of TCDD, findings from several other studies do not support this trend (Rose et al., 1976; Clark et al., 1991c; Tritscher et al., 1992).

Species and tissue-related differences exist for TCDD retention time. In rats, TCDD is more persistent in adipose tissue than in liver (Abraham et al., 1988), whereas in the mouse, TCDD has a similar half-life in adipose and hepatic tissue (Birnbaum, 1986). In nonhuman primates such as the rhesus monkey, TCDD is exceptionally persistent in adipose tissue (Bowman et al., 1989). Adding to this complexity of TCDD retention are biodisposition studies suggesting that the rate of TCDD decay from liver, adipose tissue, and other tissue may not remain constant with time (Birnbaum et al., 1980; Olson et al., 1980a; Birnbaum, 1986; Pohjanvirta et al., 1990; Neubert et al., 1990a).

Likewise, there is also evidence to suggest that TCDD retention in the rat liver may be cell-type specific. Four days after TCDD exposure, approximately 60 percent of TCDD associated with the liver was retained in parenchymal cells (half-life - 13 days) and 12 percent with nonparenchymal cells.

Metabolism

TCDD is biotransformed to water-soluble metabolites in a wide range of mammalian species (Poiger and Schlatter, 1979; Ramsey et al., 1979, 1982; Olson et al., 1980a; Poiger et al., 1982; Gasiewicz et al., 1983; Kleeman et al., 1988; Sijm et al., 1990). In a number of rodent species including the rat, mouse, hamster, and guinea pig, more than 90 percent of the TCDD that undergoes urinary and biliary excretion is in a polar biotransformed form. In fact, excretion of absorbed TCDD is metabolism dependent, with the exception of nonabsorbed compound that undergoes direct intestinal excretion. In dogs, the effects of pretreatment with mixed-function oxidase (MFO) inducers, either phenobarbital or TCDD, on the biliary excretion of subsequently administered ^{3}H-TCDD were investigated (Poiger and Schlatter, 1985). Without pretreatment, 24.5 percent of the absorbed TCDD was excreted in bile within 110 hours. Phenobarbital pretreatment produced no effect on the rate of TCDD biliary excretion, whereas TCDD pretreatment (a single 10-µg/kg dose 9 days earlier) resulted in a doubling in the biliary TCDD elimination rate. These results illustrate the important role the MFOs play in the rate of TCDD elimination.

Although the metabolism of TCDD has been somewhat enigmatic, a number of metabolites have been identified. Six TCDD metabolites were detected in the bile of dogs that had received a lethal dose (Poiger et al., 1982). The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo*p*-dioxin. Additionally, 3,7,8-trichloro-3-hydroxydibenzo-*p*-dioxin and 1,2-dichloro-4,5-hydroxybenzene

were identified as minor metabolites. The structure of the three remaining metabolites was not confirmed; however, it was believed that two of the metabolites were trichlorohydroxydibenzo-*p*-dioxins, with the third possibly being chlorinated 2-hydroxydiphenyl ether. In the rat, trichlorodihydroxydibenzo-*p*-dioxin and tetrachlorodihydroxydiphenyl ether were the major metabolites identified in bile (Poiger and Buser, 1984). Additionally, what were believed to be glucuronide conjugates were identified in rat but not dog bile. In vitro studies utilizing isolated rat hepatocytes in culture identified two glucuronide conjugates as the major metabolites of 2,3,7,8-TCDD (Sawahata et al., 1982). Deconjugation of the metabolites with β-glucuronidase yielded 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-trichloro-3-hydroxydibenzo-*p*-dioxin. It is generally believed that the major route of metabolism in the rat involves oxygenation of the unsubstituted carbon nearest the bridging oxygen in 2,3,7,8-TCDD.

Metabolic biotransformation of TCDD is generally accepted as being a detoxification reaction. This premise is supported by a number of different studies using a variety of approaches. For example, bile extracts from TCDD-treated dogs administered to guinea pigs were found to be 100 times less toxic than orally administered TCDD itself (Weber et al., 1982). Structure-activity relationship studies using synthesized congeners of known TCDD metabolites found those compounds to be toxicologically inactive even at very high concentrations (i.e., up to 5,000 µg/kg), suggesting that TCDD itself is the active species (Mason and Safe, 1986). Poland and Glover (1979), investigating the potential for in vivo bioactivation of TCDD to reactive intermediates, demonstrated that very low amounts of TCDD actually formed DNA adducts (i.e., 8 1 DNA adduct/35 cells). These findings suggest that the covalent binding of TCDD to DNA is not likely to be responsible for its oncogenic effects, and further support the premise that TCDD metabolism is primarily a detoxification mechanism.

Excretion

The rate and primary route of TCDD excretion has been found to differ among animal species. After a single dose, TCDD undergoes a first-order elimination process exhibiting very slow excretion kinetics. In the hamster, the half-life for elimination has been estimated at approximately 11 days. The mean half-life of TCDD in the guinea pig is approximately 94 days (Olson, 1986). In the rat, following repeated oral dosing (0.1-1.0 µg/kg), 5 days per week for 7 weeks, the half-life for elimination ranged from 16 to 37 days (Rose et al., 1976). From drug disposition studies in the rat, Rose and colleagues (1976) concluded that based on calculated steady-state values, it was unlikely that TCDD would continue to accumulate indefinitely in the tissues of animals exposed chronically to low levels of the compound.

However, in nonhuman primates, TCDD was found to be highly persistent (Bowman et al., 1989; Neubert et al., 1990a).

Species differences also exist with respect to the route of elimination of TCDD. In the hamster and mouse, excretion of TCDD occurs via both feces and urine (Olson et al., 1980a; Gasiewicz et al., 1983; Birnbaum, 1986). Conversely, in all other species, excretion occurs primarily through feces (Piper et al., 1973a; Allen et al., 1975; Rose et al., 1976; Gasiewicz and Neal, 1979). In virtually all rodent studies, results indicate that all of the TCDD excreted in urine and bile is in the form of TCDD metabolites. In the rat, hamster, and mouse, approximately 15-35 percent of TCDD in feces is unmetabolized, whereas in the guinea pig, approximately 81 percent was unmetabolized in feces (Olson et al., 1980a; Neal et al., 1982; Gasiewicz et al., 1983; Olson, 1986). Unmetabolized TCDD in feces is believed to be primarily a result of direct intestinal elimination since no parent form of the compound is normally observed in bile. Minimal excretion of ^{14}C-TCDD has also been reported in expired air (Piper et al., 1973a). The relationship between administered dose and excretion rate is limited; however, little change in excretion of 2,3,7,8-TBDD (2,3,7,8-tetrabromodibenzo-*p*-dioxin) was observed between 1- and 100-nmol/kg doses (Kedderis et al., 1991). Based on present data, no significant correlations have been made between metabolism and disposition of TCDD and strain- or species-specific toxicity.

Mechanism of Action

Introduction

A great deal of research has gone into determining the mechanism of TCDD toxicity in order to determine the plausible biologic activity of the molecule. Most of this research has focused on identification and characterization of the interaction of TCDD with an intracellular protein called the Ah receptor. An Ah receptor protein can interact with a TCDD molecule when it enters a cell, and then translocate to the nucleus where the TCDD-receptor complex can interact with specific sites on DNA. Interaction with specific sites on DNA may have an effect on the regulation of DNA expression, affecting a wide range of mechanisms that regulate normal cellular activity.

Receptor-mediated events are generally characterized by the following: (1) they are restricted to cells that express the receptor; (2) there is a structure-activity relationship (i.e., molecules that bind have a specific geometric configuration, and their potency varies with deviations in this geometric configuration); (3) binding occurs at relatively low concentrations of the ligand (the molecule that binds the receptor); (4) binding is reversible; and (5) the magnitude of the response is proportional to the number of receptors

occupied by the ligand. For Ah receptor-mediated events, TCDD meets many of these criteria.

Not all effects of TCDD are mediated through the binding of TCDD to the Ah receptor, including neurotoxicity and in vitro immunotoxicity to B cells. The mechanism by which these effects are elicited by TCDD is currently unknown; therefore, the emphasis in this section is on effects mediated by the Ah receptor.

Ah Receptor

Early studies by Poland demonstrated that TCDD saturably binds an intracellular protein with a high affinity. Further characterization of the binding properties indicated that the ligand binding exhibited stereospecificity (i.e., planar molecules with at least three halogen atoms were bound) (Poland and Glover, 1973; Poland and Knutson, 1982). Additional studies showed that the binding affinity of various congeners for the soluble receptor correlated well with the ability of the molecules to elicit a biological response. In addition, genetic strains of mice were identified whose Ah receptor had a lower affinity for TCDD. These mouse strains had a decreased sensitivity to the toxic effects of TCDD. Crossbreeding studies indicate that the sensitive phenotype segregates as an autosomal dominant phenotype. Further genetic studies identified the "Ah locus" as the area of the genome that encodes for the Ah receptor (Poland and Knutson, 1982; Nebert, 1989). Therefore, biochemical and genetic evidence indicates that the cytosolic protein Ah is the receptor for TCDD. Although this protein has a high affinity for TCDD, recent studies have identified possible naturally occurring high affinity ligands for the receptor (Gillner et al., 1985, 1987; Rannug et al., 1987; Bjeldanes et al., 1991).

Human cells from a variety of tissue types contain an intracellular protein that resembles the Ah receptor in animals (Manchester et al., 1987; Cook and Greenlee, 1989; Harris et al., 1989; Roberts et al., 1990; Lorenzen and Okey, 1991; Waithe et al., 1991). The isolated receptor was shown to have approximately the same sedimentation rate, molecular weight, and binding specificity as the murine Ah receptor (Harper et al., 1988). The human Ah receptor has a binding affinity 5-10 times higher than mouse (5-10 nM versus 0.8-3 nM in the murine hepa 1 cell line) (Manchester et al., 1987; Roberts et al., 1991; Waithe et al., 1991). In addition, human cells have a lower sensitivity to enzyme induction than murine cells (Harper et al., 1991; Roberts et al., 1991). The properties of this receptor have not been extensively characterized, but it is likely that, as in mice, the human population will be polymorphic with respect to the structure, function, and ligand affinity of the Ah receptor (Nebert et al., 1991).

Complementation studies conducted using variant cells that are defective

in either TCDD binding or translocation of the receptor-ligand complex to the nucleus, which occurs as part of the signal transduction process, indicate that more than one gene contributes to receptor function (Hankinson, 1983; Miller et al., 1983; Whitlock, 1990). Prior to ligand binding, the receptor is cryptic and contains the 90-Kd heat shock protein, whose release is necessary to unmask the functional activity of the receptor (Poellinger et al., 1991).

Studies were conducted to determine the ligand characteristics important for binding and eliciting a biologic response. There is good correlation between the binding affinities of various TCDD congeners for the Ah receptor and the induction of enzyme (aryl hydrocarbon hydroxylase, AHH) activity (Poland et al., 1979). Analogous structure-activity studies implicate the Ah receptor in a broad number of biochemical, morphological, immunologic, neoplastic, and reproductive effects (Poland and Knutsen, 1982; Safe, 1986). However, some responses do not have a clear relationship to Ah receptor binding and therefore may not be mediated by the Ah receptor (Rozman et al., 1993).

The Ah receptor is a soluble intracellular protein that, upon binding to TCDD, acquires a high affinity for DNA and accumulates in the nucleus (Denison et al., 1989; Hapgood et al., 1989; Nemoto et al., 1990; Saatcioglu et al., 1990a,b; Cuthill et al., 1991; Denison and Yao, 1991). The transformation of the Ah receptor into a DNA-binding form involves multiple events and interactions, including a conformational change measured by several parameters (Denison et al., 1987; Gasiewicz and Bauman, 1987; Kester and Gasiewicz, 1987; Henry et al., 1989). Evidence from a variety of sources indicates that the DNA-binding form of the receptor is composed of at least two different proteins (Elferink et al., 1990; Gasiewicz et al., 1991). One protein, termed "Arnt," that does not bind TCDD is associated with the liganded Ah receptor and may be either the DNA-binding component of the receptor or associated with translocation of the receptor from the cytoplasm to the nucleus (Hoffman et al., 1991). In addition, the ligand-binding portion of the Ah receptor appears to have been identified (Bradfield et al., 1991, Burbach et al., 1992; Ema et al., 1992).

Function of the Ah Receptor Binding of TCDD to its receptor, subsequent translocation to the nucleus, and DNA-binding result in a number of biologic effects. Many genes have elements associated with them that are responsive to TCDD (dioxin-responsive enhancers, DRE; J.M. Fisher et al., 1989; Whitlock, 1990). For example, TCDD induces AHH activity (a drug-metabolizing enzyme) by stimulating the transcription of the CYP1A1 gene, which encodes for the hydroxylase protein, through a means that does not require protein synthesis and is receptor dependent. The liganded receptor binds to a transcriptional enhancer regulatory element, DRE, upstream from

the CYP1A1 gene (Jones et al., 1986; Neuhold et al., 1986; Fujiisawa-Sehara et al., 1987; Fisher et al., 1990). This liganded receptor recognizes a specific nucleotide sequence (5'-TGCGTG-3'), which occurs in multiple copies within the enhancer region (Denison et al., 1989; Hapgood et al., 1989; Saatcioglu et al., 1990a,b). The activity of a transcriptional promotor is also required to enhance the appropriate transcription of the gene that is being affected by TCDD (Jones and Whitlock, 1990).

Studies have characterized the interaction between the liganded-Ah receptor and the DRE element. As stated above, a specific nucleotide sequence was identified that occurs multiple times within the DRE. Further studies, using either DNA fragments or intact cells, show that the binding of the TCDD-Ah receptor complex to DNA occurs within the major DNA groove and contacts four guanines of the recognition sequence (Shen and Whitlock, 1989; Neuhold et al., 1989). Binding of the TCDD-Ah receptor to DRE and the function of DRE are diminished by methylation of the cytosine nucleotide within the recognition sequence. This may be one mechanism by which differences in responsivity to TCDD occur between tissues (Shen and Whitlock, 1989). Finally, binding of the liganded receptor to the DRE may alter the configuration of the DNA and chromosome structure (Durrin and Whitlock, 1989; Elferink and Whitlock, 1990; Wu and Whitlock, 1992).

As indicated above, the induction of the transcription of a variety of genes is mediated by TCDD binding to the Ah receptor and subsequent binding of the liganded receptor to DNA at an element similar to that found upstream of the CYP1A1 gene. These genes include a cytochrome P4501A2 gene, a glutathione *S*-transferase Ya subunit gene, an aldehyde dehydrogenase gene, and a quinone reductase gene (Dunn et al., 1988; Jaiswal et al., 1988; Telakowski-Hopkins et al., 1988; Quattrochi and Tukey, 1989; Favreau and Pickett, 1991). Recent studies indicate that TCDD also induces the transcription of plasminogen activator inhibitor-2 and interleukin-1 as well as other unidentified genes (Sutter et al., 1991). However, the mechanism by which this induction occurs is not as well defined as that described above.

In response to the induction of gene expression and primary effects on the cell, compensatory effects may occur. For example, TCDD affects the levels of steroid hormones and growth factors in rodents (Umbreit and Gallo, 1988; Ryan et al., 1989; Sunahara et al., 1989; Harris et al., 1990; Choi et al., 1991). These direct alterations in gene transcription lead to a variety of effects that are not mediated directly by the Ah receptor. TCDD may therefore induce a cascade of biochemical changes and thereby produce a biological response, such as cancer, by several different mechanisms that can affect different tissues. TCDD may either induce a gene for a growth factor and directly affect tissue proliferation, or induce the gene for the growth factor receptor and increase the sensitivity of the cell to the growth factor

signal. Alternatively, TCDD may lead to tissue destruction, and the compensatory cellular proliferation may make permanent a genetic defect that allows cellular transformation and consequently neoplasia. Additional mechanisms have also been proposed for the induction of neoplastic tissue in the intact animal. Which of these mechanisms are actually involved is not clear, and they may vary among tissues and animal species.

Linearity of Response or Threshold Scientists hold vastly different opinions about the existence of a threshold effect, that is, whether there is a point below which no effect of the chemical exists, for the activity of TCDD. There are those who argue that all events that occur up to and including the induction of gene transcription have a linear dose-response curve; others argue that more complex events that require the concordance of two or more events, such as cell proliferation, may have a threshold. This seems to be the most favored view, and it is supported by the available data. This does not mean, however, that there is a threshold for the biological effects of TCDD, simply that the response is receptor-mediated (Portier et al., 1993).

From this latter view, however, two further divergent views are defined. One view is that (1) all events that occur in response to the binding of TCDD to its Ah receptor have a linear dose-response curve; (2) the observance of a threshold is due to background levels of the response, which obscure the detection of any TCDD-induced response at low doses; and (3) a linear dose-response curve would be observed if the method of measurement could be more refined (Silbergeld, 1991). Scientists who hold this view note that (1) there is a linear dose-response curve for the effects of TCDD; (2) it is not clear what causes the dose-response curve to become nonlinear as the complexity of the event mediated by TCDD increases; (3) TCDD is acting in addition to a natural ligand for the Ah receptor that has yet to be defined; and (4) the concentration of the natural ligand within the cell may influence the response to TCDD.

The other view suggests that a finite amount of TCDD is necessary to elicit detectable effects due to the possibility that (1) events subsequent to receptor binding may not have a linear response to TCDD; (2) activation of transcription of a gene does not correlate with the binding affinity for DNA; and (3) the concentration of TCDD required to produce a biological effect varies among species and among individuals within a species (Whitlock, 1991; Shen and Whitlock, 1992). Scientists who hold this view believe that a threshold amount of TCDD is required to produce any of its biological effects, but they do not believe that currently available data are sufficient to set the dose for this threshold.

Some scientists feel that there may be a threshold for the biological effects of TCDD, but are unwilling to set a level for this threshold; they feel

that the current body burden for TCDD (when added to the toxic equivalency factors for other compounds that bind to the Ah receptor) in industrialized nations may exceed this threshold, and therefore the point is of only theoretical and not practical significance.

Non-Ah-Mediated Toxicity

In order to establish that an observed toxicity is mediated through an Ah receptor, certain criteria must be met. Currently, these are (1) a structure-activity relationship when using ligands of lower affinity for the Ah receptor, and (2) differential sensitivities when using mice congenic at the Ah locus. However, some toxicologic effects do not meet these criteria and therefore cannot be considered to be mediated through the Ah locus. Examples of this are immune suppression after in vitro exposure to TCDD and neurotoxicity. The mechanism by which these events occur is unknown at this time, but this does indicate that some actions of TCDD are not mediated through the mechanism discussed above.

Health Outcomes in Animal Studies

Carcinogenicity

Carcinogenicity Bioassays Long-term carcinogenicity bioassays of TCDD have been conducted in rats, mice, and hamsters. A total of eight studies have been reported; these are summarized in Table 4-2. Routes of exposure have included oral, intraperitoneal, dermal, and subcutaneous. Increased tumor rates have been reported at several sites; the only consistent site among species and studies has been the liver. The results of each of these bioassays are described below.

Kociba et al., (1978) The Kociba and colleagues (1978) bioassay of TCDD is the most cited and that on which the Environmental Protection Agency cancer potency estimate is based. Groups of male and female Sprague-Dawley rats received 0, 0.001, 0.01, or 0.1 µg TCDD/kg body weight/day in the diet for two years. The results of this study were based on histopathologic evaluations of tissue samples performed by Dr. R.J. Kociba. These tissues were subsequently evaluated independently by Dr. R.A. Squire (Squire, 1980). Both investigators used the criteria for evaluating hepatocellular lesions described by Squire and Levitt (1975) and ILAR (1979). These criteria have been revised since their evaluations, based upon continuing studies of hepatocellular proliferative lesions (Maronpot et al., 1986; NTP, 1984). Because of these revisions, an independent panel review of the liver histopathology slides from the Kociba study has recently been conducted by PATHCO, Inc. (1990a; Goodman and Sauer, 1992). The results

of this and the Squire review are at variance with each other and with the original results, all of which are summarized for female rats in Table 4-6.

Table 4-6 shows the incidences of foci of hepatocellular proliferation, hepatocellular adenoma, and carcinoma for each dose group. It is apparent from the table that few of the lesions identified by the original investigators as malignant were confirmed as such in the more current evaluation. Increased liver tumor rates were not observed in male rats.

Hepatic toxicity was observed frequently among treated female rats (Table 4-6). These lesions included necrosis, vacuolization, cellular enlargement, multinucleated cells, infiltration of inflammatory cells, fatty changes, oval cell proliferation, and regenerative hyperplasia. Grading of the toxic lesions was performed by PATHCO, Inc. (1990b; Goodman and Sauer, 1992), based on a scale of 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked), reflecting increased incidence and severity of the lesions noted above. Nonspecific (minimal) changes were noted primarily in the control and low dose groups, although several animals in the low dose group exhibited mild hepatotoxicity as well. In the mid- and high dose groups, there was a clear increase in the incidence and severity of hepatotoxicity. Interestingly, tumors in these groups were observed only in animals exhibiting toxicity.

In addition to liver tumors in female rats, a significantly increased incidence of squamous cell carcinoma of the nasal turbinates/hard palate in both sexes, as well as of the tongue in males, was identified. These tumors are rare in Sprague-Dawley rats. A statistically significant increase in the incidence of keratinizing squamous cell carcinoma of the lung was also detected in female rats at the high dose. In addition, significantly reduced incidences in tumors of the uterus, pancreas, and the pituitary, mammary, and adrenal glands were reported.

National Toxicology Program (NTP, 1982a) In the NTP (1982a) standard carcinogenicity bioassay, groups of male and female Osborne-Mendel rats and male B6C3F_1 mice received doses of 0, 5, 25, or 250 ng TCDD/kg body weight by gavage twice weekly for two years; female mice received 0, 20, 100, or 1,000 ng TCDD/kg. These doses corresponded to average daily doses of 0, 1.4, 7.1, or 71 ng/kg for rats and male mice, and 0, 5.7, 28.6, or 286 ng/kg for female mice. Survival was not affected, and there was an increased incidence of both adenomas and carcinomas of the liver at the high dose in male and female mice. In female rats, the increased incidence of hepatocellular adenomas and carcinomas was significant when combined. Other positive results included increased rates of thyroid follicular cell adenoma in male rats at all dose levels, of combined adenomas and carcinomas of the adrenal gland in high dose female rats, of subcutaneous fibrosarcomas in high dose rats and female mice, and of lymphoma in high dose female mice.

National Toxicology Program (NTP, 1982b) Male Swiss-Webster mice received doses of 0.001 µg TCDD applied to the skin 3 days per week for

TABLE 4-6 Results of Kociba et al. (1978) TCDD Bioassay in Female Rats: Liver Lesions

		Dose (ng/kg/day)			
Pathologist	Lesion	0	1	10	100
Kociba	Hyperplastic nodule	8/86 (9.3)	3/50 (6)	18/50 (36)	23/50 (46)
	Hepatocellular carcinoma	1/86 (1.2)	0/50 (0)	2/50 (4)	11/50 (22)
Squire	Hyperplastic nodule and hepatocellular carcinoma	16/86 (18.6)	8/50 (16)	27/50 (54)	33/47 (70.2)
PATHCO (Goodman and Sauer)	Severity and incidence of hepatoxicity[a, b]	0.6 (57)	1.2 (88)	2.3 (96)	3.6 (100)
	Foci of cellular alteration (eosinophilic foci)[c]	31/86 (36)	23/50 (46)	37/50 (74)	40/45 (80)
	Hepatocellular adenoma[d]	2/86 (2.3)	1/50 (2)	9/50 (18)	14/45 (28)
	Hepatocellular carcinoma	0/86 (0)	0/50 (0)	0/50 (0)	4/45 (8.9)

NOTE: Results are given as number of animals with lesion/number of animal evaluated (percent).

[a] Severity was graded on a scale of 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked).

[b] In some cases the number of animals examined for hepatotoxicity differed slightly from the number examined for other lesions due to autolysis and leukemia.

[c] May include animals with hepatocellular adenoma or carcinoma.

[d] Only the most malignant tumor in each animal was counted.

104 weeks. Female mice received 0.005 µg TCDD per application. One control group remained untreated and one received acetone instead of TCDD. Treatment with TCDD significantly increased the incidence of skin fibrosarcomas in female mice. This effect was not significant in male mice.

Rao et al. (1988) Groups of male Syrian golden hamsters received two to six doses of 50 or 100 µg TCDD/kg body weight by either intraperitoneal or subcutaneous injection over a 4-week period. Animals were sacrificed after 12-13 months. Those that received the highest dose (total dose 600 µg/kg) developed squamous cell carcinomas of the facial skin at a rate of 4/18 (22 percent) by intraperitoneal injection and 3/14 (21 percent) by subcutaneous injection. The tumors were large, with extensive necrosis and some metastasis to the lung. This tumor is so rare that none could be identified in a study of control hamsters of this strain. No other treatment groups developed this or other exposure-related tumors.

Della Porta et al. (1987) Groups of male and female $B6C3F_1$ and $B6CF_1$ mice received intraperitoneal doses of 0, 1, 30, or 60 µg TCDD/kg body weight in corn oil once weekly for 5 weeks, starting at 10 days of age. The mice were observed until they reached 78 weeks of age. Only the liver, kidney, and organs with gross pathologic changes were examined histologically. Thymic lymphomas occurred at a statistically elevated rate in both sexes of both strains receiving the highest dose. In addition, an increased rate of hepatocellular adenomas and carcinomas occurred in $B6C3F_1$ males. In another study, groups of male and female $B6C3F_1$ mice received 0, 2.5, or 5.0 µg TCDD/kg body weight by gavage once weekly, starting at 6 weeks of age, for 52 weeks. Mice were observed until 110 weeks of age, and complete histopathology was performed. An increased incidence of hepatocellular carcinomas was observed for both sexes at both doses.

Van Miller et al. (1977) The Van Miller study was intended to be a range-finding study and, as such, used few rats and was poorly reported (U.S. EPA, 1985). Male Sprague-Dawley rats were fed a diet containing 0, 0.001, 0.005, 0.05, 0.5, 1.0, 5.0, 50, 500, or 1,000 parts per billion (ppb) of TCDD for 78 weeks. This regimen corresponded to approximate dose levels of 0, 0.0003, 0.001, 0.01, 0.1, 0.4, 2.0, 24, 240, and 500 µg TCDD/kg body weight/week. Surviving animals were observed for an additional 17 weeks. All animals receiving 1 ppb TCDD or more were dead by week 90. Survival at lower doses was unaffected, although degenerative changes in the kidneys were observed. A statistically elevated total tumor incidence was reported in rats receiving 0.005 ppb or more, along with an elevated incidence of squamous cell tumors of the lungs, hepatic neoplastic nodules, and cholangiocarcinomas in the 5-ppb dose group. However, no tumors were reported to have occurred in control animals, which is very unusual for Sprague-Dawley rats. The validity of these results is thus questionable.

Toth et al. (1979) Groups of male Swiss/H/Riop mice received doses of

0, 0.007, 0.7, or 7.0 µg TCDD/kg body weight weekly by gavage for one year. There were two vehicle and two untreated control groups. Animals were observed for their life spans. Liver tumors (combined adenomas and carcinomas) occurred at a high spontaneous rate in control groups (18-33 percent) and were positively correlated with survival. A statistically elevated liver tumor incidence was observed in the middle-dose group, although the ratio of benign to malignant tumors was unaffected. A nonstatistically elevated liver tumor incidence was observed in the highest dose group. The latter group also had reduced survival, however, and time-to-tumor data, if they had been available, were likely to have shown a decrease in time-to-tumor compared to controls. The results of this study are thus suggestive of an effect of TCDD on liver tumor incidence.

Initiation/Promotion Bioassays Several bioassays have been performed to assess the ability of TCDD to promote the carcinogenicity of an initiating chemical or treatment. Initiation/promotion bioassays recognize that there are discrete stages in carcinogenesis, and understanding the stage or stages that are affected by an agent gives clues to its mechanism of action. The initiation/promotion paradigm for carcinogenesis postulates that a cell undergoes genetic alteration at a critical site on DNA (initiation) and that the altered cell undergoes cell proliferation and clonal expansion (promotion), which can be followed by additional rounds of DNA damage and cell proliferation (progression) until a tumor is formed. Skin tumor initiation/promotion bioassays usually start with a single dose of a genotoxic chemical, followed by repeated applications of the test chemical to determine if it is a promoter. Liver tumor initiation/promotion protocols generally involve treatment with a genotoxic chemical and partial hepatectomy to make the genetic damage permanent, followed by treatment with the test agent. An agent is considered to promote carcinogenesis if it increases tumor incidence compared to that expected to be induced by the initiator alone. Chemicals that test positive may be considered tumor promoters within the operational definition of these protocols.

Skin The skin tumor-promoting ability of TCDD was tested in the presence and absence of initiation by dimethylbenzanthracene (NTP, 1982b). Male Swiss-Webster mice received doses of 0.001 µg TCDD applied to the skin 3 days per week for 104 weeks. Female mice received 0.005 µg TCDD per application. Half the animals received a single application of 50 µg dimethylbenzanthracene one week prior to beginning TCDD treatment. One control group remained untreated, and one received acetone instead of TCDD. Treatment with TCDD significantly increased the incidence of skin fibrosarcomas in female mice, in both the presence and the absence of dimethylbenzanthracene (8/29 and 8/27, respectively, versus 2/41 in controls). This effect was not significant in male mice.

In another study, Poland et al. (1982) administered a single initiating dose of *N*-methyl-*N*-nitrosoguanidine to the skin of hairless mice, followed by twice weekly doses of 0, 3.75, 7.5, 15, or 30 ng TCDD for 20 weeks. Papilloma formation occurred at all doses in a dose-dependent manner. Tumors were infrequent in mice that received either compound alone.

The tumor-initiating ability of TCDD has also been tested, by using an initiation/promotion protocol with phorbol ester (TPA) as the promoter. TCDD had weak or no initiating activity in this system (DiGiovanni et al., 1977).

The skin tumor-promoting activity of TCDD is dependent on the presence of the Ah receptor and segregates with the hr locus (Poland and Knutson, 1982; Poland et al., 1982).

Liver In a bioassay reported by Pitot and colleagues (1980), female Sprague-Dawley rats received an initiating dose of diethylnitrosamine followed by 0.14 or 1.4 µg TCDD/kg body weight subcutaneously once every two weeks for seven months (equivalent to 10 and 100 ng/kg/day, the same as the medium and high doses in the Kociba et al. 1978 bioassay). No liver tumors occurred in groups of rats that received either diethylnitrosamine or TCDD alone, or in the group that received diethylnitrosamine and the low dose of TCDD. Five of seven rats that received diethylnitrosamine and the high dose of TCDD developed hepatocellular carcinomas, however. Altered hepatic foci (eosinophilic) also occurred much more frequently in this treatment group than in the others. These results have been confirmed by other studies, which have also demonstrated that TCDD's promoting effect in rats is dependent on the presence of ovaries (Graham et al., 1988; Clark et al., 1991b; Flodstrom and Ahlborg, 1991; Lucier et al., 1991, 1992; Dragan et al., 1992). This finding is consistent with the results of long-term studies showing that TCDD is a hepatocarcinogen in female but not male rats. Interestingly, although the absence of ovaries appeared to protect against TCDD-induced liver tumors, lung tumors appeared only in ovariectomized rats treated with diethylnitrosamine and TCDD. Complex hormonal interactions clearly are involved in the site specificity of TCDD-induced carcinogenesis.

Mechanistic Studies TCDD has a wide range of effects on growth regulation, hormone systems, and other factors associated with the control of cell proliferation and differentiation. Several of those effects, and the roles they may play in TCDD-mediated carcinogenesis, are described below.

Genotoxicity The preponderance of data indicates that TCDD is not genotoxic: it does not form detectable adducts with DNA (U.S. EPA, 1985; Turteltaub et al., 1990); it has produced negative results in batteries of tests for genotoxicity (NTP, 1984); and although it is an effective tumor promoter (see previous discussion), it is only a weak initiator. There is no

consistent evidence for increased frequencies of chromosomal aberrations among humans exposed to TCDD either occupationally or accidentally (Shu et al., 1987). The results of specific genotoxicity tests of TCDD have been thoroughly reviewed by Shu and colleagues (1987).

Enzyme Induction The induction of cytochrome P450 isoenzymes CYP1A1 and CYP1A2 by TCDD has been studied extensively (Whitlock, 1990). Information is available on isoenzyme specificity; the time-course and dose-response aspects of induction; molecular mechanisms of transcriptional activation; and species, tissue, and cell specificity. Several studies have shown that TCDD also induces the activity of at least one isoenzyme of uridine 5'-diphosphate glucuronosyltransferase (UDPGT) (Lucier et al., 1986). This effect, like that on P450, is Ah receptor dependent (Bock, 1991). Both P450 and UDPGT are responsible for conjugating numerous substrates, both endogenous and exogenous, rendering them water-soluble and excretable in urine. A mechanistic relationship among cytochrome P450, UDPGT induction, and carcinogenesis, or any other end point of toxicity, has not been established, however.

The controversy regarding the relevance of P450 induction to carcinogenesis is based on the notion that inducing P450 would enhance the rate at which it activates other carcinogens to form DNA-reactive metabolites. However, P450 also deactivates reactive metabolites, and most evidence indicates that the carcinogenic potency of many chemicals is diminished by P450 induction. For example, both the carcinogenic potencies of benzo[*a*]pyrene and dimethylbenzanthracene are decreased by TCDD exposure, as are their rates of DNA adduct formation, presumably because deactivation of their reactive metabolites is enhanced (Cohen et al., 1979). While it is possible that P450 induction could increase the formation of reactive metabolites and lead to increased rates of DNA adduct formation, in general, P450 induction diminishes the carcinogenicity of a wide variety of chemicals (Miller et al., 1958; Wattenburg, 1978, 1985). Generalizations are not possible, however, and predictions cannot be made for specific chemicals in the absence of experimental data.

Another possible role of P450 in TCDD-mediated carcinogenesis is the ability of CYP1A2 to convert estrogens to catechol estrogens in the liver (Graham et al., 1988). Catechol estrogens are thought to possess DNA-damaging capability as a consequence of free-radical generation (Metzler, 1984; Li and Li, 1990). Inducing CYP1A2 may thus increase DNA damage by estrogens, which may play a part in TCDD-induced carcinogenesis in the female rat liver. This possibility is consistent with the observations that CYP1A2 is found only in the liver, that ovariectomy protects against TCDD-induced hepatocarcinogenesis in female rats, and that male rats are not susceptible.

Evaluating the possible role of P450 induction in TCDD-mediated carcinogenesis

is complicated by the heterogeneity of hepatocyte responses to TCDD: P450 activity in some hepatocytes is maximally induced by low doses of TCDD, whereas others do not respond. Increasing the dose of TCDD increases the number of cells responding, not the amount of induction in responding cells (Bars and Elcombe, 1991; Tritscher et al., 1992). In addition, cells that exhibit TCDD-mediated increases in P450 induction are different from those that exhibit TCDD-induced increases in DNA replication (Lucier et al., 1992).

Estrogen Receptor-Mediated Responses The results of the Kociba and colleagues (1978) bioassay indicated that TCDD can increase liver tumor incidence in rats while it decreases tumor incidence in organs such as the mammary gland, uterus, and pituitary gland. These results may be affected by the interaction of TCDD and the estrogen receptor. The estrogen receptor-ligand complex is capable of reversibly binding with DNA; this interaction is responsible for the transcriptional activation of estrogen-responsive genes. TCDD decreases the binding capacity of estrogen receptors in the liver and uterus, leading to decreased estrogen concentrations and activity (Romkes et al., 1987). This effect is also Ah receptor dependent (Lin et al., 1991a). The role of this response in TCDD-mediated carcinogenesis is unknown, however.

Cell Proliferation TCDD-mediated hepatocarcinogenesis may be related to its ability to increase rates of hepatocellular proliferation in that organ. Both of these effects are dependent on the presence of ovaries (Clark et al., 1991b). Increased rates of cell proliferation may be related to TCDD's effects on the epidermal growth factor (EGF) receptor. EGF is a potent mitogen that affects both normal and neoplastic cells (Stoschek and King, 1986). The EGF receptor and its ligands also play a role in cell transformation and tumorigenesis (Marti et al., 1989; Velu, 1990). Several studies have shown that TCDD mimics EGF, decreasing the binding capacity of the plasma membrane EGF receptor for its ligand without changing its binding affinity (Hudson et al., 1985; Abbot and Birnbaum, 1990; Astroff et al., 1990), although TCDD does not bind with the receptor itself. TCDD produces a functional change by decreasing EGF-stimulated autophosphorylation of the receptor (Clark et al., 1991b). In addition, TCDD induces the production of transforming growth factor- α (TGF- α), which does bind to the EGF receptor, leading to increased mitogenic signals (Choi et al., 1991). TCDD also has effects on a number of other EGF receptor pathways (Cochet et al., 1984; Beguinot et al., 1985; Astroff et al., 1990). Increasing cell proliferation rates occur at higher doses than loss of the EGF receptor (Lucier et al., 1992), however, so TCDD's effects on the EGF receptor probably have a number of consequences in addition to cell proliferation. Nonetheless, these responses are consistent with the observation that TCDD has mitogenic effects, which may play a role in its tumor-promoting ability.

Immune Suppression As discussed further below, there is ample evidence that administration of TCDD to animals results in suppression of the immune response. Some studies in animals and humans have shown that the exposure to immunosuppressive agents increases the incidence of tumors. As discussed further below, administration of TCDD did increase the incidence of tumors in animals injected with tumor cells. This may add another mechanism by which exposure to TCDD could increase the incidence of tumors in treated animals.

Conclusions Chronic rodent bioassays have demonstrated that exposure to TCDD can enhance tumor rates in a variety of target organs. TCDD is not a genotoxic carcinogen, although it may enhance the DNA-damaging properties of other chemicals. In multistage models of carcinogenesis, TCDD acts as a tumor promoter and has little, if any, tumor-initiating activity. TCDD mediates carcinogenesis through a variety of biochemical effects that are dependent on the presence of the Ah receptor. These effects involve multiple pathways that play roles in regulating cell proliferation and differentiation. The multiple site specificity of TCDD-mediated carcinogenesis is likely to reflect its multiple mechanisms of action.

Immunotoxicity

Introduction The immune system is pivotal in the maintenance of health, resistance to infection, and surveillance for some types of altered cells. Suppression of the immune system can result in an increase in the incidence and severity of infectious disease, as well as an increase in the incidence of some types of neoplasia. Enhancement of the immune response can result in the development or exacerbation of allergy and autoimmune disease. Therefore, an alteration in the level of immune responsiveness, either increased or decreased, could result in an increase in susceptibility to disease and should be considered toxic.

The immune system is a complex network that involves the interaction of different types of cells and soluble mediators. Because of the consequences of alterations in immune function, this system is highly regulated in the extent and duration of the response to a given antigen. Since a chemical may act at a given step in the generation of an immune response, the timing of exposure to the chemical or antigen to which a response is generated is critical, and must be considered in determining the applicability of animal studies on chemical exposure to epidemiologic evaluation of the effects of environmental toxicants on the human immune system.

Numerous studies in several species have indicated that the immune system is highly sensitive to the effects of exposure to TCDD and related compounds. However, because of the wide variation in the experimental

design, exposure protocols, and immunologic assays used, these studies are only summarized.

In Vivo Immunotoxicity Following exposure to relatively high levels of TCDD, the amount of lymphoid tissue is depleted, with the thymus being most sensitive to its effects (Vos et al., 1973; Vos, 1977; McConnell and Moore, 1979; Exon et al., 1987; Van Loveren et al., 1991). Alterations in immune effector function and host resistance occur at doses well below those that cause lymphoid tissue depletion. Multiple cellular targets, which are described below, have been implicated in the immunotoxic effects of TCDD. In addition, there is evidence that some of the effects of TCDD on the immune system are due to indirect actions on nonlymphoid tissues.

Cellular Immunity Results of studies of immune function in mice, rats, guinea pigs, and nonhuman primates indicate that TCDD suppresses cell-mediated and humoral immune functions in a dose-related manner (reviewed by Kerkvliet, 1984; Exon et al., 1987; Vos and Luster, 1989; Holsapple et al., 1991a,b). Because there are extensive scientific reviews available of the effects of TCDD on specific immune functions, this information is only summarized here.

A common observation in rodents following adult and pre- or postnatal exposure to TCDD has been the suppression of cell-mediated immunity. Immune functions suppressed following exposure to TCDD include delayed-type hypersensitivity, proliferative responses to mitogen and allogeneic lymphocytes, graft-versus-host disease, allograft rejection, and generation of cytotoxic T lymphocytes to allogeneic tumor (Vos et al., 1973; Faith and Moore, 1977; Sharma and Gehring, 1979; Thomas and Hinsdill, 1979; Luster et al., 1980a,b; Mantovani et al., 1980; Vecchi et al., 1980; Clark et al., 1981; Kerkvliet et al., 1990a). Of interest, especially in light of the effects observed in human studies, proliferative responses of rodent splenocytes to mitogen were shown to be elevated following exposure to low doses of TCDD (Luster et al., 1980a,b).

Humoral Immunity Multiple studies have shown that TCDD suppresses the generation of a humoral immune response, which results in the generation of monospecific antibodies from terminally differentiated B cells, in mice and guinea pigs (Vos et al., 1973; Thomas and Hinsdill, 1979; Hinsdill et al., 1980; Vecchi et al., 1980, 1983; Davis and Safe, 1988; Kerkvliet and Brauner, 1990; Kerkvliet et al., 1985, 1990b). Either the generation of specific serum antibody titer or the number of plaque-forming cells produced after immunization with sheep red blood cells (SRBCs), a T cell-dependent antigen, is markedly suppressed following TCDD exposure. In adult mice, the generation of a humoral immune response appears to be more sensitive to TCDD than a cell-mediated immune response. There is a differential sensitivity in the generation of humoral immune responses to

antigens (SRBCs, dinitrophenyl-Ficoll, and trinitrophenyl-lipopolysaccharide) that correlates with the degree of T cell involvement in the generation of the immune response (House et al., 1990; Kerkvliet et al., 1990b). Although a direct effect on B cell function has been shown after in vitro exposure or in vivo exposure and ex vivo immunization, these data suggest that TCDD may affect regulatory T cell function when the humoral immune response is generated in vivo (Holsapple et al., 1986; Dooley and Holsapple, 1988; Luster et al., 1988; Morris et al., 1991).

Macrophage and Polymorphonuclear Neutrophil Function and Inflammation Macrophage functions have also been examined following TCDD exposure and generally found to be resistant to suppression by TCDD when assessed ex vivo (Vos et al., 1978; Mantovani et al., 1980). In contrast, there is a selective inhibition of the anti-tumor cytolytic and cytostatic activity of polymorphonuclear neutrophils (PMNs) (Ackermann et al., 1989).

On the other hand, the pathology associated with TCDD toxicity includes neutrophilia and an inflammatory response characterized by activated macrophage and PMN accumulation (Vos et al., 1973, 1974; Weissberg and Zinkl, 1973; Puhvel and Sakamoto, 1988). This effect may be due to a normal inflammatory response to tissue injury or to a specific effect on inflammatory cells. Recent studies indicate that TCDD increased the respiratory burst of rat peritoneal macrophages (Alsharif et al., 1990). In addition, the inflammatory response that occurs following intraperitoneal injection of SRBCs, as measured by infiltration of PMNs and macrophages and alterations in macrophage cell surface proteins, was increased (Kerkvliet and Brauner, 1990; Kerkvliet and Oughton, 1993). One mechanism by which TCDD and related halogenated aromatic hydrocarbons may augment the inflammatory response is through enhanced production of inflammatory mediators such as interleukin-1 and tumor necrosis factor (TNF). On the other hand, serum complement has been reported to be suppressed in TCDD-treated mice (White et al., 1986). Recent studies indicate that the hypersensitivity of TCDD-treated mice to endotoxin may be due to an increase in the production of these inflammatory cytokines (Thomas and Hinsdill, 1978, 1979; Vos et al., 1978; Loose et al., 1979; Taylor et al., 1990; Clark et al., 1991a; Hoglen et al., 1992). In addition, the wasting syndrome and the mortality observed following exposure to TCDD were reduced after treatment of mice with pharmacologic levels of anti-TNF antibodies, suggesting a role for this inflammatory mediator in the process (Taylor et al., 1992). Recent studies show that TCDD may affect the transcription of the interleukin-1 gene directly through interaction with a DRE (Sutter et al., 1991).

Interspecies Sensitivity Studies have indicated that there is a difference in the sensitivity of various species to the immunotoxic effects of TCDD; however, most studies use different species and antigens, and are therefore not completely comparable. In one report, the effects of TCDD on the

immune system of rats, mice, and guinea pigs were examined, and even then, different immunologic parameters were assessed and different antigens were used (Vos et al., 1973). For example, the delayed-type hypersensitivity (DTH) response was the cell-mediated immune response parameter measured in rats and guinea pigs, and graft-versus-host response was measured in mice. From this study, it was shown that guinea pigs were more sensitive to the effects of TCDD than rats, which is consistent with other toxic effects of TCDD (McConnell et al., 1978a; Poland and Knutson, 1982). The mouse cell-mediated immune system also seems to be more sensitive to TCDD than the rat's; however, different antigens and end points were tested, and therefore the results cannot be compared. Studies by Clark and colleagues (1981) indicated that the sensitivities of immune responses of different antigens to TCDD in the same species and the same study are not equivalent.

In Utero Exposure Several studies have examined immune function in rodents following exposure to TCDD during fetal development (Vos et al., 1973; Faith and Moore, 1977; Thomas and Hinsdill, 1979; Luster et al., 1980a; Clark et al., 1983). These studies indicate that the fetus is much more sensitive to the effects of TCDD than the adult animal. The most sensitive indicator of TCDD immunotoxicity in these studies was a decrease in the host resistance to tumor. The ability of the offspring of TCDD-exposed dams to generate a cell-mediated and humoral immune response to antigen was also suppressed. When the mice were exposed only in utero and not during lactation, significant thymic atrophy and suppressed cell-mediated immunity were observed (Holladay et al., 1991).

Host Resistance Models The ability of an animal to eliminate viral, bacterial, and parasitic infections, as well as neoplastic diseases, is determined by both nonspecific and specific immunologic functions. A decrease in the functional activity of any immunologic compartment may result in increased susceptibility to disease. Host resistance models allow investigators to determine whether alterations in specific immunologic parameters result in increased susceptibility to disease.

TCDD exposure increases the susceptibility to challenge with gramnegative bacteria (Thigpen et al., 1975; Hinsdill et al., 1980). In addition, increased mortality was observed in animals infected with *Streptococcus pneumonia* after subchronic administration of TCDD (White et al., 1986). Enhanced susceptibility to viral and parasitic disease has also been reported after TCDD administration (Clark et al., 1983; Tucker et al., 1986; House et al., 1990). An increase in the growth of transplanted tumors has been demonstrated in mice treated with TCDD in utero (Luster et al., 1980a,b). This exposure protocol resulted in an increased incidence of PYB6 tumors in pups from dams receiving repeated doses of TCDD.

Role of Ah Locus in Immunotoxicity Many studies have been conducted to define the involvement of the Ah receptor, the protein that is thought to mediate the translocation of TCDD from the cytosol to the nucleus and its binding to DNA, in the mediation of TCDD immunotoxicity. Two lines of investigation have been undertaken to assess this contribution: comparison of the potency of the immunotoxicity of various congeners; and studies using mice of different genetic background known to differ at the Ah locus. Though the use of TCDD-sensitive (B6) and resistant (D2) mice, which differ in the affinity of their Ah receptor for TCDD, and mice that are congenic at the Ah locus, the generation of cell-mediated and humoral immune responses in vivo seems to be Ah receptor-mediated (Silkworth and Grabstein, 1982; Vecchi et al., 1983; Kerkvliet et al., 1990a,b). The involvement of the Ah receptor in the immunotoxic effect of TCDD was further confirmed by structure-activity relationships (Kerkvliet et al., 1985; Davis and Safe, 1988-1990). These studies all involved acute or subacute exposure to TCDD in vivo.

The contribution of the Ah receptor to the immunotoxicity observed after chronic exposure to TCDD is less clear. Recent studies showed that the sensitivity of D2 mice to TCDD-induced immunosuppression increased when TCDD was administered daily over 14 days rather than as a single dose (Morris et al., 1992). The effects of this treatment regime on the sensitivity of B6 mice are unclear in this study. In addition, the early studies of Vecchi, which indicated that the Ah receptor was involved in the immunotoxicity of TCDD, were conducted following multiple exposures to TCDD (Vecchi et al., 1983). Therefore, further studies are required to define the contribution of the Ah receptor to immunotoxicity after chronic exposure.

Some, but not all, in vitro studies of the effects of TCDD on the generation of a humoral immune response indicate that the Ah receptor may not be involved in the observed immunosuppression (Holsapple et al., 1986; Tucker et al., 1986; Davis and Safe, 1991). The basis for these variable effects following in vitro exposure to TCDD is unknown at this time, and the relevance of these negative data in light of the in vivo results is uncertain.

Contribution of the Endocrine System Many studies of the effects of TCDD and congeners that bind the Ah receptor following in vivo administration and immunization indicate that there may be an indirect component to the immunosuppression observed. Studies by Kerkvliet and colleagues (1990a) indicated a measurable elevation in serum corticosterone levels that correlated with the suppression of the generation of a cytotoxic T lymphocyte (CTL) response after exposure to hexachlorobiphenyl. However, further studies using adrenalectomized mice or rats and a glucocorticoid receptor antagonist suggest that this increase in serum corticosterone

is probably not involved (Van Logten et al., 1980; DeKrey et al., 1990). Alternatively, comparison of the sensitivity of male and female mice to hexachlorobiphenyl immunotoxicity, and the partial protection of male mice to the immunotoxicity of hexachlorobiphenyl by castration, suggest a role for testosterone in the suppression of the CTL response (Kerkvliet and Baecher-Steppan, 1988; DeKrey et al., 1992). These data suggest that the hormonal status of the animal may contribute to the immunotoxic effects observed after exposure.

Immunotoxicity of TCDD in Nonhuman Primates Some studies have been conducted on the effects of TCDD on the immune system of nonhuman primates. Immunologic effects were described in rhesus monkeys and their offspring chronically exposed to TCDD (Hong et al., 1989). In the mothers, the total number of T cells and CD8+ cells was increased and the number of CD4+ cells was decreased. No effect was observed on immune function. The ability of the offspring to generate a humoral immune response to tetanus toxoid was significantly increased.

Acute administration of TCDD to marmosets resulted in a decrease in CD4 + T cells (especially CD4+CDw29+) and CD20+ B cells, and an increase in the percentage of CD8+ cells and CD4+45RA+ (Neubert et al., 1990b). Chronic exposure of marmosets to a low dose of TCDD resulted in the opposite effects on the subpopulation of CD4+ cells (i.e., CD4+CDw29+ cells increased, and CD4+45RA+ cells decreased; Neubert et al., 1992). On the other hand, when the dose of TCDD was increased in this study, the results were consistent with those observed after acute administration of TCDD. These data are extremely important with regard to observations in the human population after chronic low dose exposure to TCDD. As the authors state, "Extrapolations of the results obtained at higher doses to very low exposures is not justified with respect to the effects induced by TCDD on the immune system of marmosets" (Neubert et al., 1992).

Hepatotoxicity

Introduction In animal species that exhibit sensitivity to TCDD and related halogenated aromatic hydrocarbons (HAHs), the liver represents one of the primary target organs. From animal studies, a characteristic profile of liver-associated changes at the cellular, biochemical, and molecular levels has been identified. It is important to emphasize that almost without exception, structure-activity studies indicate that liver alterations produced by HAH congeners are associated with the Ah locus. The most extensive characterization of liver-associated alterations in response to TCDD and related compounds has been in rodents, primarily mice and rats; however, some hepatotoxicity data are available in other mammalian species. This

section describes the profile of hepatic changes induced by TCDD as identified in laboratory animals.

Morphologic Changes Significant inter- and intraspecies variation has been observed in the severity of hepatic lesions produced by TCDD. Without exception, all mammalian species investigated experimentally exhibit some degree of hepatomegaly even at TCDD doses that are sublethal (Kociba et al., 1976; McConnell, 1985). Hepatomegaly appears to be a direct result of hyperplasia (an increase in number) and hypertrophy (increase in size) of parenchymal cells (hepatocytes) (Fowler et al., 1973; Hinton et al., 1978). Additionally, administration of lethal doses of TCDD has been shown to produce degenerative and necrotic changes in a variety of rat strains, which can be accompanied by mononuclear cell infiltration, multinucleated giant hepatocytes, an increase in hepatic smooth endoplasmic reticulum, and increased numbers of mitotic figures (Gupta et al., 1973; Jones and Butler, 1973; Kociba et al., 1976; Hinton et al., 1978). Other lesions varying among species include focal, centrilobular lesions in the mouse (Vos et al., 1974) and widespread necrosis in the rabbit (Vos and Beems, 1971). Neither the guinea pig nor the hamster displays severe morphological alterations in the liver following TCDD administration. In light of this, although liver lesions may contribute to TCDD lethality in laboratory animals, the cause of death cannot be explained on the basis of liver lesions alone.

Changes in Hepatic Function Alterations in liver morphology following TCDD treatment are accompanied by impaired hepatobiliary function including increased microsomal monooxygenase activity, liver enzyme leakage, impaired plasma membrane function, porphyria, hyperlipidemia, hyperbilirubinemia, hyperproteinemia, and increased regenerative DNA synthesis. As stated above, in most cases, the magnitude of these changes is Ah related and has been demonstrated in numerous studies using Ah-responsive and nonresponsive mouse strains (C57/BL/6J and DBA/2J, respectively).

Hyperlipidemia Recently, studies comparing the hepatotoxic effects of TCDD in Ah-responsive and nonresponsive mice demonstrated that C57/BL/6J mice, administered a single dose of 3 μg/kg TCDD, developed mild to moderate hepatic lipid accumulation but no inflammation. Severe fatty change, mild inflammation, and necrosis were observed in C57/BL/6J mice following a single dose of 150 μg/kg (Shen et al., 1991). Conversely, DBA/2J mice that received a single dose of 30 μg/kg developed hepatocellular necrosis and inflammation, and exhibited no hepatic lipid accumulation; only slight lipid accumulation was observed at high doses of TCDD (600 μg/kg). Induction of fatty liver following exposure to TCDD and related compounds has also been demonstrated in a number of other animal species including rat, chicken, and human. Sublethal doses of TCDD in rats have been reported to produce an increase in total hepatic lipid content.

Specifically, triglycerides and free fatty acids increased while sterol esters decreased. Lethal doses of TCDD increased cholesterol esters and free fatty acids (Albro et al., 1978). Dose-dependent increases in total and high-density serum lipoproteins in rats administered sublethal concentrations of TCDD have also been reported (Poli et al., 1980). No difference in lipid synthesis between control and TCDD-treated rats has been found (Cunningham and Williams, 1972). Studies with polychlorobiphenyl-treated rats, which were also found not to have increased lipid synthesis, provided evidence that hepatic lipids had an increased half-life (Hinton et al., 1978). Further evidence supporting a decrease in lipid utilization following TCDD treatment was obtained in studies by Albro and coworkers (1978) in which ultrastructural changes in liposomes and reduced levels of ester hydrolases were reported. Taken together, these studies suggest that fatty liver following exposure to TCDD is due to decreased lipid utilization rather than increased lipid biosynthesis.

Porphyria Accumulation of porphyrins (a family of compounds derived from tetrapyrrole, which is found in hemoglobin, myoglobin, and most cytochromes) in liver as well as spleen and kidney is stimulated by subchronic or chronic treatment with TCDD and related compounds, and is also accompanied by an increase in urinary porphyrin excretion (Goldstein et al., 1982). Cantoni and colleagues (1981) demonstrated that oral exposure of rats to 0.01, 0.1, or 1 µg TCDD/kg body weight/week for 45 weeks produced an increase in coproporphyrin concentrations at all dose levels.

Porphyria cannot be induced by a single dose of TCDD or related compounds. The mechanism responsible for HAH-mediated hepatic porphyria, although not elucidated, may occur partially through a decrease in uroporphyrinogen decarboxylase activity as suggested from work by Elder (1978). Jones and Sweeney (1977) also reported a decrease in this enzyme activity as well as elevated levels of carboxylated porphyrins following several weeks of TCDD treatment in mice. Additionally, TCDD-mediated induction of hepatic porphyria and inhibition of uroporphyrinogen decarboxylase activity in C57/BL6 mice were markedly reduced by iron deficiency (Sweeney et al., 1979). In contrast to these studies, Yao and Safe (1989) recently demonstrated in C57/BL6 mice that 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), an inactive HAH congener, was capable of antagonizing the induction of porphyria by TCDD; however, MCDF was incapable of antagonizing the inhibition of uroporphyrinogen decarboxylase or the induction of both AHH and ethoxyresorufin *O*-deethylase (EROD) activity. These studies suggest that inhibition of uroporphyrinogen decarboxylase is not the primary event responsible for porphyria. Another enzyme also implicated in the mechanism associated with TCDD-induced hepatic porphyria is δ -aminolevulinic acid (ALA) synthetase, the enzyme responsible for the initial and rate-limiting step in heme biosynthesis. ALA

synthetase is markedly induced by TCDD exposure. In rodents, ALA synthetase is not increased following acute TCDD treatment but is elevated following subchronic or chronic exposure. Goldstein and colleagues (1982) reported that after 16 weeks of chronic exposure, ALA synthetase was increased only in animals that were porphyric. However, the role of ALA synthetase in the mechanism of porphyria is questionable, according to several reports. Goldstein and colleagues (1974) reported that elevation in ALA synthetase occurs only after the onset of porphyria. Additionally, studies by Jones and Sweeney (1980) demonstrated that porphyria could be produced by TCDD in mice without increased ALA synthetase activity. In light of these findings, the induction of ALA synthetase does not appear to be a necessary event in the induction of porphyria.

Impaired Cell Membrane Function The effects of TCDD on hepatocyte plasma membrane function have been investigated in a number of experimental systems. In rodents, TCDD markedly inhibited the binding and/or expression of a number of receptors for regulatory factors in hepatocytes including epidermal growth factor receptor (Madhukar et al., 1988; Lin et al., 1991a), glucocorticoid receptor (Lin et al., 1991b), estrogen receptor (Lin et al., 1991b), and low-density lipoprotein (LDL) receptor (Bombick et al., 1984). TCDD produced an 80-90 percent decrease in the maximum binding capacity (both high- and low-affinity sites) of the hepatic EGF receptor in female Ah-responsive and Ah-nonresponsive mice. However, the ED50 (dose at which 50 percent of animals experience the effect) for the effects of TCDD on EGF binding was tenfold higher in the Ah-nonresponsive mice (Lin et al., 1991a). Additionally, TCDD did not affect the hepatic content of EGF receptor MRNA, indicating that the effects on the EGF receptor are not pretranslational. TCDD administered to mice also was found to produce approximately a 30 percent decrease in the maximal binding capacity of both the hepatic glucocorticoid and the estrogen receptors. A concomitant 30 percent decrease in tyrosine aminotransferase activity, which is regulated by the glucocorticoid receptor was also observed (Lin et al., 1991b). A greater sensitivity to inhibition by TCDD of the binding of estrogen to the hepatic estrogen receptor was demonstrated in Ah-responsive than in Ah-nonresponsive mice. Conversely, TCDD produced a comparable inhibition of glucocorticoid receptor binding and tyrosine aminotransferase activity in Ah-responsive and nonresponsive mice. These results suggest that the Ah receptor regulates the effects of TCDD on the binding of estrogen to the hepatic estrogen receptor, but the decreased binding of the hepatic glucocorticoid receptor does not appear to be mediated directly by the Ah locus. Acute TCDD exposure of guinea pigs and rats also produces a significant reduction in binding of LDL to its receptor on the hepatic plasma membrane (Bombick et al., 1984). It is important to emphasize that this reduction in LDL binding was not caused

by a decrease in food intake by treated animals as demonstrated by comparison to pair-fed control animals.

Biliary Excretion A number of studies have investigated the effect of TCDD and related compounds on bile flow. Structure-activity studies indicated that treatment of rats with HAH congeners decreased bile flow as measured by the clearance of ouabain, a model compound for neutral nonmetabolized substrates (Yang et al., 1983). The magnitude of depression by HAH congeners of ouabain excretion was closely associated with their potency in inducing AHH activity. Bile duct cannulation of TCDD-treated rats followed by segmented retrograde intrabiliary injection of radiolabeled morphine, imipramine, or ouabain demonstrated that TCDD inhibited the canalicular transport of glucuronide metabolites of morphine and imipramine into the bile (Berman et al., 1986). Likewise, TCDD inhibited the canalicular transport of ouabain into bile. Acute TCDD exposure in rats was also found to produce a greater than twofold increase in hepatic copper (compared to pair-fed controls; i.e., animals possessing the same dietary intake), a trace metal whose homeostasis depends on its biliary excretion (Elsenhans et al., 1991). These data suggest that TCDD and related compounds decrease bile flow.

Enzyme Induction The most extensively studied biochemical effects on hepatic function associated with exposure to TCDD and related compounds are the marked changes this class of chemicals produces on enzyme activity. The most profound effect is an increase in microsomal mixed-function oxidase activity in parenchymal cells. HAHs markedly induce the cytochrome P450 isozyme CYP1A1, which is most often monitored experimentally through the measurement of AHH and EROD activity. Numerous in vivo and in vitro structure-activity studies have investigated the relative potency of HAH compounds, including the chlorinated dioxins, biphenyls, and dibenzofurans, in their ability to induce AHH and EROD activities (reviewed: Safe, 1990). A structural relationship was identified between the arrangement of halogen atoms on the dibenzo*p*-dioxin molecule and the ability of these congeners to induce AHH and EROD. The sites identified as confirming the greatest biological potency were the four lateral ring positions. Additionally, congeners that were halogenated at all four lateral ring positions, the 2,3,7,8-sites, demonstrated more biological activity than those that were halogenated at only three of the four positions. Halogenation at only two of the four lateral positions results in loss of biological activity. On a molecular basis, 2,3,7,8-TCDD is the most potent inducer of AHH activity among the HAH congeners. A strong correlation for HAHs has also been established between AHH induction and their Ah-binding affinity. A similar correlation has been established between AHH induction by chlorinated dibenzo-*p*-dioxins, chlorinated dibenzofurans, and polychlorinated biphenyls and their respective acute toxicities (Poland and Glover, 1973; Safe, 1990).

There are two obvious concerns pertaining to the relevance of MFO induction. First, MFO induction can result in a greater rate of metabolism of endogenous substrates. Secondly, and perhaps of even greater significance is the potential for increased metabolism of exogenous compounds that undergo metabolic bioactivation to a more toxic form. However, detoxification of exogenous substrates is also an important role played by MFO, so inducing MFO can also reduce the toxicity of a variety of compounds.

The most widely studied experimental models of MFO induction have been murine (i.e., rats and mice). In rats, TCDD has been shown to increase a variety of oxidative and conjugative enzymes involved in drug metabolism and elimination including CYP1A1 and CYP1A2, aniline hydroxylase, AHH, biphenyl hydroxylase, ethoxycoumarin *O*-deethylase (ECOD), EROD, and UDPGT. Additionally, a number of non-drug metabolizing enzymes have also been identified as being inducible by TCDD (ornithine decarboxylase, prostaglandin synthetase, porphyrinogen carboxylase, transglutaminase, aldehyde dehydrogenase, δ-aminolevulinic acid synthetase).

In the mouse, AHH induction by TCDD and related compounds is under the control of the Ah gene locus, which is believed to encode a soluble receptor that binds to inducers and mediates an increase in cytochrome P450 gene transcription (Taylor, 1984—see mechanism of action section above). Identification of the Ah locus was achieved primarily from enzyme induction studies using various mouse strains differing at this locus. TCDD as well as other inducers of AHH (i.e., 3-methylcholanthrene, benzo[*a*]pyrene, etc.) are 10 times as potent, or more, in inducing hepatic CYP1A1 in C57BL/6J mice than in DBA/2J mice (Poland and Knutson, 1982; Nebert, 1989).

Data on enzyme induction in other species are less well-characterized. The guinea pig, which is the most sensitive species to TCDD, at least in terms of lethality, shows only a very slight induction of MFO (Beatty and Neal, 1977). Similarly, enzyme induction in the rabbit is not well-characterized. In the Syrian golden hamster, TCDD was found to induce MFO and to reduce NAD (P):menadione oxidoreductase [NAD(P) = nicotinamideadenine dinucleotide (phosphate)] and ECOD (Gasiewicz et al., 1986).

Reproductive and Developmental Toxicity

Introduction TCDD has been found to have a number of effects on reproductive and developmental function in laboratory animals. Although a number of studies have addressed the effects of TCDD on the fertility of male laboratory animals, there are no data relating male animal exposure to TCDD and developmental end points such as congenital anomalies, cancer, and growth retardation, which are the end points of interest in humans. Extrapolating observations from laboratory animals to humans is not straightforward, however, due to important species determinants for both reproductive

and developmental end points. In addition, little information is available on the cellular and molecular mechanisms of action of TCDD that play a role in these effects. This section summarizes the information that is available in laboratory animals for each end point.

Reproductive Toxicity

Male A number of studies have demonstrated that overtly toxic doses of TCDD can affect several parameters that play a role in the reproductive function of male laboratory animals. TCDD administration to adult animals can decrease the weight of testes and accessory sex organs, affect testicular morphology, decrease spermatogenesis, and reduce fertility (Allen and Lalich, 1962; Allen and Carstens, 1967; Khera and Ruddick, 1973; Kociba et al., 1976; Van Miller et al., 1977; McConnell et al., 1978a; Moore et al., 1985; Chahoud et al., 1989, 1992; Morrisey and Schwetz, 1989; Rune et al., 1991a,b). These effects have been reported in rats, mice, guinea pigs, marmosets, monkeys, and chickens, but have generally been observed only at doses that also decrease body weight and feed intake.

TCDD's effects on spermatogenesis include loss of germ cells, appearance of degenerating spermatocytes and spermatozoa, and reduced numbers of seminiferous tubules containing mature spermatozoa. The lowest total dose at which effects on spermatogenesis have been observed was 3 µg TCDD/kg, which was administered as a single subcutaneous injection to rats and reduced the number of spermatids per testis to 60 percent that of controls (Chahoud et al., 1992). No other toxic effects were reported at this dose. In another study in rats, daily gavage doses totaling 65 µg TCDD/kg, administered over 13 weeks, also reduced spermatogenesis (Kociba et al., 1976); at this dose, however, body weights and feed consumption were reduced as well.

The effects of TCDD on fertility were evaluated in male rats exposed to either a single subcutaneous dose of 25 µg/kg followed by weekly maintenance doses of 5 µg/kg, or a single dose of 75 µg/kg followed by maintenance doses of 15 µg/kg. Rats were treated for 10 weeks prior to mating, as well as throughout the 12-week mating period. Female rats were untreated. Mortality was 93 percent in the high dose group, and body weights were significantly decreased in both groups. At the low dose, 15 percent of animals were found to be sterile, morphological changes of the testes were apparent, and the mating and fertility indices were somewhat reduced (84 percent and 95 percent of controls, respectively). The pregnancy index was not affected, however (Chahoud et al., 1991).

At lower doses of TCDD (15 µg/kg), androgenic deficiencies in rats have been detected, including decreased plasma testosterone levels, decreased testicular responsiveness to luteinizing hormone, and increased pituitary responsiveness to feedback inhibition by androgens and estrogens (Moore et

al., 1989, 1991, 1992; Bookstaff et al., 1990a,b; Kleeman et al., 1990). Decreased testosterone levels apparently result from inhibition of testosterone biosynthesis due to inhibition of the mobilization of free cholesterol, a testosterone precursor (Moore et al., 1991). Normally, an increase in plasma levels of luteinizing hormone would be expected to facilitate testicular compensation for decreased plasma testosterone; in TCDD-treated rats, however, this increase does not occur, due to an enhanced ability of testosterone and its metabolites to inhibit luteinizing hormone secretion (Moore et al., 1989; Bookstaff et al., 1990a,b). In addition, TCDD treatment prevents the increase in the number of pituitary gland receptors for gonadotropin-releasing hormone that normally occurs in response to decreased plasma androgen concentrations (Bookstaff et al., 1990b).

In general, both the morphologic and the biochemical effects of TCDD on fertility-related parameters occur in laboratory animals at doses that also induce overt toxicity. Only one study demonstrated an effect on spermatogenesis in rats at a dose that was not associated with other toxicity (Chahoud et al., 1992); the consequence of this effect on fertility was not evaluated. Much higher doses of TCDD did not affect male rat fertility (Chahoud et al., 1991). The reproductive systems of adult male laboratory animals thus appear to be relatively insensitive to TCDD.

Female Reduced female fertility has been demonstrated in a number of studies of rodents exposed to TCDD. Murray and colleagues (1979) exposed both male and female rats to 0, 0.001, 0.01, or 0.1 µg TCDD/kg body weight/day in the diet over three generations. The female fertility index, defined as the ratio of the number of females confirmed pregnant to the number of females for which mating was confirmed, varied among both control and exposed animals, with a reduced number of impregnated animals reported in the exposed groups. Reduced fertility and litter size were reported in the F_0 generation at the 0.1- µg/kg daily exposure level and in the F_1 and F_2 generations at the 0.01- µg/kg daily exposure level. Kociba and colleagues (1976, 1978) reported suppression of the estrous cycle, absence of ovulation, and signs of ovarian dysfunction in female rats exposed to 1-2 µg TCDD/kg/day for 13 weeks, but not in rats exposed to 0.001-0.01 µg/kg/day for two years.

Reduced female fertility, detected as a reduced ability to conceive and give birth, has also been demonstrated in a series of studies in rhesus monkeys, although many of those exposed also experienced maternal toxicity (Allen et al., 1977, 1979; Barsotti et al., 1979; Schantz et al., 1979; Bowman et al., 1989; Schantz and Bowman, 1989). In these experiments, female monkeys received TCDD in their diets at levels ranging from 5 to 500 ppt, and were mated to unexposed males after seven months of exposure. No effects on reproductive function were detected at the lowest dose. A reduced ability to give birth was also seen when monkeys were fed a total

dose of 1 µg TCDD/kg during the first trimester of pregnancy; no effect was observed at lower doses (McNulty, 1984).

These limited experiments on the effects of TCDD on female reproductive function indicate that in laboratory animals, TCDD can decrease fertility, decrease the ability to remain pregnant throughout gestation, and in the case of rats, decrease litter size, although some of these effects may have been secondary to maternal toxicity. Mechanistic information is confined to the effects of TCDD on ovarian function suggested by Kociba and colleagues (1976), and to studies of serum hormone levels in monkeys reported by Allen and colleagues (1979) and Barsotti and colleagues (1979), in which reductions in estradiol and progesterone concentrations were correlated with dietary levels of TCDD as well as with reduced fertility and increased spontaneous abortion. No such alterations in hormone levels were detected in rats, however, which suggests species differences (Shiverick and Muther, 1983). Whether TCDD's effects on hormone levels result from their increased metabolism (due to induced hepatic P450 levels) or to an effect on the responsiveness of gonadal tissue itself is unknown. Antiestrogenic effects of TCDD in rats and mice include decreased uterine weight and a decreased concentration of tissue progesterone receptor levels; when TCDD and 17 β -estradiol were coadministered, TCDD prevented the usual 17 β -estradiol-induced increases in uterine weight and progesterone receptor levels (Gallo et al., 1986; Astroff et al., 1990; Safe et al., 1991). This effect is age dependent and is not observed in immature animals (Safe et al., 1991).

Developmental Toxicity Prenatal exposure to TCDD has been associated with both increased fetal mortality and increased developmental abnormalities in laboratory animals. Increased fetal mortality is dose related and depends on the timing of exposure: embryos are more susceptible to TCDD-induced mortality at certain stages of development than others (Couture et al., 1990a). The embryolethal dose of TCDD is generally one to two orders of magnitude lower than the dosage that is lethal to adults; however, increased embryolethality generally occurs at doses that also induce nonlethal maternal toxicity (Sparschu et al., 1971; Khera and Ruddick, 1973; Courtney, 1976; Giavini et al., 1982). Other common fetotoxic effects of TCDD are thymic hypoplasia, subcutaneous edema, decreased growth, and hematologic alterations. Species-specific effects include cleft palate formation and hydronephrosis in the mouse, extra ribs in the rabbit, and intestinal hemorrhage in the rat (Courtney and Moore, 1971; Courtney, 1976; Giavini et al., 1982; Couture et al., 1990b). Evidence in the mouse indicates that the developmental effects of TCDD may be mediated by the Ah receptor (Poland and Knutson, 1982). Mouse strains with Ah receptors that have a relatively high affinity for TCDD respond to lower doses than strains with a relatively low-affinity (Poland and Glover, 1980; Hassoun et al., 1984).

The tissue specificity of the response to TCDD cannot be attributed solely to the presence of Ah receptors, however; other as yet unidentified factors also play a role.

Neurotoxicity

Introduction The physiological and structural function of the nervous system is preserved across species in terms of the mechanisms by which neurons generate and propagate action potentials, the mechanisms that transmit chemically encoded information across synaptic clefts, and the manner in which organized functional groups of neurons or CNS nuclei communicate and influence each other. Behavioral actions and interactions at their most basic levels are also conserved phylogenetically. Thus there are common themes that are apparent in the ways in which laboratory animals and humans sense, integrate, and respond to external stimuli. These similarities include similarities in cognitive function, learning, and memory. Because of the structural and functional similarities of the nervous system across species, extrapolation of experimentally produced neurotoxicity from laboratory animals to humans is easier than for many other end points of toxicity.

Exposure Levels Although TCDD and related chemicals have an affinity for accumulating in lipid-rich tissues, the concentrations of these materials in the brain after systemic exposure are low (Piper et al., 1973a; Van Miller et al., 1976; Gasiewicz and Neal, 1979; Olson et al., 1980a; Gasiewicz et al., 1983; Abraham et al., 1990). Although different methods have been used to investigate the TCDD concentrations in the brain, the actual levels reported are quite close among rodent species. For example, within 1-3 days after receiving a dose of TCDD, the concentrations of TCDD (percentage of dose per gram of tissue) in the brain were rat—0.06; hamster—0.05; guinea pig—8 0.25; and mouse—0.1-0.4. TCDD levels in adult primates have been reported to be lower than those found in the rat (0.006 versus 0.13 percent of dose/g tissue), but infant monkeys have higher levels than adults (0.018 versus 0.006 percent of dose/g tissue) (Van Miller et al., 1976). Brain TCDD concentrations do not appear to differ significantly in TCDD-sensitive mice when compared to TCDD-insensitive mice (Gasiewicz et al., 1983). Initial brain levels of TCDD do not appear to be affected by redistribution of TCDD from adipose tissue during the weeks following a single systemic exposure (Piper et al., 1973a; Olson et al., 1980a; Gasiewicz et al., 1983).

Direct injection (8 μg/kg) of TCDD into the lateral ventricle of Sprague-Dawley rats results in total brain concentrations that are 100 times greater than those achieved after intravenous injection (72 μg/kg) (Stahl and Rozman, 1990). Total brain concentrations of 356 ppb TCDD following intraventricular

injections of 8 µg/kg were not associated with systemic toxicity or neurotoxicity (Stahl and Rozman, 1990).

Ah Receptors in the Nervous System Relatively little work has been done to quantify the concentration of Ah receptors in the nervous system (Gasiewicz and Rucci, 1984). In the CNS of the guinea pig, a species that is sensitive to TCDD-induced acute toxicity, Ah receptors have been identified in the cerebrum and cerebellum at similar concentrations (11-12 fmol/mg cytosolic protein). The hamster, which is resistant to TCDD-induced acute toxicity, showed a similar concentration of receptors in the cerebrum, but none were detected in the cerebellum. No Ah receptors were detected in the midbrain, medulla, or hypothalamus of either species. The CNS Ah receptor concentrations (where detectable) were approximately 20 percent of those seen in the livers of the guinea pig and hamster. The presence of Ah receptors in the peripheral nervous system has not been investigated, and Ah receptors have not been detected in skeletal muscle tissue (Gasiewicz and Rucci, 1984). Because high concentrations of Ah receptors have been associated primarily with epithelial structures (Gasiewicz and Rucci, 1984), the most likely region of the brain to have higher concentrations of Ah receptors would be the epithelium of the choroid plexus.

Neurotoxicity Studies Following Acute and Subchronic Exposure A large number of acute toxicity studies have been conducted with TCDD, but most of these studies were not designed specifically to investigate neurotoxicity. None of these studies specifically noted frank signs of neurotoxicity associated with TCDD exposure.

Sirkka and colleagues (1992) studied a series of neurological end points in male Han/Wistar rats given 1,000 µg/kg of TCDD intraperitoneally (i.p.). This dose level is equivalent to one-third of the i.p. LD_{50} for this strain of rat. Controls included both ad libitum and pair-fed controls for the majority of end points examined. The tests were conducted at varying times after exposure and were designed to detect changes in motor control, nociception, anxiety level, and learning. TCDD exposure had no effect on the ability of rats to complete these tests, with the possible exception of a transient decrement in coordination on the narrow-bridge portion of the horizontal-bridge test 16 hours after exposure. At 8 days, there was no performance decrement, and at 16 hours, performance on the broad-bridge portion of the test and on the more difficult rotating-rod test were normal. Overall, these results indicated no significant neurological impairment in animals that developed significant decreases in body weight following TCDD exposure.

Allen and colleagues (1977) studied the clinical and morphological effects associated with feeding diets containing TCDD [500 parts per trillion (ppt)] to female rhesus monkeys for nine months. The only morphological effect reported in the CNS was hemorrhage into the Virchow-Robin space.

This effect was associated with significant bone marrow toxicity and hemorrhage in many other organ systems. In this study, hemorrhage was probably an agonal lesion since no other effects were reported in the adjacent neuropil.

Role of Neurotoxicity in the Wasting Syndrome Because appetite is significantly regulated by CNS processes (Morley and Levine, 1985), there has been interest in trying to determine if CNS dysfunction is involved in causing TCDD-induced hypophagia. The TCDD-induced wasting syndrome has been observed in several different species (Kelling et al., 1985), but the relationship between the syndrome and central nervous system function has been studied primarily in rats. Seefeld and colleagues (1984) showed that hypophagia induced by TCDD in Sprague-Dawley rats was associated with a change in feeding behavior (increased spillage) that occurred in a dose-dependent fashion 7 days after TCDD administration and continued to increase for 15 days after dose administration. By 15 days, TCDD-treated rats were spilling 2 to 3 times more feed than the controls. The TCDD-induced depression of feed intake also began immediately after dosing. Hypophagia in these animals was not associated with an inability to feed, since rats given TCDD and placed on a feed-restricted diet prior to TCDD administration exhibited a significant degree of hyperphagia and a greater rate of weight gain than unrestricted animals (Seefeld et al., 1984). Thus TCDD did not appear to impair the rats' ability to feed but may have affected the set point at which body weight was maintained, which is under CNS control.

In light of pharmacokinetic data suggesting that little administered TCDD reaches the brain (Piper et al., 1973a; Lakshman et al., 1986), Pohjanvirta and colleagues (1989) compared the differences in toxicity of TCDD when administered intracerebrally (left ventricle) or subcutaneously via implanted minipumps in TCDD-susceptible Long-Evans rats and TCDD-resistant Han/Wistar rats. The pumps were set to deliver 20-21 μg/kg/day of TCDD. In both rat strains, intracerebral TCDD was more effective at reducing feed intake and body weight gain than subcutaneous TCDD. While the authors interpreted these data to imply that the CNS has a specific role to play in induction of TCDD toxicity, this interpretation is highly speculative in the absence of mechanistic or pharmacokinetic data linking the route of administration to a particular tissue target site or showing that the administered TCDD was not transported out of the brain.

Pohjanvirta and Tuomisto (1990) reported that TCDD-resistant Han/Wistar rats given a sublethal dose of TCDD showed residual alterations in feeding behavior following a variety of feeding regulatory challenges. After groups of 11 male rats were given a single high dose of TCDD (1,000 μg/kg body weight) or corn oil (vehicle), the TCDD-dosed rats began losing

body weight by about day 6 and continued to lose body weight for approximately 5 weeks, at which time the body weight reached a plateau (12.5 percent maximum loss) that was maintained until the end of the study (week 15). Both the TCDD-treated and the control groups were apparently fed ad libitum. Because the TCDD-dosed animals would be expected to reduce their feed consumption in response to TCDD and the controls were not pair-fed, a confounding variable may have been introduced into this study. In response to a sodium chloride challenge (1 percent of body weight of 1 M NaCl), water consumption was stimulated equivalently in both groups. In response to glucose deprivation induced by 2-deoxyglucose (400 mg/kg body weight), TCDD-treated rats ate little, while controls increased their feed consumption 10 times. The response to insulin-induced (10 μg/kg body weight) hypoglycemia was reduced in TCDD-treated rats, two of which died because they failed to eat following insulin dosing. Deprivation of rat chow induced feeding in both control and TCDD-treated rats, but naloxone (an antagonist of the endogenous opioid peptidergic feeding system) suppressed the feeding response to fasting in controls and to a lesser extent in the TCDD-treated rats. TCDD-dosed rats, deprived of rat chow for 24 hours and then fed, showed a suppression of feeding when given glucose (1.36 g/kg body weight) or fructose (1.36 g/kg body weight) i.p. Feed intake was not similarly suppressed in control rats. A challenge with sodium 2-mercaptoacetate (MA), an inhibitor of mitochondrial β-oxidation of fatty acids, following feeding with a high-fat diet resulted in a decrease in intake of a high-fat diet rather than an expected increase. When TCDD-dosed rats were challenged with both MA and 2-deoxyglucose, feed consumption was increased, although the response was delayed and attenuated. The abnormal responses imply that TCDD is capable of producing long lasting impairments to metabolic challenges, which are at least in part mediated by the CNS. The data are not sufficient to determine whether there was a CNS component involved in causing the effects observed, however.

The impact of pharmacologic agents on TCDD-induced wasting was investigated by Pohjanvirta and colleagues (1988b). The TCDD model that was used in this study included TCDD-sensitive Long-Evans rats given a dose of TCDD (20 μg/kg body weight i.p.) that induced hypophagia and death. TCDD-dosed rats were exposed to a series of pharmacologic agents for periods of 3-14 days. The agents were given at dose levels and for time periods that were expected to increase feed consumption in TCDD-dosed animals. Because aminergic and serotonergic neurotransmission are involved in control of feed consumption, agents affecting one or the other of these systems were included in the treatment program.

The treatments included dopamine antagonists (haloperidol and amperozide), an α-adrenoceptor-blocking agent (phenoxybenzamine), a 5-hydroxy tryptophan (HT) synthesis inhibitor [*p*-chlorophenylalanine (PCPA)], a β-blocker

of sympathetic stimulation of brown adipose tissue (sotalol), a steroidal inducer of gluconeogenesis (dexamethasone), a hypoglycemia-inducing agent (insulin), doses of other modifiers of aminergic neurotransmission that have been reported to increase feeding behavior (amphetamine, morphine, reserpine, chlordiazepoxide, and clonidine), and indomethacin at a dose level that has been reported to increase food consumption. None of the drug treatments lessened or prevented the induction of hypophagia or weight loss leading to death. None of the treatments affected the nocturnal feeding reduction induced by TCDD, although some of the treatments altered the daytime TCDD-induced hypophagia. For example, reserpine and PCPA further reduced feeding in TCDD-treated rats, and insulin, which increased daytime feeding in TCDD-treated rats, was associated with aphagia on discontinuation of treatment. None of the treatments were regarded as suggesting a crucial role for aminergic or serotonergic regulatory systems in the mechanism of action of TCDD-induced hypophagia.

To investigate further the role of aminergic neurotransmission in TCDD-induced hypophagia and lethality, Tuomisto and colleagues (1990) determined the levels of noradrenalin, dopamine, dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, tryptophan, and histamine in several areas of the brain at various times (1-76 hours) after i.p. administration of a lethal dose (50 µg/kg body weight) of TCDD to TCDD-sensitive Long-Evans rats. Control rats were treated with the vehicle used to deliver the TCDD (corn oil) and were apparently fed ad libitum. Except for a slight increase in tryptophan concentrations in several areas of the brain, there were no consistent or readily interpretable changes in other parameters.

Bestervelt and colleagues (1991) investigated the role of hypothalamic endorphin and mu opioid receptor levels in Sprague-Dawley rats given a high but sublethal oral dose of TCDD (50 µg/kg body weight). Two control groups received the vehicle (acetone and corn oil 1:2); one group was fed ad libitum and one was pair-fed with the TCDD group. Pair feeding did not alter receptor levels. TCDD-dosed rats initially showed a relative increase in hypothalamic β-endorphin (β-E) concentrations followed by depressed concentrations 2 and 3 days after dosing. The mean absolute β-E concentrations were 6.0, 12.4, and 11.6 pg in the controls on postdosing days 1, 2, and 3, respectively, and 10.0, 4.8, and 5.6 pg in the TCDD-dosed rats. Thus the actual concentrations showed a significant amount of overlap and were not unequivocally different. Three days after TCDD administration, the brain mu receptor number was 60 percent higher in the TCDD-dosed rats than in controls, but the binding affinity of the mu opioid receptor was not changed. The changes in receptor number coincided with the beginning of hypophagia in the rats and may indicate that TCDD can act as an opioid antagonist.

The effects of TCDD on serum levels of melatonin and the morphology of the pineal gland by light and electron microscopy were investigated by Linden and colleagues (1991) in TCDD-resistant Han/Wistar rats. Serum melatonin levels decreased within 24 hours after an i.p. dose of 50 µg/kg TCDD and remained reduced for 28 days. The change in serum melatonin level was not associated with morphological changes in the pineal glands even when rats were given a higher dose of TCDD (1,000 µg/kg i.p.). The reduction in serum melatonin levels (˜50 percent of control values) could not be correlated with hypophagia or changes in body weight.

Russell and colleagues (1988) investigated hypothalamic factors that could account for the reduction of serum concentrations of prolactin in Sprague-Dawley rats given a lethal dose of TCDD (50 µg/kg body weight) i.p. This dose level of TCDD results in a marked inhibition of prolactin release within 4 hours of dosing. Exposure to pimozide, a dopamine receptor antagonist, 30 minutes prior to TCDD administration prevented TCDD-induced suppression of hormone release. The pimozide effect was presumed to have been the result of antagonism of the inhibitory effect of dopamine on prolactin release from the anterior pituitary (Russell et al., 1988). Measurement of dopamine concentrations in the median eminence of TCDD-dosed rats indicated that TCDD increased dopamine levels by 15 percent. The rate of dopamine synthesis and the rate constant for decline of dopamine in the median eminence were increased. Similar changes in norepinephrine levels were not observed. Exposure of pituitary tissue to TCDD in vitro did not alter the secretion rate of prolactin. Taken together, these data imply that exposure to TCDD at dose levels that can produce the wasting syndrome can increase dopamine synthesis and turnover in the hypothalamus and that, through increased release of dopamine, TCDD can inhibit the release of prolactin.

In a series of papers Stahl and Rozman (1990), Weber et al. (1991), Rozman et al. (1991), and Stahl et al. (1991) have shown that the hypophagia induced by TCDD is most likely not due to a direct effect of TCDD on the brain, but is related to changes that occur in the liver affecting the peripheral feedback mechanisms involved in appetite control. Stahl and Rozman (1990) attempted to induce hypophagia in Sprague-Dawley rats by direct intraventricular injection of 3H-labeled TCDD (8 µg/kg body weight) into the brain, leading to TCDD levels in various regions of the brain that were increased 3-400 times. For a comparison, a second group of rats was given 72 µg/kg TCDD intravenously. Levels of TCDD in the brain after intraventricular injection ranged from 30 to 1,220 ppb and after intravenous injection from 2.4 to 5.2 ppb. Levels in the liver were much higher in rats given TCDD intravenously. In spite of the much higher brain levels of TCDD after intraventricular injection, hypophagia and lethality were only seen in the group given TCDD intravenously. These data demonstrated that TCDD

accumulation in the brain is not associated with appetite suppression or the wasting syndrome.

Rozman and colleagues (1991) found that administration of 125 µg/kg body weight TCDD i.p. to Sprague-Dawley rats increased plasma tryptophan (a serotonin precursor) and brain levels of tryptophan, serotonin, and 5-hydroxyindoleacetic acid (a serotonin metabolite). No changes were observed in brain levels of norepinephrine and dopamine in the hypothalamus. The increase in the plasma levels of tryptophan and the brain concentrations of serotonin, its precursor, and its metabolite was later shown to be associated with impairment of several enzymes involved in gluconeogenesis. The most important of these enzymes appears to be phosphoenolpyruvate carboxykinase (PEPCK), although pyruvate carboxylase and glucose-6-phosphatase were also altered. Inhibition of PEPCK results in increased tryptophan levels in the blood and brain. Increases in tryptophan levels can result in increased levels of serotonin, which can act as an appetite suppressant. These data indicate that peripheral mechanisms may play an important role in the wasting syndrome.

Stahl and colleagues (1991) showed that central serotonergic pathways may not be involved in the wasting syndrome. Prior to dosing with TCDD, rats were given a dose of 5,7-dihydroxytryptamine to cause central 5-HT (a serotonin metabolite) depletion (up to 90 percent). The 5-HT depletion did not affect the outcome of TCDD exposure, suggesting that although TCDD increases 5-HT levels in the brain, this may not be the cause of the wasting syndrome.

While lethal or near lethal dose levels of TCDD may be associated with neurochemical alterations or changes in the responsiveness of neurochemical processes in the central nervous system, the changes observed may also be regulatory responses occurring secondary to changes induced in other organ systems. The available data imply that CNS alterations play at most a secondary role in the pathogenesis of the wasting syndrome induced by TCDD.

Metabolic Toxicity

Toxicity Associated with Intermediary Metabolism Animal studies, primarily those performed in the rat, have identified a number of changes in intermediary metabolism following treatment with TCDD. These include effects on body temperature and serum and tissue concentrations of glucose, insulin, glycogen, somatostatin, and thyroid hormones. There is some evidence suggesting that TCDD-mediated alteration in intermediary metabolism may be linked to the wasting syndrome described in a number of rodent species following treatment with TCDD and related compounds.

Blood Glucose Lethal doses of TCDD produce a rapid onset of severe

hypoglycemia in the rat (Zinkl et al., 1973; Gasiewicz et al., 1980; Potter et al., 1983; Gorski and Rozman, 1987; Gorski et al., 1988a,b). The direct cause of hypoglycemia, although initially suspected to be the progressive starvation stress associated with TCDD treatment in rodents, cannot be attributed to a decrease in food consumption. Gasiewicz and coworkers (1980) demonstrated that TCDD-treated rats that received continuous intravenous feeding exhibited hypoglycemia as well as other signs of TCDD toxicity, including lethality, while maintaining normal weight gain. These findings suggest that rats treated with TCDD are capable of efficiently utilizing glucose and amino acids. Similarly, pair-feeding studies clearly showed that although TCDD-treated rats developed hypoglycemia (Christian et al., 1986a), control rats in a comparable state of nutrition maintained normal blood glucose levels.

In pair-fed control studies, concomitant to hypoglycemia, TCDD-treated rats also exhibited a decrease in serum and pancreatic insulin (Potter et al., 1983; Gorski and Rozman, 1987). This decrease is unusual because hyperglycemia normally would be expected in the presence of hypoinsulinemia. Comparable onset kinetics were observed with respect to both decreased insulin and decreased serum glucose following TCDD treatment (Gorski and Rozman, 1987). TCDD treatment of rats was also found to induce insulin hypersensitivity. Injection of nontoxic doses of insulin 3 days after administration of 125 μg/kg TCDD resulted in 80 percent mortality of rats within 24 hours. Insulin hypersensitivity preceded both hypoglycemia and hypoinsulinemia.

Blood glucose is derived from three sources: dietary intake, glycogen, and gluconeogenesis. Prior to TCDD-induced death, rats cease to eat, which results in an exhaustion of glycogen stores. However, neither the reduction in glycogen stores nor the decrease in dietary intake appears to account for the decrease in blood glucose. As described above, although TCDD-treated rats exhibited severe hypoglycemia, pair-fed control rats remained normoglycemic (Christian et al., 1986a). There is evidence to suggest that decreased blood glucose following TCDD treatment in rodents may be mediated through the disruption of gluconeogenesis. TCDD treatment of rats was found to produce an elevation in serum alanine, suggesting a decrease in the conversion of alanine to glucose (Christian et al., 1986a). At a lethal dose of TCDD (125 μg/kg), conversion of ^{14}C-alanine to ^{14}C-glucose was significantly reduced compared to pair-fed controls (Gorski et al., 1990). Following a lethal dose of TCDD, rats also maintained a low respiratory quotient (the ratio of CO_2 output to oxygen usage), whereas pair-fed control rats showed an enhanced respiratory quotient (Muzi et al., 1989), suggesting protein utilization for maintenance of normal blood glucose concentration in untreated rats. Additionally, a congener of TCDD, 3,3',4,4'-tetrachloroazobenzene, was found to inhibit markedly several key enzymes required

for hepatic gluconeogenesis (Hsia and Kreamer, 1985), further supporting a role for altered gluconeogenesis as a mechanism for TCDD-induced hypoglycemia.

Somewhat paradoxically, one laboratory using two independent methods has reported corticosterone, a stimulator of gluconeogenesis, to be greatly elevated in TCDD-treated hypoglycemic rats (Gorski et al., 1988d). This observation is contrary to results reported by Balk and Piper (1984), who found approximately a 70 percent decrease in corticosterone 14 and 21 days after a single oral dose of TCDD (25 μg/kg) in rats. A second major effect of corticosterone on intermediary metabolism, in addition to stimulating gluconeogenesis, is to decrease peripheral utilization of glucose. In light of this, corticosterone reduction in rats by adrenalectomy significantly increased the rate of TCDD lethality, as compared to rats without this surgical alteration (Neal et al., 1979; Gorski et al., 1988a). Likewise, corticosterone replacement offered partial protection to TCDD-treated adrenalectomized rats, as compared to TCDD-treated adrenalectomized rats not receiving corticosterone (Gorski et al., 1988a). The protective effects of supplemental corticosterone may be mediated through an increase in peripheral glucose utilization and therefore a reduction in hypoglycemia.

Somatostatin is known to decrease serum glucose, as well as various hormones including insulin and glucagon. A single nonlethal dose of TCDD (45 μg/kg) in rats had no effect on somatostatin concentrations in serum, liver, or pancreas (Potter et al., 1983), thus ruling out a role for somatostatin of TCDD-induced hypoglycemia.

Fatty Acid Biosynthesis Thyroid-derived hormones are known to regulate de novo fatty acid synthesis. Within 7 days after TCDD treatment (45 μg/kg), rats exhibited hypothyroidism (Potter et al., 1983) followed by changes in serum concentrations of certain thyroid-derived hormones. TCDD treatment has no known direct effect on thyroid-stimulating hormone. Conversely, total thyroxine (TT_4) and free thyroxine (FT_4) were decreased somewhat dose dependently beginning around 2-4 days after a single dose of TCDD and returned to control levels by day 32. Similar findings were observed for reverse triiodothyronine (Rt_3) following TCDD administration. One study has reported no effect of TCDD on total triiodothyronine (TT_3) (Potter et al., 1983), whereas a second study found that TCDD increased TT_3 (Bastomsky, 1977). One role of thyroid-derived hormones is the regulation of de novo fatty acid synthesis, especially by triiodothyronine (T_3), which is stimulatory. Following TCDD treatment, rats exhibited an increase in plasma fatty acids and de novo fatty acid synthesis in liver. Conversely, there was a decrease in de novo fatty acid synthesis in interscapular brown adipose tissue. The mechanisms for differences in de novo fatty acid synthesis between liver and interscapular brown adipose tissue are unknown; however, these findings may be related to findings by Bianco and Silva (1987),

which indicated that T_4 (thyroxine), but not T_3, penetrates interscapular brown adipose tissue. Once T_4 has penetrated into interscapular brown adipose tissue, it is converted to T_3, which in turn stimulates de novo fatty acid synthesis. In light of this, Gorski et al. (1988c) have speculated that a decrease in T_4 by TCDD may account for the decrease in de novo fatty acid synthesis in interscapular brown adipose tissue.

Body Temperature Potter and colleagues (1983) reported that within 2 weeks of a single 90-μg/kg i.p. dose of TCDD, rats exhibited a body temperature of less than 35°C, with the lowest mean value of 34.5°C recorded on day 16. Mean body temperatures for control rats ranged from 36.8 to 37.5°C.

Gastrointestinal Toxicity

Although research in this area has been limited, gastrointestinal (GI) associated changes have been reported following TCDD administration in a number of animal models (i.e., rat, rabbit, monkey, chicken, hairless mouse).

Stomach-related effects of TCDD have been among the most widely studied. A greater than threefold decrease in gastric antrum somatostatin, a hormone possessing moderate inhibitory effects on gastric secretion, was observed in rats following a single nonlethal dose of TCDD (45 μg/kg) and was accompanied by a 29 percent increase in stomach dry weight (Potter et al., 1983). The mechanism for the increase in stomach dry weight is unknown. Theobald and colleagues (1991) also reported a decrease in both antral gastrin and somatostatin in rats following TCDD treatment (100 μg/kg) and a seven- to tenfold elevation in serum gastrin on day 14 posttreatment. Histologic examination revealed increased antral mucosal height on day 14 in TCDD-treated rats and increased antral wet weight. Because the TCDD ED_{50} values that produced decreased antral somatostatin concentrations were less than those that produced increased gastrin—and, secondly, the decrease in antral somatostatin in TCDD-treated rats occurred one week before the hypergastrinemia—it is unlikely that decreased antral somatostatin levels initiate the increase in gastrin. It has been suggested that the main cause of TCDD-induced hypergastrinemia is decreased gastric acid secretion (Mably et al., 1990).

Chronic exposure to TCDD and related compounds has been associated with hyperplasia and metaplasia within the oxyntic gland of the stomach of monkeys and rats (Norback and Allen, 1973; Becker and McNulty, 1984). The height of the oxyntic gland mucosa in TCDD-treated monkeys is greatly enhanced, with extensions of gastric glands into the submucosa and formation of submucosal cysts (Norback and Allen, 1973; Becker et al., 1979; Becker and McNulty, 1984). Likewise, TCDD has been shown to cause hyperplasia of the ileal mucosa in the hamster (Olson et al., 1980b).

TCDD has also been reported to interfere with intestinal absorption. In pair-fed control studies, TCDD-treated rats (125 μg/kg) exhibited decreased glucose absorption as measured in isolated perfused jejunal segments. The greatest magnitude of inhibition was measured 2 days following TCDD treatment (30 percent), with partial resolution occurring by day 14 (14 percent inhibition) (Richter et al., 1992), compared to vehicle controls (ad libitum fed or pair-fed). No effect was observed on sodium or calcium transfer; however, potassium transfer was markedly increased, which paralleled the inhibition in glucose absorption (Richter et al., 1992). Measurements of 3-*O*-methylglucose uptake in mucosal tissue suggested that TCDD inhibition of glucose uptake occurred at the basolateral membrane.

Respiratory Tract Associated Toxicity

The effects of TCDD on the respiratory tract have not been widely studied and have focused primarily on enzyme induction, most notably cytochrome P450 in animal lung tissue. As discussed earlier, one of the primary toxicologic issues associated with the phenomenon of enzyme induction pertains to the fact that a wide variety of carcinogenic agents, although relatively inert in their parent form, can be metabolically activated to a carcinogenic form by cytochrome P450 isozymes. Hence, an increase in cytochrome P450 enzyme activity can allow a tissue (e.g., lung) to activate carcinogens more efficiently. Conversely, increasing P450 activity can also increase the rate at which carcinogens are detoxified. This is especially a concern in terms of the respiratory tract because it is a major site of deposition of inhaled xenobiotics.

TCDD treatment in both the rabbit and the rat resulted in significant induction of cytochrome P450 isozymes in the lung (Hooker et al., 1975; Domain et al., 1986; Overby et al., 1992). Examination of rat lung cytosol revealed the presence of a high affinity, low-capacity TCDD-binding complex (Kurl et al., 1986). In the rabbit, immunochemical examination and in situ hybridization have identified a number of different cell types containing the TCDD-inducible isoform of P450, P4501A1, including endothelial cells of the entire vascular bed of rabbit lung, Clara cells at all levels of airway, type 2 cells, alveolar macrophages, and, to a lesser degree, ciliated cells (Overby et al., 1992). Interestingly, cytochrome P450 reductase, which is required for P450 activity, although present in Clara cells, alveolar macrophages, and type 2 cells (Domain et al., 1986), was not present in endothelium of rabbit lung, which raises the question of what role this isozyme may play in this tissue (Overby et al., 1992). Intraperitoneal treatment of rats with TCDD also produced a twofold induction of AHH activity in rat nasal tissue. These studies indicate that exposure to TCDD causes a modest induction of AHH enzyme activity in the rat and rabbit respiratory tract.

Cardiovascular Toxicity

Currently, there is a very limited amount of information pertaining to whether administration of TCDD to laboratory animals can produce cardiovascular toxicity. To date only two studies have been published in this area, whose results are described below.

Mechanical responses of isolated atria to (–)-isoproterenol were assessed in rats 7 days following TCDD treatment (6.25, 25, or 100 µg/kg) compared to vehicle-treated rats with unlimited access to feed. In TCDD-treated rats (100 µg/kg), the basal rate of spontaneously beating right atria was significantly decreased, whereas for the left atria, maximal inotropic responses to (–)-isoproterenol and 1-methyl-3-isobutylxanthine were enhanced to the same degree (Kelling et al., 1987). The changes in a trial function were not secondary to loss in body weight as determined by pair-fed controls. There was no effect of TCDD exposure on the ratio of heart ventricular mass to body weight or on the activities of pyruvate kinase and citrate synthase in homogenates prepared from heart ventricular muscle. The authors concluded that overtly toxic doses of TCDD in the rat did not depress mechanical function of the heart.

As discussed earlier, TCDD treatment in many animal species induces an increase in serum triglyceride concentrations. In rabbits, a single i.p. injection of TCDD (1 or 50 µg/kg) produced elevated triglycerides by day 10 (Brewster et al., 1988). By day 20 in the 50-µg/kg treated rabbits, electron microscopic examination of aortic arches revealed alterations resembling preatherosclerotic lesions, which included ruffling, denudation, and sloughing off of the cell surface. Additionally, there was an appearance of macrophage-like structures in the intima and the media of endothelial cells.

Corticosteroids

Many of the toxic effects observed after acute exposure to near lethal levels of TCDD (e.g., alterations in metabolic homeostasis and hypercholesterolemia) may be mediated by changes in endocrine function. Studies have included examination of the effects of high doses of TCDD on the levels of pituitary, thyroid, and adrenal hormones. The data presented in the literature are conflicting. Since the dosing regimens (i.e., acute administration of lethal doses of TCDD) used in such studies are not relevant to the questions being examined in this report and there is no obvious explanation for the conflicting data (although variations in protocol such as the age of the animal, feeding regimen, length of time between dosing and sacrifice, and time of sacrifice, may contribute), a short summary of the data is presented with no attempt to resolve this controversy.

The effect of TCDD on serum levels of corticosteroids is variable. Both an increase and a decrease in the level of corticosteroids have been observed (Neal et al., 1979; van Logten et al., 1980; Balk and Piper, 1984; DiBartolomeis et al., 1984, 1987; Gorski et al., 1988d). This variation may be due to the time at which the serum was harvested (diurnal variations) (DiBartolomeis et al., 1987). DiBartolomeis and colleagues observed that administration of TCDD depresses the evening peak in corticosteroid secretion and elevates the peak observed early during the light phase. These authors also felt that the early rise in corticosteroid levels was due to nutritional deprivation. However, this same diurnal variation was not observed by other laboratories (Gorski et al., 1988d; Kerkvliet et al., 1990a).

There is also controversy as to the mechanism by which the level of serum corticosterone is altered. Again, this controversy may be due to the length of time between the dosing and the test. At least two laboratories indicate that the mechanism by which corticosterone levels are altered is an indirect response to other physiologic alterations. Jones and colleagues (1987) observed a delay in the evening peak and an increase in the early peak on day 1 after dosing. These alterations were preceded by a decrease in prolactin, which may regulate the response of the adrenals to adrenocorticotropin. On the other hand, as discussed in more detail above, Rozman and colleagues (1992) and Stahl and colleagues (1992) showed a decrease in the MRNA, protein, and enzyme activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase, within 4-8 hours after oral dosing with TCDD. The alterations in insulin and the increase in corticosterone 4-8 days after TCDD administration occurred as a response to this primary defect in PEPCK activity. Although the alterations observed in insulin and corticosterone (key regulators of PEPCK activity) should act to increase PEPCK activity, this does not occur. The authors suggest that TCDD alters the responsivity of the PEPCK gene to these endogenous regulatory agents.

Other investigators (DiBartolomeis et al., 1986a-c) suggest that the alterations in corticosterone levels (from the laboratory that observes a decrease in levels) are due to alterations in cholesterol accumulation and enzyme levels involved in adrenal hormone synthesis. Synthesis of adrenal steroids employs a series of distinct cytochrome P450-dependent mixed-function oxidases located in the mitochondria and endoplasmic reticulum of cells. In the rat, TCDD may alter adrenal steroidogenesis by modulating the level of perturbation of adrenal cellular cholesterol homeostasis and the inhibition of cholesterol side chain cleavage (DiBartolomeis et al., 1986a-c).

Dermal Toxicity

Chloracne is considered the most consistent response to—and the hallmark of—TCDD toxicity in humans. Although chloracne is considered a

symptom of exposure to a certain class of chemicals, including TCDD, other polychlorinated dibenzodioxins, and polychlorinated biphenyls, the absence of chloracne does not indicate that no exposure occurred. Early on, it was established that these compounds are capable of eliciting a chloracnegenic response in animals (rabbit ear bioassay) as well as humans (Jones and Krizek, 1962). However, the skin of laboratory animals generally does not exhibit a similar sensitivity to chlorinated compounds. In fact, cutaneous changes are often seen as the last manifestation of systemic toxic effects following exposure to a compound that generates chloracne (Kimbrough, 1974).

The biological response of the cutaneous tissue to TCDD observed in certain animals (but not all) is the elicitation of hyperkeratosis and comedone formation. The formation of an acnegenic response after exposure to TCDD was shown to occur in the rabbit, monkey, and hairless mouse (Jones and Krizek, 1962; Knutson and Poland, 1982; Puhvel et al., 1982). The skin areas affected all lacked major hair growth, which suggested to some that longer hair shafts may reduce the formation of chloracne lesions (Kociba and Schwetz, 1982).

Structure-activity relationships and genetic studies by Knutson and Poland (1982) indicate that in hairless mice (hr/hr, hairless and nu/nu, nude), the ability of TCDD to produce epidermal hyperplasia, hyperkeratosis, and sebaceous gland metaplasia is mediated through the Ah receptor described above. However, in this study, an additional genetic locus (hr) seems to regulate the expression of the chloracne lesion. In these mice, the formation of chloracne was observed on a gross level if the animal was homozygous for the hr gene. However, if the animals were heterozygous at this gene (i.e., only a single genetic difference between the two mouse strains and therefore the same at the Ah locus), no visible scaliness or hyperkeratosis was observed. Although the dermal reaction was not observed, the binding and persistence of TCDD on the skin and the induction of liver enzymes by TCDD were the same in both strains. These studies indicate that TCDD is biologically active in the hr/+ mouse; however, additional events must occur (which, in this case, may be mediated by the hr gene locus) for the chloracne lesion to be evident.

Further studies show that some proliferation of the epidermal cells (hyperplasia) following histopathologic examination of the hr/+ mice was observed after TCDD exposure, but this proliferation alone did not result in the formation of a chloracne lesion. TCDD generated sebaceous gland metaplasia, inflammation, and hyperkeratosis only in mice homozygous at the hr locus (Molloy et al., 1987). Biochemical analysis of the keratins in the skin of TCDD-treated mice from the hr/+ and hr/hr strains indicated a difference between these two strains in the changes in keratins that occur after TCDD exposure. The alterations in keratin observed in the hr/+ mice

were consistent with an increase in the proliferative response to TCDD. On the other hand, the alterations in keratin observed in the hr/hr mice were consistent with an increase in proliferation and differentiation in these mice.

Studies of the generation of hyperkeratosis after TCDD administration in neonatal mice (which have hair in both hr/+ and hr/hr strains) show that this genetic control at the hr locus is evident even prior to hair loss (Puhvel and Sakamoto, 1988). This observation suggests that the differential response to TCDD is under genetic control and not a secondary effect due to phenotypic differences. However, other studies (Puhvel and Sakamoto, 1987) showed that the response to TCDD of keratinocytes from hr/+ and hr/hr mice after in vitro exposure could not be distinguished. The authors suggest that physiologic factors beyond the epidermal cells themselves may be involved in the expression of the different responses observed in the skin of mice exposed to TCDD in vivo. However, as described above with regard to the immunotoxicity of TCDD and the Ah receptor, the concentration of TCDD to which the cells are exposed in vitro may be so high compared to the amount in vivo that any difference due to strain sensitivity and gene loci may be overwhelmed by the amount of compound available.

Lastly, one study examined the contribution of diet to the ability of TCDD to generate chloracne lesions in hairless mice (Puhvel et al., 1991). In this study, mice of both the +/+ and the hr/hr strains were placed on a diet deficient in vitamin A. Depletion of vitamin A from the diet did not lead to an observable response in the +/+ mice, but did increase the hyperkeratinization observed in hr/hr mice. These studies show that the toxicity of TCDD can be modulated by the diet of the animal.

In summary, the formation of chloracne lesions after administration of TCDD is observed in some laboratory animals. The lesions are formed in areas of reduced hair growth or in strains of animals in which hair growth is reduced. Studies indicate that there may be an additional gene locus, besides the Ah locus, that regulates the differentiation (but not proliferation) of keratinocytes and the formation of a chloracne lesion in response to TCDD exposure. In addition, deficiency of vitamin A in the diet may increase the expression of chloracne lesions in animals genetically predisposed to the formation of such lesions.

TOXICITY PROFILE OF 2,4-DICHLOROPHENOXYACETIC ACID

Introduction

2,4-D (2,4-dichlorophenoxyacetic acid; Chemical Abstracts Service (CAS) No. 94-75-7; Figure 4-1) has been used commercially in the United States since World War II to control the growth of broadleaf plants and weeds on

range lands, lawns, golf courses, forests, roadways, parks, and agricultural land (CCT, 1987). Formulations used include 2,4-D amine and alkali salts and esters, which are mobile in soil and easily absorbed through both the leaves and the roots of many plants.

2,4-Dichlorophenoxyacetic acid is an odorless (when pure), white to yellow (the yellow color is due to phenolic impurities) crystalline powder. The melting point of 2,4-D is 138°C, and the free acid is corrosive to metals. It is soluble in water and a variety of organic solvents (e.g., acetone, alcohols, ketones, ether, and toluene).

Pharmacokinetics

The pharmacokinetics of 2,4-D resemble those of other phenoxy acid herbicides. Absorption of oral doses is rapid and complete, whereas absorption of dermal doses is much slower (Arnold and Beasley, 1989). Only 4.5-6.4 percent of a dermal dose of 2,4-D is thought to be absorbed by humans (Feldmann and Maibach, 1984; H.L. Fisher et al., 1989; Harris and Solomon, 1992). Absorption following inhalation is less well-studied, but experiments in rats indicate that inhaled 2,4-D is absorbed rapidly (WHO, 1984). Studies with human volunteers have also demonstrated that single doses of ingested 2,4-D are absorbed rapidly (Sauerhoff et al., 1977). Experiments in rats confirm that dermal absorption of 2,4-D is much slower than gastrointestinal absorption (Knopp and Schiller, 1992). Accumulation of 2,4-D in the brain has been reported in rats, mice, and rabbits (Kim et al., 1988; Schulze and Dougherty, 1988).

2,4-D undergoes no notable transformations in animals. Its amines and salts are hydrolyzed rapidly, whereas its esters are hydrolyzed more slowly (Arnold and Beasley, 1989). Some conjugation with amino acids can occur, and 2,4-D in serum is protein bound (Arnold and Beasley, 1989). 2,4-D is not metabolized to reactive intermediates capable of interacting with DNA.

2,4-D is excreted rapidly in urine, predominantly in its unmetabolized form (Sauerhoff et al., 1977), although both dose and formulation can affect elimination rate (Bjorklund and Erne, 1966). Human urinary excretion of 2,4-D is diurnal and continues for many days after the initial exposure, so one sample is unlikely to be representative (WHO, 1984). In addition, its distribution and elimination are dose-dependent, characterized by a sigmoidal relationship; extrapolation from high to low exposure doses is thus problematic (Gehring and Betso, 1978). A half-life in humans following single doses of 2,4-D has been estimated to be approximately 18 to 20 hours (Sauerhoff et al., 1977; WHO, 1984); a half-life in humans based on multiple doses has not been estimated with certainty (Ibrahim et al., 1991).

Carcinogenicity

Several studies of the carcinogenicity of 2,4-D have been performed in laboratory animals. In contrast to more recent studies, those conducted before 1986 are considered inadequate because they do not meet current guidelines for bioassay quality (Sontag et al., 1976; Huff, 1982). The earlier studies were universally negative, whereas the later studies provide limited but not conclusive evidence of carcinogenicity. These studies are described below.

Bionetics (1968a) and Innes and colleagues (1969) reported a study in which 18 male and 18 female mice of strain (C57BL/6×C3H/Anf)F_1 or strain (C57BL/6×AKR)F_1 received commercial (90 percent pure) 2,4-D according to the following dosing regimen: a dose of 46.4 mg/kg body weight by gavage at 7 days of age, the same amount unadjusted for increasing body weight daily up to 28 days of age, then 149 mg/kg of diet until about 78 weeks of age. Another group of 18 males and 18 females of strain (C57BL/6×AKR)F_1 received 100 mg/kg by gavage from days 7 to 28 of age, followed by 323 mg 2,4-D/kg diet until about 78 weeks of age. These doses were considered to be the maximum tolerated doses. No differences in tumor rates were detected compared to untreated or vehicle controls.

The same investigators also administered single doses of 215 mg/kg body weight of commercial (90 percent) 2,4-D in dimethyl sulfoxide (DMSO) subcutaneously or by gavage on day 28 of age to groups of 18 male and 18 female mice of strain (C57BL/6×C3H/Anf)F_1 or of strain (C57BL/6×AKR)F_1 (Bionetics, 1968a). Animals were observed up to 78 weeks of age. Again, no differences in tumor rates were detected compared to untreated or vehicle controls.

In another study, groups of 25 male and 25 female Osborne-Mendel rats received 0, 5, 25, 125, 625, or 1,250 parts per million (ppm) 96.7 percent pure 2,4-D/kg diet for two years (Hansen et al., 1971). No effect on growth rate, survival, organ weights, or hematologic parameters was observed. There were no statistically significant elevations in tumor rates at any site, although complete pathologic examinations were not performed on every animal and the maximum tolerated dose was not achieved.

In the first study that conformed to guidelines for the conduct of bioassays (Sontag et al., 1976; Huff, 1982), groups of 60 male and 60 female Fischer 344 rats received doses of 0, 1, 5, 15, or 45 mg 2,4-D/kg body weight in the diet for two years (Hazleton, 1986). Six male rats receiving the highest dose developed brain tumors (astrocytomas), compared to one control rat. None of the other treated rats had an excess incidence of this tumor, and no other excess tumor incidences were observed. In a second study, groups of 60 male and 60 female B6C3F_1 mice received 0, 1, 15, or 45 mg 2,4-D/kg body weight in the diet for two years (Hazleton, 1987). No

excess tumor incidences of any kind were observed. These studies have been criticized for possibly failing to identify and use the maximum tolerated dose, although body weights of the high dose females were significantly reduced. An expert panel that reviewed the results of these studies concluded that they provided weak evidence that exposure to 2,4-D causes astrocytomas in male rats, noting that the background incidence of this tumor is variable and that there was no apparent explanation for the absence of such tumors in female rats because there are no known sex differences in its absorption, distribution, metabolic fate, or elimination (Ibrahim et al., 1991). It is possible that high doses of 2,4-D overwhelm the male rat's excretion capability and no tumors would be expected at lower doses that did not affect normal excretion rates. Pharmacokinetic studies of this possibility are needed. Other factors that precluded a strong association between 2,4-D exposure and astrocytoma formation were the absence of preneoplastic lesions or target organ toxicity, decreased tumor latency, and certain histologic characteristics that usually distinguish agent-induced brain tumors from those that occur spontaneously (CCT, 1987; Munro et al., 1992).

A recent study evaluated the ability of 2,4-D to enhance lung tumor initiation by urethan in mice (Blakley et al., 1992). Groups of 25 male CD-1 mice received Weed-no-More in their drinking water for 15 weeks at concentrations of 0, 0.0325, 0.08125, or 0.163 percent (equivalent to doses of 0-50 mg 2,4-D/kg body weight/day). After 3 weeks of treatment, 20 of the mice in each group received a single intraperitoneal dose of 1.5 mg urethan/kg body weight. 2,4-D had no effect on the metabolism of urethan or on the size of the lung adenomas induced. The number of adenomas was somewhat enhanced, although not in a dose-related manner, suggesting that 2,4-D may have a weak co-carcinogenic effect on urethan-induced adenomas in mice. In a simultaneous study, the effect of 2,4-D on spontaneous leukemia incidence in CD-1 mice was evaluated (this strain has a spontaneous leukemia incidence of 50 percent). After one year of treatment with the same dose levels used in the urethan study, no effect on mortality or survival was seen, and more untreated mice died of leukemia than did treated mice.

Hayes and colleagues (1991) reported the results of a case-control study in which the incidence of malignant lymphoma among dogs kept as pets was found to have a positive association with owners' use of 2,4-D on lawns. Information from a self-administered questionnaire and telephone interviews was used to show that households with dogs that developed malignant lymphoma used 2,4-D or a commercial lawn service somewhat more frequently than control households (odds ratio = 1.3). The risk rose to a twofold excess in households with four or more annual applications of 2,4-D. These results are of interest because of the histologic and epidemiologic similarity between canine and human malignant lymphoma, and because of

suggestive epidemiologic evidence linking human lymphoma with 2,4-D exposure. The results do not establish a clear association between 2,4-D exposure and lymphoma in dogs, however, because of the likelihood that both owners and lawn services used a number of chemicals in addition to 2,4-D, and because no actual exposure data were available. In addition, the etiology of malignant lymphoma is unknown. Also, given the nature of the data collection methodology, the possibility of recall bias cannot be excluded. The results are in contrast to those of Hansen and colleagues (1971), who administered 2,4-D to 3 male and 3 female beagle dogs in their diet for two years (0, 10, 50, 100, or 500 ppm) and reported no malignant tumors. Two years is generally not long enough to obtain tumors in dogs, however.

In conclusion, studies in animals indicate that there is suggestive, but not compelling, evidence that 2,4-D exposure may be associated with the development of astrocytoma in male rats and malignant lymphoma in dogs. 2,4-D has equivocal genotoxic effects (see below), is not metabolized to an active intermediate, is rapidly eliminated, and does not accumulate in body tissues (Munro et al., 1992). There are no significant mechanisms of action that indicate a likely risk of tumorigenesis from 2,4-D. 2,4-D thus presents a possible, but not probable, risk of cancer to humans.

Genotoxicity

A variety of in vitro tests of the mutagenicity and clastogenicity of 2,4-D have been conducted, with both positive and negative results reported. 2,4-D is not a classic mutagen. The majority of test results for both 2,4-D and its compounds are negative, including those in *Salmonella typhimurium* and *Escherichia coli* (IARC, 1977; Mortelmans et al., 1984; Ibrahim et al., 1991). Some positive results for mutagenicity have been reported at high doses (> 10 μg/ml) in plant cells and in V79 Chinese hamster fibroblast cells (Khalatkar and Bhargara, 1982; Pavlica et al., 1991), but the relevance of positive results in plants or in immortalized cell lines in vitro to normal human cells in vivo is not known. An in vivo study in mice found that topically applied 2,4-D was weakly positive in the hair follicle nuclear aberration assay and negative in the bone marrow micronucleus test (Schop et al., 1990). In contrast, 2,4-D was weakly clastogenic to the bone marrow cells of rats treated twice by i.p. injection with doses of 35 or 70 mg/kg body weight (Adhikari and Grover, 1988). Weak positive results in tests for sister chromatid exchanges in cultured human lymphocytes have also been reported (Linnainmaa, 1984; Turkula and Jalal, 1985), but the majority of such tests, conducted in rat, mouse, hamster, and human cells, have been negative (IARC, 1977; Ibrahim et al., 1991). In vivo tests for sister chromatid exchange and chromosomal aberrations have also been negative, including in exposed humans (Linnainmaa, 1983; Mustonen et al., 1986, 1989;

Galloway et al., 1987, Ibrahim et al., 1991). This positive result is of some interest in view of the suggested target sites observed in epidemiologic studies, although the negative results in the overwhelming majority of both in vitro and in vivo studies of lymphocytes and bone marrow cells make conclusions difficult to support. In addition, the phenomenon of sister chromatid exchange has not been correlated to carcinogenicity and its clinical significance is unknown.

Acute Toxicity

The acute toxicity of 2,4-D varies a great deal among species, with the variation apparently related to its plasma half-life (Nielsen et al., 1965). Its LD_{50} ranges from 100 mg/kg in dogs to 540 mg/kg in chickens and 375-2,000 mg/kg in rats (Bjorklund and Erne, 1966; Nielsen et al., 1965; IARC, 1977). The target organ for acute toxicity in humans is the central nervous system (Flanagan et al., 1990). In a case report of attempted suicide, Friesen and colleagues (1990) noted prominent CNS depression and muscle damage, but no liver or kidney damage or electrocardiogram (EKG) abnormalities, at a dose sufficient to produce a serum level of 392 mg/liter (approximately 50 g). Studies in cats and dogs also indicate that the CNS is the principal target organ for acute 2,4-D toxicity in mammals, and suggest that the primary site of action is the cerebral cortex or the reticular formation (Dési et al., 1962a,b; Arnold et al., 1991).

Chronic Systemic Toxicity

Studies in laboratory animals have demonstrated that chronic exposure to 2,4-D can elicit effects in a number of organs. For example, chronic exposure to 2,4-D has produced a wide variety of hepatotoxic effects in rodents, including subacute toxic hepatitis, local necrosis, centrilobular atrophy, elevated peroxisomes, elevated mixed-function oxidases, and other enzyme and glycogen changes (IARC, 1977; WHO, 1984). Other studies have shown that 2,4-D is only a weak inducer of mixed-function oxidases, and that the pattern of induction differs from that of TCDD (Mustonen et al., 1989; Chaturvedi et al., 1991; Knopp and Schiller, 1992). Some renal and hematologic effects of 2,4-D have also been shown in rodents: increased kidney weights and cortical and subcortical pathology were seen at doses of 15 mg/kg/day or higher, and reductions in mean hemoglobin, mean hematocrit and red blood cell levels, and mean reticulocyte levels were observed at doses of 5 mg/kg/day or higher in rats and at 15 mg/kg/day in mice (Hazleton, 1986; Gorzinski et al., 1987a). Renal toxicity in rats was also reported in the Hazleton (1986) study described in the carcinogenicity section; a no-observed-effect level of 1 mg 2,4-D/kg body weight was identified.

Lukowicz-Ratajczak and Krechniak (1988) concluded that the renal toxicity of 2,4-D in rats is due to damage to the loop of Henle and not to the proximal tubule.

Neurobehavioral toxicity was reported in rats that received daily subcutaneous injections of 150-250 mg 2,4-D/kg body weight over four successive 14 day periods (Schulze and Dougherty, 1988). The extent of toxicity was affected by the formulation used and by food deprivation (Schulze, 1987). This toxicity was reversible, however, due to an apparent tolerance that developed with repeated exposure. A functional/cellular mechanism was thought to be responsible for the developed tolerance.

Reproductive and Developmental Toxicity

Tests of the developmental toxicity of 2,4-D have produced both positive and negative results. Administration of 90 percent pure 2,4-D, its isopropyl or butyl ester (99 percent pure), or its isooctyl ester (97 percent pure) to pregnant BL6, AKR, or C3H mice during days 6-14 of gestation increased the incidence of fetal anomalies. No effects were seen in B6AK or A/Ha mice (Bionetics, 1968a,b). No malformations or effects on litter size were observed among the offspring of Sprague-Dawley rats that received doses of 1,000 mg/liter drinking water 2,4-D (purity unspecified) during pregnancy and for an additional 10 months. The mothers also remained normal. The offspring then received 2,4-D for up to two years, and exhibited retarded growth and increased mortality; no unequivocal clinical or morphologic changes were observed, however (Bjorklund and Erne, 1966). Similar results were obtained by Hansen and colleagues (1971), who fed diets containing 0, 100, 500, or 1,500 ppm 2,4-D (96.7 percent pure) to male and female Osborne-Mendel rats over three successive generations (further detail not provided). No effects on fertility were reported, and average litter sizes were normal, although offspring mortality was increased and growth rates were decreased at the highest dose.

In another study, female rats received 0, 1,000, or 2,000 ppm 2,4-D (purity unspecified) in the diet for 95 days and were then mated with untreated males, continuing on the diet throughout pregnancy and lactation. The offspring of the rats fed the highest dose were small, and most died before weaning (IARC, 1977). In contrast, Schwetz and colleagues (1971) reported no effects on fertility, gestation, lactation, or mortality when Sprague-Dawley rats received the maximum tolerated dose (87.5 mg/kg/day) of 98.7 percent pure 2,4-D or its esters on days 6-15 of gestation. This dose was not teratogenic but was lethal to embryos, produced some delayed ossification, and had growth retarding effects on the offspring, however. No effect on fertility was noted in a multigenerational study in rats that received 0, 5, or 20 mg 2,4-D/kg/day (Mullison, 1986).

When 2,4-D (purity unspecified) was fed at a dose of 500 mg/kg diet to a pig throughout its pregnancy, anorexia was observed and the piglets were underdeveloped and apathetic; most died within 24 hours of birth (Bjorklund and Erne, 1966). No consistent embryotoxic effects were observed when 2,4-D was administered orally to hamsters on days 6-10 of gestation at doses up to 100 mg/kg (Collins and Williams, 1971).

Taken together, these studies suggest that 2,4-D does not affect fertility and is not a teratogen in laboratory animals, but it can reduce growth rates and increase mortality among exposed offspring. Very high doses are required to elicit these effects, however.

Immunotoxicity

Little work has been performed to evaluate the potential immunotoxicity of 2,4-D. There have been a number of case reports of allergic skin reactions involving 2,4-D exposure (Cushman and Street, 1982). One clinical study suggested that 2,4-D could produce contact sensitization among exposed farmers: 3 of 30 farmers patch-tested with a 1 percent solution of 2,4-D (purity unspecified) tested positive, whereas no positive reactions were observed among dermatitis-free controls (Sharma and Kaur, 1990).

Blakley (1986) administered single doses of 2,4-D butyl ester at 50-200 mg/kg body weight to female BDF1 mice by gavage and reported enhanced antibody production against SRBCs, as well as a stimulated lymphoproliferative response to lipopolysaccharide. These doses also produced clinical signs of toxicity and CNS pathology, however. In contrast, after dermal application of similarly toxic doses, a suppressed antibody response to SRBCs was noted (Blakley and Schiefer, 1986). Repeated administration of lower doses by either route for 3 weeks failed to produce either toxicity or immunologic effects. In these cases, the immunologic effects appear to have occurred as an indirect consequence of the clinical toxicity of 2,4-D.

TOXICITY PROFILE OF 2,4,5-TRICHLOROPHENOXYACETIC ACID

Introduction

Like 2,4-D, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid; CAS No. 93-76-5; Figure 4-1) was developed during World War II as an herbicide to control the growth of broadleaf plants and weeds on range lands, lawns, golf courses, forests, roadways, parks, and agricultural land (CCT, 1987). Most of these uses were banned by the U.S. Department of Health, Education and Welfare, the Department of Agriculture, or the Environmental Protection Agency between 1969 and 1972 because of the suspicion that 2,4,5-T

might be a human health hazard. The subsequent discovery that 2,4,5-T is generally contaminated with TCDD has led many to conclude that the reported adverse effects were due to this contaminant (Lilienfeld and Gallo, 1989). The registration for 2,4,5-T was canceled by the EPA in 1978.

When purified, 2,4,5-T is an odorless, white to light-tan solid with a melting point of 158°C. This compound is noncorrosive, and is soluble in alcohol and water. 2,4,5-T reacts with organic and inorganic bases to form salts, and with alcohol to form esters. As stated, commercial formulations of 2,4,5-T used during the Vietnam war contained TCDD as a contaminant from the manufacturing process.

Pharmacokinetics

The pharmacokinetics of 2,4,5-T resemble those of other phenoxy acid herbicides. Experiments in rats, dogs, and humans show that absorption of oral doses is rapid and complete, whereas absorption of dermal doses is much slower (Leng et al., 1984; Arnold and Beasley, 1989). Pharmacokinetic modeling has indicated that 97 percent of the 2,4,5-T absorbed through the skin is cleared, primarily through the urine, within one week (Leng et al., 1984). The pharmacokinetics of inhalation exposure have not been studied.

Distribution of 2,4,5-T occurs quickly, and the parent compound is eliminated via the urine, undegraded, as the free acid (Gehring et al., 1973; Piper et al., 1973b). Salts of 2,4,5-T are hydrolyzed prior to excretion, and a small amount may be conjugated (Gehring et al., 1973). Rates of clearance from plasma and urinary excretion depend on dose and are species-specific. Doses greater than 50 mg/kg body weight saturate the renal clearance mechanism for 2,4,5-T in rats (Piper et al., 1973b). Following a single oral dose in humans, 2,4,5-T was found to have a plasma half-life of about 19-23 hours, although interindividual variation was substantial; its urinary excretion was relatively rapid and fluctuated diurnally (Gehring et al., 1973; Kohli et al., 1974). Gastrointestinal absorption kinetics, plasma clearance, and urinary elimination are all first-order processes (Gehring et al., 1973; Piper et al., 1973b). Because the distribution and elimination of 2,4,5-T are dose-dependent, characterized by a sigmoidal relationship, extrapolation from high to low exposure doses is problematic (Gehring and Betso, 1978).

Carcinogenicity

Several studies of the carcinogenicity of 2,4,5-T have been performed in laboratory animals; these are described below. Only one study conforms to current standards for the conduct of carcinogenicity bioassays (Sontag et al., 1976; Huff, 1982). All produced negative results. There appears to be no evidence of or mechanistic basis for the carcinogenicity of 2,4,5-T in laboratory animals, although thorough testing has not been performed.

Bionetics (1968a) and Innes and colleagues (1969) reported a study in which 18 male and 18 female mice of strain (C57BL/6×C3H/Anf)F_1 or strain (C57BL/6×AKR)F_1 received commercial (98 percent pure) 2,4,5-T according to the following dosing regimen: a dose of 21.5 mg/kg body weight by gavage at 7 days of age, the same amount unadjusted for increasing body weight daily up to 28 days of age, then 60 mg/kg of diet until about 78 weeks of age. No differences in tumor rates were detected when compared to untreated or vehicle controls.

The same investigators also administered single doses of 21.5 mg/kg body weight of commercial (98 percent pure) 2,4,5-T in DMSO subcutaneously on day 28 of age to groups of 18 male and 18 female mice of strain (C57BL/6×C3H/Anf)F_1 or of strain (C57BL/6×AKR)F_1 (Bionetics, 1968a). Animals were observed up to 78 weeks of age. Again, no differences in tumor rates were detected compared to untreated or vehicle controls.

In another experiment, 20 male and 19 female 6-week-old inbred XVII/G mice received a concentration of 100 mg 2,4,5-T (containing 8 0.05 mg chlorinated dibenzodioxins)/liter of drinking water for two months, followed by 80 mg/kg diet for their life spans (Muranyi-Kovacs et al., 1976). Treated animals survived longer than did controls, and an increase in the incidence of spontaneous lung tumors was observed, although it was attributable to the longer life span of the treated animals. A group of 22 male and 25 female C3Hf mice received the same treatment, but treated males experienced reduced survival compared to controls. In addition, the total number of tumors in treated females was greater than that in female controls, although poor reporting prevented an analysis of tumor sites.

Kociba and colleagues (1979) provided groups of 60 male and 60 female Sprague-Dawley rats with diets containing 3, 10, or 30 mg 2,4,5-T/kg body weight/day for up to two years. A group of 96 males and 96 females served as untreated controls. An interim sacrifice was performed on 10 animals of each sex from each group after 118-119 days of treatment. Some toxicity was observed at the highest dose, indicating that the maximum tolerated dosage was achieved. No effect on tumor incidence was observed.

Two studies of the ability of 2,4,5-T to modulate carcinogenesis have been performed. In one, Abdellatif and colleagues (1990) used an initiation/selection/promotion protocol for the induction of liver tumors in Wistar rats to test the promoting ability of 2,4,5-T. Rats received an initiating dose of diethylnitrosamine, followed by a diet containing 2-acetylaminofluorine, and in the middle of the latter treatment, they received a necrogenic dose of carbon tetrachloride. Finally, diets containing 0.05 percent 2,4,5-T were provided for the remaining 23 weeks of the experiment. Rats receiving 2,4,5-T had an incidence of hepatocellular carcinoma of 16 percent, compared to the control incidence of 0 percent. The relevance of this result to human carcinogenesis is not known. In the second study, Mirvish and

colleagues (1991) sought to test the suspected relationship between 2,4,5-T exposure and non-Hodgkin's lymphoma in humans by providing 600 mg/kg diet to MRC-Wistar rats simultaneously with 75 mg of 2-hydroxyethylnitrosourea/ liter of drinking water. The 2,4,5-T was 98 percent pure and contained 1-4 μg/ kg each of TCDD and 2,3,7,8-tetrachlorodibenzofuran. 2-Hydroxyethylnitrosourea is a known inducer of B cell lymphoma. Coadministration of 2,4,5-T did not affect the incidence of lymphoma induced by the nitrosamine, nor did it produce tumors at any other site.

Genotoxicity

2,4,5-T is not genotoxic. Tests for mutagenicity in *Salmonella typhimurium, Escherichia coli* WP2, *Serratia marcescens* a21, and *Saccharomyces cerevisiae* D4 have been negative for both 2,4,5-T and its compounds (IARC, 1977; Mortelmans et al., 1984; Rashid et al., 1984). 2,4,5-T produced an increase in the frequency of chromosomal aberrations in Chinese hamster ovary cells and of clastogenicity in Mongolian gerbil bone marrow cells only at very high concentrations (1.75 mg/ml and 250 mg/kg for 5 days, respectively) (Majumdar and Hall, 1973; Galloway et al., 1987). It did not produce sex-linked recessive mutations in *Drosophila* (Zimmering et al., 1984) or micronuclei in bone marrow erythrocytes of mice (Jenssen and Renberg, 1976).

Acute Toxicity

The acute toxicity of 2,4,5-T varies among species, with the variation thought to be related to plasma half-life (Lilienfeld and Gallo, 1989). The LD_{50} of 2,4,5-T ranges from 100 mg/kg body weight in dogs to between 389 and 940 mg/kg in mice (Rowe and Hymas, 1954; IARC, 1977). Bjorklund and Erne (1966) gave single oral doses of 100 mg 2,4,5-T to pigs, and reported anorexia, vomiting, diarrhea, ataxia, hemorrhagic enteritis, and congestion of the liver and kidneys. Myotonia and anorexia have also been observed in dogs that received 100 mg 2,4,5-T/kg body weight (Drill and Hiratzka, 1953).

Chronic Systemic Toxicity

A number of effects have been reported in laboratory animals chronically exposed to 2,4,5-T. For example, some minor liver congestion was reported in dogs that received doses of 20 mg/kg for 90 days (Drill and Hiratzka, 1953). Hepatic inflammation, biliary hyperplasia, and renal disease were also reported in Sprague-Dawley rats that received diets containing 30 mg 2,4,5-T/kg/day for two years; only minimal changes occurred at lower doses (Kociba et al., 1979).

Reproductive and Developmental Toxicity

Several studies have indicated that 2,4,5-T can be fetotoxic in rodents. In general, doses of 2,4,5-T greater than 20 mg/kg body weight administered on days 6-15 of pregnancy can retard growth, and increase embryolethality and the frequency of cleft palates among mice, both in the presence and in the absence of maternal toxicity (Bionetics, 1968b; Roll, 1971; Neubert and Dillmann, 1972; Seidenberg et al., 1986; Seidenberg and Becker, 1987; Holson et al., 1992). Bazare and colleagues (1990) found that the half-life of 2,4,5-T is increased in pregnant mice, which may contribute to its toxicity. Malformations have also been produced in hamsters treated with 100 mg 2,4,5-T/kg body weight orally on days 6-10 of pregnancy (Collins and Williams, 1971). Doses of at least 400 mg 2,4,5-T/kg body weight were required to induce significant embryotoxicity in rats in the study of Wilson and colleagues (1971), although other studies have reported embryotoxicity and reduced fetal mortality in rats at lower dosages (Sparschu et al., 1971; Smith et al., 1981). Some behavioral toxicity has also been associated with intrauterine exposure of rats to 2,4,5-T or to a 2,4-D/2,4,5-T mixture (Rogers, 1983; Mohammad and St. Omer, 1986). No developmental or fetotoxic effects of 2,4,5-T have been reported in rabbits, sheep, or monkeys, however (IARC, 1977).

The purity of 2,4,5-T appears to affect its fetotoxicity and teratogenicity in hamsters but not in mice. In general, 2,4,5-T contaminated with TCDD was fetocidal and teratogenic to hamsters at doses of 20 mg/kg or higher, whereas that containing no detectable TCDD elicited effects only at a dose of 100 mg/kg (Collins and Williams, 1971). Both technical grade and analytical grade 2,4,5-T (differing tenfold in TCDD content) produced similar frequencies of embryolethality, cleft palate, and kidney malformations in mice at the same dose levels, however (Courtney and Moore, 1971; Nelson et al., 1992). In addition, the embryotoxicity of doses of 100 mg 2,4,5-T/kg body weight was not increased by the simultaneous administration of 1 µg TCDD/kg in mice (Courtney and Moore, 1971).

No studies of the reproductive toxicity of 2,4,5-T could be found.

Immunotoxicity

No studies of the immunotoxicity of 2,4,5-T could be found.

TOXICITY PROFILE OF CACODYLIC ACID

Introduction

Cacodylic acid (hydroxydimethylarsine oxide; dimethylarsinic acid; CAS No. 75-60-5; Figure 4-1) is a nonselective, postemergence contact herbicide.

It is currently a registered herbicide in the United States and is a List B chemical under the Federal Insecticide, Fungicide, and Rodenticide Act registration, with data development and review ongoing. The herbicide formulation used in Vietnam in defoliation and crop destruction missions (Agent Blue or Phytar 560-G) contained 26.4 percent sodium cacodylate and 4.7 percent cacodylic acid as the active ingredients (Hood, 1985). Sodium cacodylate and cacodylic acid are likely to have similar toxicologic characteristics.

The solid form is an odorless, colorless crystal with a melting point of 195-196°C. In aqueous solution, the chemical is mildly corrosive. It is soluble in alcohol, acetic acid, and solutions that are 50 percent aqueous.

Pharmacokinetics

Cacodylic acid is one of the primary metabolites of inorganic arsenic: pentavalent inorganic arsenic is reduced to the trivalent form [As(III)], then methylated to methanearsonic acid and subsequently to dimethylarsinic acid (cacodylic acid) (Yamanaka et al., 1991). There is no evidence in the recent literature that inorganic arsenic is released into the body after exposure to cacodylic acid. Cacodylic acid has been used in the past as a pharmacologic agent, and it was believed that both the efficacy of this agent and its toxicity were due to inorganic arsenic liberated by hydrolysis in the stomach. Cacodylic acid is, however, very resistant to hydrolysis, and studies with improved analytical methodology have not demonstrated the metabolism of cacodylic acid to arsenic (Hood, 1985). For example, Marafante and colleagues (1987) studied the metabolism of orally administered cacodylic acid in mice, hamsters, and humans, and found that in mice and hamsters, 80-85 percent of the dose was eliminated as unmetabolized cacodylic acid, and 13-15 percent as a cacodylic acid complex; in mice and hamsters respectively, 3.5 percent and 6.4 percent of the doses were excreted as trimethylarsine oxide (TMAO), whereas in humans, about 80 percent of the dose was excreted as cacodylic acid and 4 percent as TMAO. No demethylation of cacodylic acid to inorganic arsenic was observed in any species.

Studies in rats indicate that cacodylic acid is absorbed more slowly following oral administration than intratracheal administration, becomes bound to red blood cells, and is excreted readily in urine. Clearance from the rat red blood cell is very slow, with the half-life of cacodylic acid in rat erythrocytes being approximately the same as that of the erythrocytes (95 days), indicating irreversible binding of cacodylic acid to rat hemoglobin. Rat erythrocytes were found to bind cacodylic acid much more readily than rabbit or guinea pig erythrocytes, however, and human red blood cells had the least binding, demonstrating substantial species differences (Stevens et al., 1977).

In another study of metabolism, cacodylic acid was administered orally to hamsters (50 mg/kg). Peak blood levels were reached 6 hours after administration and declined rapidly thereafter. The cacodylic acid was excreted relatively rapidly by the hamster (80 percent after 24 hours following oral administration), primarily in urine and feces. Some of the cacodylic acid (approximately 26 percent of the total arsenic in the whole blood) was further methylated to a trimethylarsinic compound, which was excreted primarily in the urine (Yamahuchi and Yamamura, 1984). Humans also excrete cacodylic acid predominantly in the urine following oral dosing (Buchet et al., 1981).

Carcinogenicity

Very sparse animal oncogenicity data are available for cacodylic acid. The one limited study conducted in mice showed no evidence of oncogenic potential; however, conclusions should be drawn with caution because of its inadequacies. In this study, male and female pathogen-free mice (strain unspecified) were administered cacodylic acid (purity unspecified) in distilled water at a dose of 46.4 mg/kg from days 7 to 28 of age, and then received 121 ppm (approximately 18 mg/kg/day) in the diet for 18 months. EPA concluded that this dose was close to a maximum tolerated dose; however, EPA did not state the basis for this conclusion. No evidence of oncognicity was observed in this study, based on statistical analyses for the cacodylic acid treated mice versus pooled negative controls for four tumor categories, which included hepatoma, pulmonary tumors, lymphoma, and total tumors. Although this study was negative for oncogenicity, it should be noted that it was a screening test with relatively small numbers of animals (not specified) and only a single dose level. This study does not meet NTP guidelines for oncogenicity testing (Innes et al., 1969; Sontag et al., 1976; Huff, 1982).

There is speculation that pulmonary carcinogenesis could result from high dose exposure to cacodylic acid because both inorganic arsenic and cacodylic acid share dimethyl- and trimethylarsine as metabolites. Dimethylarsine has been associated with DNA damage in both rat and mouse lung tissue following high dose oral acute exposures. Excess lung cancer has been reported in epidemiologic studies of smelter workers occupationally exposed primarily to pentavalent arsenic (U.S. EPA, 1981).

Genotoxicity

In general, the genotoxicity profile for cacodylic acid is mixed. It is negative in bacterial tests for mutagenicity, positive for mutagenicity and clastogenicity in yeast, negative in the dominant lethal test in mice, negative

for sister chromatid exchange in Chinese hamster ovary cells, positive for the mouse erythrocyte micronucleus test, and negative for unscheduled DNA syntheses in human diploid fibroblasts (U.S. EPA, 1981). Positive studies suggest that cacodylic acid poses a genotoxic risk only at high doses. For example, the mouse lymphoma assay showed mutagenic effects only at cytotoxic doses, and clastogenic activity was observed in the erythrocytes of mice only at acutely toxic doses (Jotz and Mitchell, 1980; Kirkhart, 1980).

Recent data implicate the cacodylic acid metabolite dimethylarsine as a mutagen in bacterial and mammalian systems (Yamanaka et al., 1989a,b). In particular, DNA single-strand breaks were induced in rat and mouse lung after oral administration of high doses of cacodylic acid (1,500 mg/kg). In vitro follow-up experiments showed that the breaks were caused by active oxygen species (superoxide anion radical produced by the one-electron reduction of molecular oxygen) and/or dimethylarsinic peroxyl radicals, both resulting from the presence of dimethylarsine. Dimethylarsine was stated to be a volatile metabolite of cacodylic acid excreted in the expired air in the in vivo phase of the study (Yamanaka et al., 1989a,b, 1991).

Acute Toxicity

Human case studies have included reports of nausea and gastrointestinal distress following exposures to cacodylic acid (Peoples et al., 1979). Data from farm animal studies have shown diarrhea and anorexia resulting from exposure to organic arsenicals. Hemorrhaging in the intestinal tract was noted at necropsy. Cacodylic acid was the least toxic of the arsenicals evaluated, with a 10 day exposure to 25 mg/kg considered "marginally toxic" to cattle (Hood, 1985).

Sedation was noted among surviving rats in an acute LD_{50} study with cacodylic acid; however, at doses of 0.6-1.35 g/kg/day, this appears to be a nonspecific effect of the test material rather than a sign of neurotoxicity (Ansul, 1967).

An acute dermal irritation study in rabbits showed that cacodylic acid was essentially nonirritating when applied dermally in a single acute exposure (Ansul, 1967). Female rates exposed to 6.94 mg/liter air of cacodylic acid in acute inhalation toxicity experiments developed erythematous lesions on the feet and ears (Stevens et al., 1979).

Chronic Systemic Toxicity

Studies of the chronic systemic toxicity of cacodylic acid are extremely limited. Reports of early clinical experience with cacodylic acid showed renal damage, including nephritis, in some cases (Hood, 1985). Cattle given

cacodylic acid at 10-20 mg/kg/day for 4 weeks showed renal tubular degeneration in four of the five animals treated (Dickinson, 1975).

Reproductive and Developmental Toxicity

Limited data are available for assessing the potential effects of cacodylic acid on male-mediated reproductive toxicity. No studies of sperm morphology, viability, or sperm counts have been done, and reproductive toxicity studies are absent. In addition, no studies have been reported that assess potential reproductive toxicity in females or offspring.

The studies of cacodylic acid in male animals are limited to a subacute dermal study in a single male rabbit, which showed decreased spermatogenesis at a high dose level (1 g/kg), and to a 21 day feeding study in Sprague-Dawley rats, which showed seminiferous tubule atrophy and decreased spermatogenesis at 226 mg/kg/day, with a no-observed-adverse-effect level for this effect of 118 mg/kg/day (Ansul, 1967). The high dose levels at which these effects were observed suggest that there is likely to be a relatively low-risk of reproductive toxicity to humans in actual exposure situations.

Exposure to cacodylic acid has been associated with fetotoxicity in laboratory animals, although these effects occurred only at doses that also produced maternal toxicity. Fetotoxic effects of cacodylic acid in rats, mice, and hamsters include reduced fetal weight, decreased ossification, and increased incidences of cleft palate, irregular palatine rugae, micrognathia, hypoplastic lungs, and other major malformations (Chernoff and Rogers, 1975; WARF, 1976; Rogers et al., 1981; Hood et al., 1982, Kavlock et al., 1985). In each of these studies, overt maternal toxicity was also apparent.

Immunotoxicity

The potential immunotoxicity of cacodylic acid has not been evaluated.

TOXICITY PROFILE OF PICLORAM

Introduction

Picloram (4-amino-3,5,6-trichloropicolinic acid; CAS No. 1918-02-1; trade name Tordon; Figure 4-1) is a systemic herbicide used to control broadleaf and woody plants. Picloram was combined with 2,4-D to generate the formulation termed Agent White, an herbicide used during the war in Vietnam. The toxicological data base for picloram is limited. Picloram is a colorless (off-white to brown, if contaminants are present) powder or crystal with a chlorine-like odor. Its melting point is 218°C. It is soluble in water and a variety of organic solvents (e.g., acetone, alcohols, and benzene).

Pharmacokinetics

The only information that could be located on the pharmacokinetics of picloram was a study in which 15 rats received either an oral or an intravenous dose, 82 percent of which was detected in urine and 15.5 percent in feces within 48 hours (U.S. EPA, 1988a,c).

Carcinogenicity

Three somewhat limited studies of the carcinogenicity of picloram have been performed, with one study producing equivocal positive results and the rest producing negative results. The positive results were attributed to hexachlorobenzene contamination, however. These studies are described below.

Fischer 344 rats (50 of each sex per group) were administered 0, 20, 60, or 200 mg/kg/day of technical picloram in the diet for two years. No clinical signs of toxicity were observed in any dose group, indicating that the maximum tolerated dose was not reached, although a number of hepatocellular alterations were detected. No oncogenic effects were observed (Stott et al., 1990).

In another study, male and female Osborne-Mendel rats were administered 0, 10,000 (500 mg/kg), or 20,000 ppm (1,000 mg/kg) of picloram in the diet for 39 weeks; due to signs of overt toxicity, the dosages were lowered to 5,000 (250 mg/kg) and 10,000 ppm (500 mg/kg) for an additional 41 weeks. Animals were administered control diets for a recovery period of 33 weeks. An increased incidence of follicular hyperplasia, C cell hyperplasia, and C cell adenoma of the thyroid was observed in both sexes of treated rats compared to controls; however, the authors concluded that these effects were not associated with picloram treatment. An increased incidence of pituitary chromophobe adenoma was observed for females in the treated groups, compared to the matched controls but not compared to pooled controls; this lesion is common in aging rats. Focal cellular changes were noted in treated male rat livers. A greater number of liver changes and an increased incidence of neoplastic nodules were observed for females at the high dose. One hepatocellular carcinoma was observed in a low dose male and one in a high dose female. The authors concluded that picloram was carcinogenic to Osborne-Mendel female rats based on the increased incidence of liver neoplastic nodules in the high dose group (NCI, 1978). An EPA peer review committee reassessed the tumor incidence and agreed that there was a statistically significant (p 8 0.05) increase in liver adenomas and in combined liver adenomas and carcinomas for high dose females; however, the hexachlorobenzene (HCB, 130 ppm) contaminating the test material was believed to be responsible for the liver tumors observed. (It

should be noted that this study was done in 1978 with technical picloram; therefore, the picloram used in Vietnam probably contained a similar level of HCB.) EPA also concluded that the biologic significance of the thyroid lesions could not be determined because of the small control group.

Finally, male and female B6C3F$_1$ mice were administered 0, 2,500 (357 mg/kg/day), or 5,000 ppm (714 mg/kg/day) of picloram in the diet for 79 weeks and then allowed to recover for 10 weeks prior to sacrifice. Mean body weights of the treated mice were unaffected by treatment, and no oncogenic response was observed (NCI, 1978).

Genotoxicity

Information on the potential genotoxic effects of picloram is meager: it did not produce cytogenetic effects in rats exposed to single doses up to 2,000 mg/kg (U.S. EPA, 1988c), and it did not produce mutagenic effects when tested in *Salmonella typhimurium* in the absence of metabolic activation (U.S. EPA, 1988c).

Acute Toxicity

The available information on the acute toxicity of picloram is also paltry. The dermal LD_{50} for technical grade picloram and a formulation of picloram referred to as Tordon K^+ salt liquor in rabbits is greater than 2,000 mg/kg (U.S. EPA, 1988c). Some erythema, but no signs of toxicity, were observed at this dose. Some neurologic effects, including hyperactivity, ataxia, and tremors, were reported in pregnant rats exposed to 750 or 1,000 mg picloram/kg (Thompson et al., 1972).

Chronic Systemic Toxicity

Several studies have reported various effects of technical grade picloram on the livers of rats. In the carcinogenicity bioassay conducted by Stott and colleagues (1990) described above, treatment-related hepatomegaly was noted in the 60 and 200 mg/kg/day dose groups, along with hepatocellular swelling and altered tinctorial properties in the central regions of the liver lobules. In addition, increased liver weights were observed for males and females at the high dose compared to the controls. The NOEL was 20 mg/kg/day, and the lowest effect level (LEL) was 60 mg/kg/day for histological changes in centrilobular hepatocellular tissues. According to EPA, the levels of hexachlorobenzene (197 ppm), a contaminant in the technical picloram tested, were probably not responsible for the liver effects (U.S. EPA, 1988c). Gorzinski and colleagues (1987b) also reported a dose-related increase in liver weights, hepatocellular hypertrophy, and changes in centrilobular

tinctorial properties in male and female F344 rats at doses of 150 mg/kg/day and higher in the diet for 13 weeks. In a 90 day study, cloudy swelling in the liver cells and bile duct epithelium occurred in male and female F344 rats administered 0.3 or 1 percent technical picloram in the diet (U.S. EPA, 1988c).

Liver effects have also been reported in dogs exposed to picloram: increased liver weights were reported in beagles that received 35 mg/kg/day or more in the diet for six months (U.S. EPA, 1988c).

No other effects of chronic exposure to picloram have been reported.

Reproductive and Developmental Toxicity

The reproductive toxicity of picloram was evaluated in a two-generation study; too few animals were evaluated, and no toxicity was detected at the highest dose tested (150 mg/kg/day) (U.S. EPA, 1988c).

Some developmental toxicity was produced in rabbits exposed to 400 mg picloram/kg/day by gavage on days 6 through 18 of gestation. Fetal abnormalities included single litter incidences of forelimb flexure, fused ribs, hypoplastic tail, and omphalocele (John-Greene et al., 1985). Some maternal toxicity was observed at this dose, however, and EPA concluded that these malformations were not treatment related, based on the low litter incidence of these findings (U.S. EPA, 1988c).

No teratogenic effects were produced in the offspring of rats administered doses of picloram by gavage up to 1,000 mg/kg/day on days 6 to 15 of gestation, although the occurrence of bilateral accessory ribs was significantly increased at this dose (Thompson et al., 1972).

Immunotoxicity

Studies of the potential immunotoxicity of picloram are limited to dermal sensitization. In one study, 53 human volunteers were administered nine 24-hour applications of 0.5 ml of a 2 percent potassium picloram solution on the skin of both upper arms. Each volunteer received challenge doses from 17 to 24 days later. This formulation of picloram (its K^+ salt) was not a skin sensitizer or an irritant (U.S. EPA, 1988c). In a similar study, a 5 percent solution of picloram (M-2439, Tordon 101 formulation) produced slight dermal irritation and caused a sensitization response in 6 of the 69 volunteers exposed. When the individual components of M-2439 were tested separately [picloram, triisopropanolamine (TIPA) salt, and 2,4-D TIPA salt], no sensitization reaction occurred, however (U.S. EPA, 1988c). Tordon K^+, but not technical grade picloram, was also found to be a skin sensitizer in guinea pigs (U.S. EPA, 1988c).

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5

Methodologic Considerations in Evaluating the Evidence

The committee has undertaken the task of summarizing the strength of the scientific evidence concerning the association between herbicide exposure during Vietnam service and each of a set of diseases or conditions suspected to be associated with such exposure. For each disease, the committee has determined, to the extent that available scientific data permit meaningful determinations,

1. whether a statistical association with herbicide exposure exists, taking into account the strength of the scientific evidence and the appropriateness of the statistical and epidemiologic methods used to detect the association;
2. the increased risk of each disease in question among those exposed to herbicides during Vietnam service; and
3. whether there exists a plausible biologic mechanism or other evidence of a causal relationship between herbicide exposure and the disease in question.

The committee was not provided, by the legislation establishing it, with a specific list of diseases and conditions suspected to be associated with herbicide exposure. The committee staff and members developed such a list based on the diseases and conditions that had been mentioned in the scientific literature or in legal documents that came to their attention through very extensive literature searches, as described in Appendix A.

The judgments made by the committee have both quantitative and qualitative aspects, and they reflect the evidence examined and the approach taken to

evaluate it. In this chapter, the committee describes more fully how it has approached its task, in the hope that readers may then be in a better position to assess and interpret the committee's findings. By offering this information, the committee wishes to make the report useful to those who may seek to update its conclusions as new information is obtained. This chapter outlines the specific questions posed by the committee, the types of evidence it identified, its approaches to evaluating reports both singly and collectively, and the nature of the conclusions it felt that logic and evidence permitted. Against this background, details of the analysis and specific conclusions concerning each health effect appear in subsequent chapters. This chapter is based on a similar description in an Institute of Medicine report *Adverse Effects of Pertussis and Rubella Vaccines* (IOM, 1991), adapted to the current task.

Attributes of the diseases being considered, as well as the population exposed to herbicides, influenced the committee's analysis. The diseases can be characterized, for example, by their frequency, by the specificity of their symptoms, and by prior knowledge of their etiology and pathogenesis. Diseases, such as non-Hodgkin's lymphoma, that occur only rarely in exposed persons are more difficult to study than those that occur more frequently. Conditions such as soft tissue sarcoma that are ill defined, birth defects that are known to occur in the absence of herbicide exposure, or conditions that generally have unknown causes or mechanisms of development are also inherently difficult to investigate.

When the actual intensity or duration of exposure to a potential disease-causing agent is difficult to measure, as is generally true for herbicide exposure in Vietnam, comparisons between presumably exposed and presumably nonexposed persons become clouded. This is due to the misclassification of truly exposed individuals as unexposed or, more likely, the misclassification of truly unexposed people as exposed. For example, some studies compare veterans with experience in Vietnam to veterans who served during the same period of time, but not in Vietnam (Vietnam era veterans). If such a classification system is used as a surrogate for exposure to herbicide, it is likely that a substantial number of those presumed to be exposed on the basis of Vietnam service had either minimal or no actual exposure. The committee deemed the issue of exposure measurement to be so important that a separate chapter of this report is devoted to that topic. A section of Chapter 6 also addresses the assessment of exposure in epidemiologic studies.

It was because of the uncertain validity of exposure measurements in many of the studies of veterans that the committee decided to review studies of other groups potentially exposed to the herbicides contained in Agent Orange, to other herbicides, or to dioxin, the contaminant presumed by some to be the actual cause of the purported adverse effects of Agent Orange. These other groups include industrial and agricultural workers, Vietnamese

citizens, and people exposed to environmental sources as a result of residing near the site of an industrial accident. The committee felt that considering studies of other groups would help address the issue of whether these compounds *could* be associated with particular health outcomes in veterans, although that would have only an indirect bearing on the question of association in veterans themselves.

In any epidemiologic study comparing an exposed to an unexposed group, it is likely that characteristics other than exposure may differ between the two groups. For example, the group exposed to herbicide in an industrial study might have a higher or lower prevalence of cigarette smokers than the unexposed group. When the groups differ with respect to factors that are also associated with the risk of the outcome of interest, a simple comparison of the groups may either exaggerate or hide the true difference in disease rates that is due to the exposure of interest. In the example of higher prevalence of smoking in the exposed workers, a simple comparison of lung cancer rates in the exposed and unexposed would exaggerate an apparent difference in lung cancer rates, since smoking is known to cause lung cancer. If the exposed workers had a lower prevalence of smoking, the simple comparison would tend to mask any true association between exposure and lung cancer by spuriously elevating the risk of the disease in the unexposed group. This phenomenon, known as confounding, represented another major challenge to the committee.

QUESTIONS TO BE ADDRESSED

What would it mean to say that exposure to herbicides is associated with one or another type of health effect? It would not mean that exposure invariably produces the disease or adverse health outcome, or that all cases of the disease were due to the herbicide. Such complete correspondence between exposure and disease is by far the exception in public health and does not occur in this context, or the present review would not be required.

In the present review, the committee has been concerned with two kinds of questions about associations. The first of these questions about exposure to herbicides is, in general, is there a statistical association with the specified adverse condition? For example, is exposure to the herbicide 2,4-D associated with an increased incidence of soft tissue sarcoma (STS)? If the conclusion is affirmative, a second question becomes pertinent: Under the assumption that exposure to herbicides is associated with an outcome in any group, what is the association among Vietnam veterans? That is, if an association is found to exist among occupationally exposed individuals, is the association also found, or likely to be found, in Vietnam veterans? Discussion of each of these questions can help to clarify the committee's view of its task. A third question, relating to the biologic plausibility that

the outcome in question was caused by the herbicide exposure, is dealt with through an examination of the toxicological literature, as reviewed in Chapter 4.

Are Herbicides Statistically Associated with the Health Outcome?

The work of the committee necessarily focused on a pragmatic question: What is the nature of the evidence relevant to drawing its conclusion about statistical association? In pursuing this question, the committee recognized that an absolute conclusion about the absence of association may never be attained. As in science generally, studies of health outcomes following herbicide exposure are not capable of demonstrating that the purported effect is impossible or could not ever occur. Any instrument of observation has a limit to its resolving power, and this is true of epidemiologic studies as well. Hence, in a strict technical sense, the committee could not prove the absence of any possibility of a health outcome associated with herbicide exposure. Nevertheless, for some outcomes examined for which there was no evidence consistent with an association, there was limited or suggestive evidence consistent with *no* association, and the committee was able to conclude *within the limits of the current resolving power of the existing studies* that there is no association with herbicide exposure.

The evidentiary base that the committee found to be most helpful derived from epidemiologic studies of populations, that is, investigations in which large groups of people are studied to determine the association between the occurrence of particular diseases and exposure to the substances at issue. To determine whether an association exists, epidemiologists estimate the magnitude of an appropriate quantitative measure (such as the relative risk or the odds ratio) that describes the joint occurrence of exposures and diseases in defined populations or groups. Usage of "relative risk," "odds ratio," or "estimate of relative risk" is not consistent in the literature reviewed and cited in this report. In its own usage, the committee intends *relative risk* to be used to refer to the results of cohort studies, and *estimates of relative risk* or *odds ratio* to refer to the results of case-control studies (see Glossary for definitions). Values of relative risk greater than 1 may indicate a positive, or direct (harmful), association and are emphasized in the discussion in this chapter; values between 1 and 0 may indicate a negative, or inverse (protective), association. "Statistical significance," that is whether the increased risk is sufficiently greater than 1 to exclude the possibility that the apparent effect is due to chance, must also be considered.

Formally, in planning an investigation, an epidemiologist poses a hypothesis to the effect that the exposures and health outcomes under study are not associated. Under this hypothesis, the value of the measure of association used is theoretically expected to be approximately 1. This is

termed the *null hypothesis* or the hypothesis of no association. The measure of association derived from the investigation is then tested statistically. To "reject the null hypothesis," or to conclude that exposures and events are not independent, is to conclude that there is evidence of an association.

When more than one epidemiologic study has been conducted, it may be instructive to combine their results so as to reach a stronger conclusion than a single study can provide. This process, termed meta-analysis, is described more fully later in this chapter.

Determining whether an observed exposure-disease association is "real" requires additional scrutiny. This is because there may be alternative explanations, other than exposure, for the observed association. These include errors in the design, conduct, or analysis of the investigation; bias, or a systematic tendency to distort the measure of association from representing the true relation between exposures and outcomes; confounding, or distortion of the measure of association because another factor, related to both exposures and outcomes, has not been recognized or taken into account in the analysis; and chance, the effect of random variation in producing observations that can, in reality, only be approximations to the truth and can, with a known probability, sometimes depart widely from the truth.

In deciding whether associations between herbicides and particular outcomes exist then, it has been the committee's task to judge in each instance whether there is evidence of an association from the available studies and, if so, whether it is direct or inverse, and whether it is due to error, bias, confounding, or chance or, instead, most likely to be due to a true association between herbicides and outcome.

What Is the Increased Risk of the Disease in Question Among Those Exposed to Herbicides in Vietnam?

The second question, which becomes pertinent principally (but not exclusively) if the answer to the first question is affirmative, concerns the likely magnitude of the exposure-disease association in Vietnam veterans exposed to herbicides. The most desirable evidence as a basis for answering this type of question involves knowledge of the rate of occurrence of the disease in those Vietnam veterans who were actually exposed to herbicides, the rate in those who were not exposed (the "background" rate of the disease in the population of Vietnam veterans), and the degree to which any other differences between exposed and unexposed groups of veterans influence the difference in rates. When those Vietnam veterans who are actually exposed have not been identified properly, as has generally been the case in existing studies, this question becomes difficult to answer. By considering the magnitude of the association observed in other cohorts, the quality and results of existing studies of veterans related to a particular outcome, and

other principles of epidemiologic research discussed below, the committee formulated a qualitative judgment regarding the second question.

BURDEN OF PROOF

In approaching its task, the committee considered the concept of "burden of proof" and its place in such an evaluation. This concept implies that one position or another concerning association is presumed to be true unless it is offset by evidence to the contrary. The prior position might be either affirmative or negative. That is, it may be assumed that an exposure is harmful unless sufficient evidence of safety is present; alternatively, it may be assumed that an exposure is safe unless convincing evidence of harmful effects is present. In either case, it is sometimes argued that a burden of proof must be fulfilled before the presumed position is rejected. In general, it is desirable to avoid making an error in either direction—concluding either that there is or that there is not an association when the opposite is true. Reducing the chance of such mistaken conclusions depends on careful assessment of the evidence, including consideration of possible errors, bias, and confounding.

The role of chance in leading to erroneous conclusions as a result of random variation in sampling or in other respects is customarily handled through formal statistical analyses, which are based on assumptions from probability theory. Statistical measures can suggest the likelihood that conclusions as to the presence or absence of an association will each be in error. In general, a result is said to have greater statistical significance as the probability of error in accepting an association becomes smaller. More technically, the level of statistical significance is the probability of observing by chance at least as great a difference as that observed between an "experimental" (exposed) and a "control" (unexposed) group, if the risk of the disease were, in truth, identical in the two groups. The likelihood that a true association will be correctly detected in an investigation is a statistical property of the investigation, termed its *power*. Both statistical significance and power reflect the role of chance in scientific observations and the concomitant uncertainty in all scientific conclusions. One obvious implication of this understanding is that the concept of "proof" in its commonsense meaning is not strictly applicable to scientific observations. Even when scientists conclude that an experiment demonstrates ("proves") an association, they know there is a small, known, probability that the conclusion is incorrect.

Consideration of the role of chance should not be made in a vacuum. When one is addressing the issues of statistical significance and power, there is an underlying assumption that a study is free from both bias and confounding. To the extent that these assumptions may not be true in a

given situation, conclusions made on the basis of statistical considerations alone need to be tempered.

The committee began its evaluation presuming neither the existence nor the absence of association. It has sought to characterize and weigh the strengths and limitations of the available evidence. Subsequent chapters of the report summarize the evidence concerning each herbicide-health outcome relation under review and present the committee's conclusions. If the first question (Is there an association between herbicides, dioxin, or related compounds and a disease?) was answered affirmatively, and if available information permitted, the second question (If a relationship is assumed in any group of individuals, how likely is it that a relationship exists in Vietnam veterans?) was answered. The committee's task was not to judge individual cases of particular diseases or conditions, although, as described elsewhere in this report, the committee did learn of individual cases through public hearings and written testimony.

It should be noted that the committee's charge was neither to focus on questions of causation nor to focus on broader issues. Topics such as the potential costs of compensation for veterans afflicted with particular illnesses and policy regarding such compensation are not considered in this report. In addition, the committee makes no recommendations regarding individual cases. However, the report does provide scientific information for the Secretary of Veterans Affairs to consider in making determinations about compensation, but these decisions remain the responsibility of the Secretary. With this orientation to the committee's task and approach in mind, the following sections discuss the characteristics of the types of evidence that bear on the questions of association at hand.

CATEGORIES OF EVIDENCE

Experiments in Humans: Randomized Controlled Trials

Theoretically, the ideal method for assessment of causal relations (and thereby associations) between treatments and health outcomes is the randomized controlled trial because, when appropriate and feasible, it is the most scientifically rigorous method for testing such hypotheses. Randomized controlled trials are experiments in which subjects are randomly allocated, often in a masked fashion, into "treatment" and "control" groups, to receive or not to receive an intervention such as, in the present context, exposure to herbicides or dioxin; the control group receives an exposure to an inert substance (placebo) or an established alternative exposure; and both groups are followed in a strictly comparable manner to determine the relative frequencies of outcomes and diseases of interest. Although they are theoretically ideal, such trials are clearly not ethical or relevant in the case

of a potentially harmful exposure. Such experiments have not been done and thus they are not considered further.

Experiments in Animals: Animal Models

In principle, experimental studies in animals allow for both rigid control over herbicide or dioxin exposure and intensive observation of any health effect that may follow. If an animal model is to be considered valid for the study of a human disease, however, the manifestations of the disease should be similar in the two species. The starting point is generally what is currently known about the human disease. With respect to evaluation of dioxin in particular, and herbicides in general, the committee found a vast body of potentially relevant and somewhat controversial literature, which is discussed in Chapter 4. These animal studies helped the committee address one of its charges (i.e., the evaluation of biologic plausibility).

Controlled Epidemiologic Studies (Observational)

In contrast to randomized controlled trials and other experimental studies in humans, most epidemiologic investigations are "observational." This means simply that the occurrences of herbicide exposure and particular diseases are studied as they arise in the usual course of life and not under the conditions of a planned experiment.

Observational studies in populations are often "controlled," however, through various strategies of formal comparative investigation. For example, the experience of health outcomes in a group after exposure to dioxin can be compared with that in an unexposed control group. Alternatively, the prior dioxin exposure history of a group that has developed soft tissue sarcoma (STS) can be compared with that of a group free of this condition (unaffected control group). In these two strategies, the experience of the control or comparison group provides an estimate of the frequency either of disease in the absence of exposure or of exposure in the absence of the disease, as experienced in the general population. Although the contribution of the control group in such studies may seem analogous to that of the placebo group in a controlled trial, the analogy is a weak one. The lack of random assignment to "treatment" groups in observational (nonexperimental) studies makes the interpretation of such studies vastly more difficult than the interpretation of randomized clinical trials.

The most relevant types of such controlled, observational studies for the present review and their main characteristics are described in this section. Examples of studies related to herbicide or dioxin exposure and health outcomes serve for illustration.

Cohort Studies

Cohort studies track groups who are defined by common characteristics—including their exposure status, for example, Vietnam veteran or Vietnam era veteran—at the starting point of observation. The rates of occurrence of health outcomes are compared between these groups over time. All study participants are known or presumed to be free of the diseases under investigation at the start of the study. In the well-designed cohort study, reliable estimates of absolute disease rates in each group can be obtained. Especially for uncommon outcomes, such as cancer at specific sites (e.g., STS), large samples of participants, prolonged periods of observation, or both are required (Last, 1988). Such studies can provide evidence that bears on the first association question discussed earlier in this chapter. By dividing the rate in the exposed group by the rate in the nonexposed group, a measure of association termed the *relative risk* is derived, which provides a measure of the strength or magnitude of the association between an exposure and an outcome.

The starting point of the investigation can be either contemporaneous or in the past. In the first case, termed *concurrent cohort studies* , all observations, including both exposures and health outcomes, may be subject to direct observation by the investigator. In the second case, which typically depends on the availability of records of past exposures and health outcomes, the entire study may relate to experience prior to the start of the investigation. Such studies are termed *historical or retrospective cohort studies*. One potential limitation of these studies is the (lack of) completeness of records relating to the exposure of interest. Some features are common to both types of cohort studies, and others are distinct in accordance with their different temporal strategies.

Occasionally in cohort studies, especially in studies of occupational exposures, the investigators do not believe that a suitable unexposed cohort is available for comparison with the occupational cohort presumed to be exposed to a particular chemical or industrial process. In such cases, the comparison disease rates are usually drawn from a large external population, such as the population of males of a certain age in the United States. Using a technique termed "indirect adjustment" by epidemiologists, investigators calculate the number of cases of a particular disease that would be expected to occur in the observed cohort if that "exposed" cohort experienced the same disease rates as a matched group drawn from the comparison "unexposed" population. The expected number of cases is generally derived using age-, sex-, and calendar-year-specific rates of disease in the external population. The ratio of the actual observed number of cases to the expected number of cases is termed the standardized mortality (or morbidity) ratio, or SMR. The ratio is often, by convention, multiplied by 100 so

that a cohort in which the observed and expected numbers of cases are exactly equal would have an SMR of 100. An SMR greater than 100 would indicate an elevated risk of the disease in the exposed cohort. The committee has chosen to part with convention on the issue of the presentation of SMRs. In order to present the SMRs as measures of relative risk, the ratios have not been multiplied by 100 in this report.

A source of bias that is often a problem in studies with external comparison groups has been termed the "healthy worker effect" (Checkoway et al., 1989). The general population giving rise to the comparison rates contains people who are too ill to be members of the work force. Since exposed workers, by definition, have to have been healthy enough to be in the work force, they as a group may be healthier than the general population of the same age and sex. This bias would tend to dampen associations between exposure and disease. The bias is often especially evident when examining the SMR for chronic diseases such as heart disease or diabetes, but it is observed for cancers as well. The result of the healthy worker effect is to yield an underestimate of the true magnitude of association between exposure and disease. It is likely that the healthy worker effect applies to studies in which veterans are compared to nonveterans as well, and this possibility needs to be considered in interpreting some studies. The potential for this type of bias is far greater for studies of mortality than it is for studies of incidence. The healthy worker effect, like other biases, cannot be removed in the analysis of data but may be avoided with an appropriate study design. The comparison group should be selected to be similar to the study group in socioeconomic and employment status in order to avoid the healthy worker effect. Strictly speaking, the healthy worker effect is not limited to studies with external comparison groups—studies with internal comparison groups may be affected as well (Breslow and Day, 1987).

As an example of a historical cohort study of mortality (which, incidentally, is being continued as a prospective cohort study), the Air Force identified all members of Operation Ranch Hand (the "Ranch Hands")—the personnel involved in aerial herbicide spray operations in Vietnam during the period 1962-1971. The comparison group consisted of all Air Force veterans who concurrently were assigned to a variety of C-130 aircraft cargo units throughout Southeast Asia during the same period, but who were not occupationally exposed to herbicides (Michalek et al., 1990). This particular comparison group was chosen because of its large numbers and its similar training and psychological background to the Ranch Hands. It seems reasonable to imagine that health status at the time of enlistment was comparable in these two groups, so the healthy worker effect is not likely to introduce serious bias. There were 1,261 Ranch Hands who were compared

with 19,080 Air Force veterans, experiencing 91 and 1,241 deaths, respectively, as of December 31, 1989 (AFHS, 1991).

In the statistical analysis of the Ranch Hand mortality study, the investigators based results on the cumulative mortality [i.e., all deaths as of the end of 1989 (AFHS, 1991)]. The measure of increased risk used in this study was the SMR. The rates in the comparison group were used to generate the numbers of deaths that would be expected in the Ranch Hand group if the rates for the Ranch Hands were the same as those in the comparison group. This analysis should have been done by using actual rates and relative risks. Instead, the SMR was used because in the words of their report, it "appropriately treats the comparison population death rates as fixed rather than as unknown parameters. …" In other words, the sampling variability in the comparison group was ignored. The SMRs calculated were adjusted for differences between the groups in age and calendar year of observation, rank (officer versus enlisted), and occupation (flyer versus nonflyer).

The attempt to investigate STS in the study of Michalek and colleagues (1990) illustrates another feature of cohort studies generally: the fact that especially rare conditions may not be detectable within the limits of sample size and duration that characterize many such studies. The average annual age-adjusted incidence rate of STS for white males, cited by Kang and colleagues (1987), is 3.8 per 100,000. Thus, in a given year, slightly less than four cases of STS for every 100,000 people would be expected to be diagnosed. Even if the nearly 20 year period of observation for some individuals in the Ranch Hand study is considered, not even one case of STS would be expected among the 1,261 Ranch Hands. Because the outcomes in question are generally rare, the case-comparison or case-control design has more often been used in the investigation of health outcomes considered in this report.

Despite the rarity of many of the cancers considered by the committee, in a group of people as large as the group who served in Vietnam, *some* cases of rare diseases would be expected to occur even if herbicides used there had no deleterious effects. For this reason, in the sections of this report covering the basic epidemiology of various cancers, the committee has presented a calculation of the *approximate* number of cases of each disease that might occur in Vietnam veterans. Numerous assumptions are necessarily involved in such a computation, given the limitations of the available data. These assumptions, the limitations of the data, and the methods used for performing the calculations are explained in Chapter 8.

Another potential weakness of cohort studies is demonstrated by the study of Michalek and colleagues (1990). It is generally accepted that carcinogenesis can sometimes require a long period to produce clinically apparent disease: that is, there is sometimes a long period, known as the

latency period, between the exposure to even a known carcinogen and the appearance of clinical disease. Thus, for some cancers, exposure to the cancer-causing agent might not result in the appearance of disease for 5 to 20 years, or longer, depending on the disease. Conversely, cases of a particular cancer occurring in the cohort shortly after exposure are unlikely to be due to that exposure. This consideration is important in evaluation of the earlier studies of veterans, which may not have allowed sufficient latency for the development of disease to take place.

With respect to the issue of latency in the Ranch Hand study, consider a hypothetical subject. A Ranch Hand exposed to Agent Orange in 1969 at age 25, would be 45 years old in 1989, and 20 years would have elapsed since his exposure. This person would still be relatively young for STS to develop and might not have had enough time for an exposure-related cancer to develop. As another example, consider a possible association between exposure and prostate cancer. The rates for that disease are so low, given the relative youth of the Vietnam veterans' cohort (with respect to the age-specific rate of occurrence of prostate cancer), that there is virtually no possibility of observing an effect at this time.

Proportionate Mortality Studies

In some cohort studies, investigators have no accurate data on the composition of the cohort, but they do have access to sets of death records. The proportion of deaths due to each cause in a particular cohort is available, but not the actual mortality rates. This situation often leads to the conduct of a proportionate mortality study, in which comparisons are made between the proportions of deaths due to particular causes in the study cohort and in a presumably comparable cohort or in the general population. Occupational studies that utilize this comparison method often obtain information on causes of mortality from death certificates.

In analytical epidemiology, proportionate mortality studies are generally considered most valuable in the initial stages of an evaluation (Breslow and Day, 1987). They provide an inexpensive and rapid way of taking an early look at a set of data. Results of proportionate mortality studies must be interpreted carefully, since a proportionate excess can reflect either an excess in the absolute rate for the disease in question or a deficit in the absolute rates for some of the other causes. Large proportionate excesses, however, are unlikely to be produced in that way. Although a proportionate mortality study is usually considered a type of retrospective cohort study, it is convenient to consider the proportionate mortality study as equivalent to a case-control study in which the cases have died from the cause of interest and the controls are selected from deaths from all other causes (Breslow and Day, 1987; Miettinen and Wang, 1981).

The basic strategy in a proportionate mortality study is to compare the proportion of deaths due to a particular cause with the corresponding proportion in a (usually large) reference group. The ratio of the cause-specific proportion of deaths in the observed cohort to the proportion of deaths from that same cause in the reference population is known as the proportionate mortality ratio, or PMR. Since cause-specific death rates, and thus the proportions of deaths for different causes, are known to depend on age, the expected number of deaths from a particular cause is usually calculated on an age-specific basis and then summed across ages (Checkoway et al., 1989).

A major potential source of bias in proportionate mortality studies is the fact that some other causes of death may also be affected by the exposure, thus reducing the strength of the association between exposure and the cause of interest. It has been suggested, therefore, that one should exclude from the analysis causes of death that are also related to the exposure in question. If a chemical is thought to cause lung cancer, for example, lung cancer deaths should be excluded from a proportionate mortality study of the association between that chemical and other cancers. An extreme example would be an exposure that elevates the risk of every cause of death. In such a situation, none of the proportionate mortality rates might appear unusual, although all absolute mortality rates might be elevated relative to an unexposed group. The most appropriate analysis of proportional mortality data is to treat them as though they represent a case-control study in which the subjects who died of the cause of interest are the cases and those who died from other causes are the controls (Breslow and Day, 1987; Miettinen and Wang, 1981). The usual analytic methods for case-control studies (i.e., estimation of odds ratios) would then be applied.

An example of a PMR study is provided by the study by Bullman and colleagues (1990) of Army Vietnam veterans who served in Military Region I. This group, known as the I Corps, was believed for various reasons to have a relatively high potential for exposure to herbicides. Proportionate mortality in the I Corps was compared against the experience of 27,917 Army Vietnam era veterans who served in the military between 1965 and 1973, and had died as of the end of 1984. The principal finding was an excess of deaths from motor vehicle accidents. The study was analyzed by using proportionate mortality ratios, not as a case-control study. There was no excess mortality from either non-Hodgkin's lymphoma (NHL) or STS; however, there were only 10 deaths from STS compared with 11.4 expected. If the methods suggested above were applied, veterans who died of NHL and STS would be compared to the others who died to see if the NHL and STS cases were more likely to have been in I Corps. The question of latency is addressed somewhat in the analysis of this study, but there were very few expected deaths from NHL and STS in the longest latency (16+ years) category.

Case-Control Studies

Controlled epidemiologic studies in which the subjects are selected on the basis of their disease status (e.g., with or without STS) and investigated to determine their prior histories of exposure, are termed *case-control studies*. Unlike cohort studies, case-control studies do not provide direct estimates of health outcome rates in the study groups because the groups are defined by the presence or absence of disease, and the cases are not ordinarily drawn from a defined population in a way that permits calculation of disease rates. Investigators generally determine the ratio of controls to cases in the study, thus making it impossible to calculate disease rates directly.

Instead, the result of the case-control study is expressed as the ratio of the odds of having been exposed as a member of the case group versus the odds of having been exposed as a member of the control or comparison group. When the cases and controls are defined and selected properly, the odds ratio may be thought of as an estimate of relative risk. When the disease of interest is uncommon, which is true for many of the diseases suspected of being related to herbicide exposure, the odds ratio will generally provide a reasonable estimate of the relative risk (Breslow and Day, 1980). As such, the *odds ratio* is a measure of association that can contribute to answering whether exposure is associated with the disease under consideration. If there is no association between exposure status and the disease, the expected odds ratio is 1. If, on the other hand, the risk of the disease is higher in the exposed group, even though the risk cannot be observed directly, the expected odds ratio is greater than 1. Because this strategy of investigation begins with the identification of cases, such as those in existing hospital or other medical records, it is not dependent, as is the cohort study, on the gradual accumulation of sufficient numbers of rare cases for analysis. Therefore, results can often be obtained in much less time and at a lower cost than they can with the cohort approach.

As examples, consider the Selected Cancers Study (SCS) performed by the Centers for Disease Control (CDC, 1990a-c). In a study of non-Hodgkin's lymphoma, cases were defined as all men first diagnosed between December 1, 1984, and November 30, 1988. The cases lived in the geographic regions covered by population-based cancer registries for five metropolitan areas. The ages were restricted to those individuals born between 1929 and 1953 who would have been eligible for service in Vietnam (ages 15-39 years in 1968).

In the Selected Cancers Study, the primary exposure variable was defined as military service in Vietnam or off the coast of Vietnam. The "exposed" group is thus likely to contain many individuals who were not actually exposed to herbicides. The interpretation of a finding of no elevated risk for STS, for example, is therefore problematic. On the other

hand, an elevated odds ratio for NHL, for example, could be a result of some form of bias (see below) and might really represent no underlying elevation of risk due to herbicides or, in this case, due to military service. If the Selected Cancers Study was otherwise free of bias, an elevated odds ratio could theoretically represent an underestimate of the true odds ratio, if the only major problem were the misclassification of exposure status.

A key concern in interpreting the results of case-control studies such as the SCS is the potential for bias in the selection of cases and controls. Evaluation of an association depends on having valid estimates of the exposure frequencies in both the case and the comparison groups. An inappropriate case or control group may seriously bias the odds ratio. Several other important types of bias relate to the information about exposure collected from cases and controls. These sources of bias are discussed in Chapter 6 with exposure assessment issues. It is helpful to note, at this point, that these types of bias may exist in *all* populations, not just in veterans. Furthermore, bias is a result of a problem with the study design and not the study participants.

Case Reports and Case Series

The medical literature frequently contains reports of an individual or groups of individuals who have experienced a particular health problem and have also been exposed to some substance in the environment that is thought (by the exposed individual or his or her physician) to be responsible for the health problem in question. Information of this sort is also frequently available in individuals' health care records. Individual medical records have been presented to the committee, as were summaries of large numbers of individual cases. Thousands of such individual reports are available to the Department of Veterans Affairs (formerly the Veterans Administration) in the form of claims for compensation.

In some circumstances, such case reports can provide valuable information about an association between an environmental exposure and a particular health outcome. This is most likely when (1) the health outcome in question is both unusual and relatively specific for the exposure in question (i.e., the health outcome is unlikely to be the result of other exposures or causes); and (2) there is a close temporal relationship between the exposure and the health outcome (i.e., the outcome becomes apparent soon after, and not before, the exposure) (Kramer and Lane, 1992). Neither of these conditions is true for outcomes reported to the committee. Most of the outcomes thought to be associated with herbicide exposure did not become apparent until years or decades after the veteran returned from Vietnam. Most are relatively common outcomes in a cohort of individuals who are now generally 40 years of age or older. Although this does not mean that the outcomes

are not caused by herbicides, cases of this sort offer no positive evidence for a statistical association and were not considered by the committee except in special instances. In many of these reports, a physician has stated that the disease was caused by Agent Orange. The committee believes that such a determination is usually not possible to make in individual cases, and that statements of this sort reflect judgments based in part on the epidemiologic evidence that is the major subject of this report. Thus, since the purpose of this report is to evaluate the epidemiologic evidence, including data from individual cases would be circular reasoning.

Case series, that is, compilations of the range of diseases experienced by a group of individuals who report such problems to a central collection point, offer more promise for detecting an association between an exposure and a disease, but again only in special circumstances. In particular, if a group of individuals exposed to herbicides was diagnosed with a unique pattern of diseases and symptoms (common to all in the group, but in a combination that is rare in others not exposed to the herbicide), this might be taken as evidence of an association or causal relationship. However, the case series of which the committee is aware (see Appendix B) exhibit a wide range of health outcomes that are not at all specific to the group. If no unique pattern emerges, it must be shown that some outcomes are more common in the exposed group than in a comparable nonexposed group. In other words, an epidemiologic study of some type discussed above is necessary. Reports of health outcomes in veterans that are adequately compared to an appropriate control group have been considered by the committee, but case series without an appropriate comparison group were not regarded by the committee as having evidentiary value.

Information from Death Certificates

Data used in many studies involving mortality experience are obtained from death certificates; these studies tend to be conducted among occupational groups, and medical records are not always reviewed. Death certificates can provide occupation as well as cause of death information; the occupation as listed is used by investigators as a surrogate for potential exposures under investigation. However, there are methodologic concerns that need to be considered when dealing with death certificates. If occupation is obtained from the death certificate, the job listed may be the decedent's final job or *usual* job. When occupation is used as a surrogate for potential exposures, the usual or final job listed may not account for other jobs that involve the exposure of interest, especially if the possible exposure was years or decades before the time of death. Also, next of kin or family members may not be aware of the usual job held by the decedent. In most cases, "exposure" is underestimated if based on death certificate

information, and the magnitude of the association under consideration is attenuated.

Initial completion of death certificates for cause of death information may also lead to over- or underascertainment of the causes of interest. This is especially true for cancers that are difficult to diagnose and classify, such as STS (Sinks, 1993). The accuracy of cause of death data from death certificates also depends on a number of factors, such as whether an autopsy has been performed, the age of the deceased, whether the physician filling in the death certificate knows the deceased, the recording physician's attention to detail, avoidance of reporting a cause of death if "stigma" is attached, incorrect coding of disease, and variations in the quality of medical care in regions or over time. If death occurred by accidental means, underlying disease may or may not be evident and recorded. Thus, among a cohort of exposed or potentially exposed individuals, the effect of underascertainment of disease is most likely to bias the magnitude of the association under investigation toward the null.

Integration of Collective Results

Inferences concerning association are commonly made on the basis of epidemiologic and related biomedical evidence. Many policy decisions and practical actions are based on such inferences made by persons with widely varied backgrounds—professional and nonprofessional, technical and nontechnical. The process of reaching such conclusions is ordinarily personal and often private. By contrast, when this process is conducted formally in the manner of the present review, it is collective and interactive. As indicated earlier in this chapter, it is also desirable that reasoning be made explicit, in order that others may be enabled to evaluate the committee's conclusions independently.

Two aspects of the integration of evidence used by the committee are explained here. First is the quantitative approach of meta-analysis, whose principles and methods are discussed briefly below. A more complete discussion of the application of meta-analytic techniques to the evaluation of epidemiologic questions has recently been published (Dickersin and Berlin, 1992). In those instances in which it was deemed appropriate, this approach was applied and the results are presented in the corresponding section below. The quantitative meta-analyses were viewed by the committee only as supporting the more qualitative conclusions drawn from the second aspect. No decisions as to the adequacy of evidence favoring or not favoring a positive association were made solely on the basis of a quantitative meta-analytic result. Nevertheless, the fact that, in several instances, the meta-analysis was consistent with the qualitative conclusion, served to reinforce that conclusion.

The second aspect is the partially quantitative, but largely qualitative, process based on what is sometimes termed "causal inference," for which a general approach has long been recognized in the epidemiologic literature. Although the committee's primary charge was not the strict evaluation of causality, it was felt that considerations applied to causal inference were relevant to evaluation of the strength of the scientific evidence. This is also discussed below, to indicate the committee's view of that approach and its application to the present evaluation.

Meta-Analysis

A review of the major studies on which this report is based suggests that the sample sizes of many studies are insufficient to detect important excess risks. As can be seen in Chapter 8, for instance, a number of the studies on cancer describe so few cases that large relative risks cannot be ruled out.

When a number of sufficiently similar studies of the same health outcome are available, it is sometimes possible to pool statistical information from the studies to develop an estimate of the relative risk, or odds ratio, of the outcome in question that is more precise than estimates from individual studies. An important consideration in the decision as to whether a meta-analysis is appropriate, is the degree of similarity of the component studies with respect to design features. Studies with vastly different definitions or levels of exposure might not be considered similar enough to be combined. On the other hand, even studies that appear to be designed similarly can often produce vastly different estimates of the relative risk. In this setting, meta-analysis can be used to explain the differences among study results on the basis of possibly subtle features of design, study populations, or statistical analysis.

The committee found a high degree of variability among epidemiologic studies with regard to the nature and extent of exposure to herbicides and dioxin, the specific health outcomes studied, and study design. After considering the studies available, the committee judged that data adequate for a formal meta-analysis were available only for several small groups of studies on particular cancers. The degree of variability (heterogeneity) of relative risks or odds ratios was tested statistically in all instances, and pooled estimates were obtained as weighted combinations of the individual estimates using the method developed by DerSimonian and Laird (1986).

Considerations in Assessing the Strength of Scientific Evidence

For each health outcome for which evidence indicated the presence of an association with herbicides or a related exposure, the committee assessed the applicability of each of six general considerations, patterned after those

attributed to Hill (1971). These criteria were originally proposed in the context of causal inference in chronic disease epidemiology. Strictly speaking, assessing causality was not the charge of this committee. However, in a more general sense, the criteria helped the committee evaluate the strength of the scientific evidence for or against associations between disease and herbicide exposure. Reflecting epidemiologic thought that had evolved over many years, Hill proposed the following guidelines for judgment: strength of association, dose-response relations, temporally correct association, consistency of association, specificity of association, and biologic plausibility. These considerations were applied, where possible, to aid interpretation in both directions.

Three of these considerations (strength of association, dose-response relation, and temporally correct association) can be applied to the findings of single studies and can therefore be regarded, in part, as measures of internal validity of the study design. Any of these considerations can be satisfied in some, but not necessarily all, studies testing a particular causal hypothesis. The other three considerations (consistency of association, specificity of association, and biologic plausibility) are not necessarily study specific and depend to varying degrees on prior knowledge.

Strength of Association

Strength of association is usually expressed in epidemiologic studies as the magnitude of the measure of effect, for example, relative risk or odds ratio. Generally, the higher the relative risk, the greater is the likelihood that the exposure-disease association is causal or, in other words, the less likely it is to be due to undetected error, bias, or confounding. Measures of statistical significance such as *p*-values are not indicators of the strength of association. Small increases in relative risk that are consistent across a number of studies, however, may also provide evidence of an association (see "Consistency of Association," below).

Dose-Response Relation

The existence of a dose-response relation—that is, an increased strength of association with increasing intensity or duration of exposure or other appropriate relation—strengthens an inference that an association is real. Conversely, the lack of an apparent dose-response relation does not rule out an association, as in the case of a threshold level of exposure beyond which the relative risk of disease remains constant and highly elevated. If the *relative* degree of exposure among several studies can be determined, indirect evidence of a dose-response relation may exist. For example, if studies of presumably low-exposure cohorts show only mild elevations in risk, whereas

studies of presumably high-exposure cohorts show more extreme elevations in risk, such a pattern would be consistent with a dose-response relation.

Temporally Correct Association

If an observed association is real, exposure must precede the onset of the disease by at least the duration of disease induction. The committee, in addition, considered whether the disease occurred within a time interval following herbicide exposure that was consistent with current understanding of its natural history. The committee interpreted the lack of an appropriate time sequence as evidence against association, but recognized that insufficient knowledge about the natural history and pathogenesis of many of the diseases under review limited the utility of this consideration.

Consistency of Association

Consistency of association requires that an association be found regularly in a variety of studies, for example, in more than one study population and with different study methods. The committee considered findings consistent across different categories of studies as being supportive of an association. Note that the committee did not interpret "consistency" to mean that one should expect to see exactly the same magnitude of association in different populations. Rather, consistency of a positive association was taken to mean that the results of most studies were positive and that the differences in measured effects were within the range expected on the basis of all types of error including sampling, selection bias, misclassification, confounding, and differences in actual exposure levels.

Specificity of Association

Specificity of association is the degree to which a given exposure predicts the frequency or magnitude of a particular outcome; if the association between the exposure and the health outcome is unique to both, a positive finding seems more strongly justified than when the association is nonspecific to both the exposure and the health outcome. The committee recognized, however, that perfect specificity could not be expected given the multifactorial etiology of many of the diseases under examination. In addition, the committee recognized the possibility that herbicides (or, more specifically, dioxin) might be associated with a broad spectrum of diseases.

Biologic Plausibility

Biologic plausibility is based on whether a possible causal association fits *existing* biologic or medical knowledge. Chapter 4 lays out the basic

scientific and animal evidence that the committee used to assess the biologic plausibility of an association. The existence of a possible mechanism, such as established carcinogenic potential based on animal studies (whether at the same tumor site as in humans or not), or known capacity to affect DNA, was thought to increase the likelihood that the exposure-disease association in a particular study reflects a true association. In addition, the committee considered factors such as (1) evidence in humans of an association between the exposure in question and diseases known to have similar causal mechanisms as the one in question; (2) evidence that certain outcomes (usually particular types of cancer) are commonly associated with occupational or environmental chemical exposures; and (3) knowledge of routes of exposure, storage in the body, and excretion that would suggest that some organs rather than others might be affected. Given the limitations of existing biological or medical knowledge, however, lack of *specific* biologic support for a given health outcome did not rule out a conclusion of sufficient evidence.

Other Considerations

As noted above, it is important also to consider whether alternative explanations—error, bias, confounding, or chance—might account for the finding of an association. If an association could be sufficiently explained by one or more of these alternate considerations, there would be no need to invoke the several considerations listed above. Because these alternative explanations can rarely be excluded sufficiently, however, assessment of the applicable considerations listed above almost invariably remains appropriate. The final judgment is then a balance between the strength of support for the association and the degree of exclusion of alternatives.

The Role of Studies of Occupational and Environmental Exposures

Because the issues surrounding the measurement of actual exposure to herbicides in Vietnam are so complex, the committee also evaluated studies of environmental and occupational exposures. In many cases, the classification of exposure status in such studies was thought to be valid and well documented or assessed, particularly relative to such classifications in the studies of veterans. The improved assessment of exposure would lead to less bias. In addition, the actual levels of exposure to dioxin or related compounds in the occupational and environmental studies were generally higher than the exposure levels in many veterans and, in fact, were high in absolute terms, as well. Higher exposure levels would lead to an increased ability of occupational and environmental studies to detect effects of exposure. Primarily for these reasons, occupational and environmental studies

were taken under serious consideration in addressing the question of whether the compounds in question are associated with specific diseases.

NATURE OF THE CONCLUSIONS

This chapter has demonstrated that judgments about the possible association between health outcomes and exposure to herbicides, or related dioxin compounds, reflect both quantitative and qualitative reasoning. Some final observations will help to clarify the nature of the committee's conclusions.

Quantification

Resolution

Resolution refers to the fineness or sharpness of detail that can be discriminated by a particular mode of observation. In light microscopy, for example, observations are described by reference to the optical properties of the lens, such as 10×, 100×, or higher magnification. Electron microscopy, with very much higher resolution, distinguishes structural features not detectable with light microscopy.

Resolution in epidemiologic studies concerns the capacity of a study to discriminate the frequencies of health outcomes or exposures between groups in order to determine the presence or absence of associations. By analogy, resolution in epidemiology also depends in a sense on magnification, that is, on the order of magnitude of the numbers of participants—for example, from tens to hundreds of cases and controls in case-control studies, and from hundreds to thousands of exposed and unexposed subjects in cohort studies. With equally valid observations, results based on the experience of increasing numbers of persons, from single individuals to tens, hundreds, or thousands of individuals, provide successively greater resolution. Because of differences in the intrinsic nature of their designs, case-control studies generally require far fewer subjects than cohort studies do, for an equivalent degree of resolution. However, the principle still holds that increasing numbers of people, within a given type of study design, provide increasing resolution.

The resolution or discriminating capacity of epidemiologic studies could theoretically be increased indefinitely through ever larger study populations. However, there are many constraints on the feasibility of large studies. Rarity of exposures or events, or other circumstances, may limit the resolution even of large studies. For example, NHL and STS are both very rare, and workers showing increased rates of these diseases are likely to have been exposed to large amounts of dioxin. Such high exposures are not

common. Meta-analysis can, under the appropriate circumstances discussed above, be used to offset the limited size of individual studies, but the collective magnitude of the contributing studies may still be lower than desired. It should be emphasized that in all such studies the potential for bias is a key problem, and that enlarging the study, or combining the results of several studies in a formal meta-analysis, reduces only random error, not systematic error. Therefore, if bias is present, a firmer but still erroneous conclusion will result from a larger study (or meta-analysis) than from a smaller one.

Power calculations indicate the probability of achieving discrimination of a predetermined degree under the design of a given study. Power is thus a quantitative measure of the capacity of a study to achieve a given degree of resolution. In particular, it provides guidance against overconfidence in the absence of an association when a study with relatively low power has failed to demonstrate one. As such, they help reviewers appreciate the nature of the evidence about association.

As discussed earlier in this chapter, two types of error must be taken into account in designing and interpreting statistical tests. Epidemiologic studies are often designed to provide statistical tests that minimize *type I error*, the probability that the null hypothesis of no association is falsely rejected. Commonly, such tests are designed so that there is less than a 5 percent chance that the test will incorrectly indicate an association between a chemical exposure and a disease if no association truly exists. On the other hand, for any given test and sample size, there is some chance that the test will err in failing to find an association when one truly exists. This is called a *type II error*. The chance of making such an error increases when both the true excess risk and the sample size are small. From another perspective, given a particular sample size and a specified probability of a type I error, one can calculate the power of a test to detect an assumed association of a given magnitude. Because the power of a test is the opposite (technically, the complement) of the probability of making a type II error, the power of a test increases when both the true excess risk and the sample size are large.

For example, in CDC's Selected Cancers Study (1990b), the lack of a significant statistical association between soft tissue sarcoma and the Vietnam experience may reflect the small sample size (310 cases) rather than a true absence of association. In other words, if this investigation were replicated with more cases of STS than occurred in the CDC study, a statistically significant difference might be detected if there truly was an association.

Power calculations are also valuable in interpreting apparently conflicting results of multiple studies of the same exposure-disease combination. If findings of no association were concentrated in the low-power studies, for instance, the suggestion that no association exists would be weakened.

Uncertainty and Confidence

All science is characterized by uncertainty. Scientific conclusions concerning the result of a particular analysis or set of analyses can range from highly uncertain to highly confident. As discussed earlier in this chapter, the theoretical concept of proof does not apply in evaluating actual observations. In its review, the committee attempted to assess the degree of uncertainty associated with the results on which it had to base its conclusions.

For individual studies, confidence intervals around estimated results such as relative risks represent a quantitative measure of uncertainty. Confidence intervals present a range of results that, with a predetermined level of certainty, is consistent with the observed data. The confidence interval, in other words, presents a statistically plausible range of possible values for the true relative risk. When it is possible to use meta-analysis to combine the results of different studies, a combined estimate of the relative risk and confidence interval may be obtained.

For an overall judgment about an association between an exposure and a disease based on a whole-body of evidence, beyond the results of single studies or of meta-analyses, no quantitative method exists to characterize the uncertainty of the conclusions. Thus, to assess the appropriate level of confidence to be placed in the ultimate conclusions, it may be useful to consider qualitative as well as quantitative aspects.

Qualitative Aspects of the Review Process

Comprehensiveness

An important aspect of the quality of a review such as the present one is comprehensiveness—to ensure against the possibility of any serious omission or inappropriate exclusion of evidence from consideration. If any such omission should be identified, a determination would be needed of whether its inclusion would likely affect the overall results and, if so, in what way.

In this report, the committee has documented in detail its approach to seeking and identifying the evidence to be reviewed (see Appendices A and B). Numerous parties were invited to supplement the materials already under review and to notify the committee of any recognized omissions of importance.

The phenomenon known as publication bias was also of concern to the committee. It has been well documented (Begg and Berlin, 1989; Berlin et al., 1989; Dickersin, 1990; Easterbrook et al., 1991; Dickersin et al., 1992) in biomedical research that studies with a statistically significant finding are more likely to be published than studies with nonsignificant results. Thus, evaluations of disease-exposure associations based solely on published

literature could be biased in favor of showing a positive association. Interestingly, this bias seems to be generated by the potential authors of papers, rather than by the editors of journals (i.e., it is the authors who choose not to submit papers). In two studies (Dickersin, 1990; Easterbrook et al., 1991), the likelihood of publication seemed to depend on the author's perception of the importance of the work, and this perception was related to the statistical significance of the findings. The committee did not consider the risk of publication bias to be high among studies of herbicide exposure and health risks for several reasons:

1. there were numerous published studies showing no positive association,
2. the committee did examine a fair amount of unpublished material, and
3. the committee felt that the publicity surrounding the issue of exposure to herbicides, particularly regarding Vietnam veterans, has been so intense that any studies showing no association would be unlikely to be viewed as unimportant by the investigators. In short, the pressure to publish such "negative" findings would be extreme.

Neutrality

To ensure a fair weighing of all the evidence, neutrality is another important consideration in the quality of conclusions such as those presented by the committee. In this connection, the committee avoided the posture of the burden of proof approach, as discussed earlier in this chapter. The essential evidence, its main strengths and limitations, and the conclusions that follow are stated for each health outcome considered.

Judgment

The evaluation of evidence to reach conclusions about statistical associations goes beyond quantitative procedures, at several stages: assessing the relevance and validity of individual reports; deciding on the possible influence of error, bias, or confounding on the reported results; integrating the overall evidence, within and across diverse areas of research; and formulating the conclusions themselves. These aspects of the review required thoughtful consideration of alternative approaches at several points. They could not be accomplished by adherence to a prescribed formula.

Rather, the approach described here evolved throughout the process of review and was determined in important respects by the nature of the evidence, exposures, and health outcomes at issue. Both the quantitative and the qualitative aspects of the process that could be made explicit were

important to the overall review. Ultimately, the conclusions expressed in this report about causation are based on the committee's collective judgment. The committee endeavored to express its judgments as clearly and precisely as the available data allowed.

SUMMARY OF THE EVIDENCE

The committee's specific mandate was to determine, if possible,

1. whether there is a statistical association between the suspect diseases and herbicide use, by taking into account the strength of the scientific evidence and the appropriateness of the methods used to detect the association;
2. the increased risk of disease among individuals exposed to herbicides during service in Vietnam; and
3. whether there is a plausible biologic mechanism or other evidence of a causal relationship between herbicide exposure and a disease.

The committee addressed the first part of this charge by categorizing each of the health outcomes under study into one of the four categories described below on the basis of the epidemiologic evidence that it reviewed. Considerations of biologic plausibility did not enter into the committee's decision about how to categorize these outcomes, but plausibility is discussed separately after the assessment of the epidemiologic evidence. The question of increased risk in Vietnam veterans is also addressed for each health outcome, subject to the considerations discussed below.

Categories of Association

The categories used by the committee were adapted from those used by the International Agency for Research on Cancer in evaluating the evidence for carcinogenicity of various agents (IARC, 1977). Consistent with the charge to the Secretary of Veterans Affairs in P.L. 102-4 (which is stated in terms of statistical association rather than causality) the distinctions between the categories are based on "statistical association," not on causality as is common in scientific reviews. The distinctions reflect the committee's judgment that a statistical association would be found in a large, well-designed epidemiologic study of the outcome in question in which exposure to herbicides or dioxin was sufficiently high, well-characterized, and appropriately measured on an individual basis.

- *Sufficient Evidence of an Association*: Evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between herbicides and the outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.

For example, if several small studies that are free from bias and confounding show an association that is consistent in magnitude and direction, there may be sufficient evidence for an association.

- *Limited/Suggestive Evidence of an Association*: Evidence is suggestive of an association between herbicides and the outcome but is limited because chance, bias, and confounding could not be ruled out with confidence. For example, at least one high-quality study shows a positive association, but the results of other studies are inconsistent.
- *Inadequate/Insufficient Evidence to Determine Whether an Association Exists*: The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association. For example, studies fail to control for confounding, have inadequate exposure assessment, or fail to address latency.
- *Limited/Suggestive Evidence of No Association*: There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, that are mutually consistent in not showing a positive association between exposure to herbicides and the outcome at any level of exposure. A conclusion of "no association" is inevitably limited to the conditions, level of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.

Increased Risk in Vietnam Veterans

The categories related to the association between exposure to chemicals and health outcomes, not to the likelihood that any individual's health problem is associated with or caused by the herbicides in question. As stated early in this chapter, the most desirable evidence as a basis for answering this type of question involves knowledge of the rate of occurrence of the event in those Vietnam veterans who were actually exposed to herbicides, the rate in those who were not exposed (the "background" rate of the event in the population of Vietnam veterans), and the degree to which any other differences between exposed and unexposed groups of veterans influence the difference in rates. When those Vietnam veterans who are actually exposed have not been properly identified, as has generally been the case in existing studies, this question becomes difficult to answer. Although there have been numerous health studies of American and other Vietnam veterans, most have been hampered by relatively poor measures of exposure to herbicides and/or dioxin and other methodological problems. Indeed, most of the evidence on which the findings in this report are based comes from studies of people exposed to dioxin or herbicides in occupational and environmental settings rather than from studies of Vietnam veterans.

The committee found the available evidence sufficient for drawing conclusions

about association between herbicides and health outcomes, but the lack of good data on Vietnam veterans per se, especially with regard to exposure, complicates the second part of the committee's charge, to determine the increased risk of disease among individuals exposed to the herbicides during service in Vietnam. By considering the magnitude of the association observed in other cohorts, the quality and results of the existing studies of veterans related to a particular outcome, and other principles of epidemiologic research discussed above, the committee formulated a qualitative judgment regarding the second question.

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6

Exposure Assessment

Assessment of individual exposure to herbicides and 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD) or other chemical compounds found in the herbicides used in Vietnam is a key element in determining whether specific health outcomes are associated with exposure to these compounds. For a variety of reasons, the committee placed particularly heavy emphasis on exposure assessment issues.

First, the committee's primary task is to review and evaluate the scientific literature to determine, if possible, whether there is a statistical association between various health effects and herbicide use, taking into consideration the strength of the scientific evidence and the appropriateness of the methods used to detect the association. Estimation of health risks associated with herbicide exposure consists of two primary activities: (1) exposure assessment and (2) assessment of the health effects in exposed individuals. The committee has found in its review that the weakest methodologic aspects complicating the interpretation of the available epidemiologic studies are the definition and quantification of exposure. This chapter describes the criteria used by the committee in assessing the quality and validity of exposure measures. It begins with a description of the role of exposure assessment in epidemiology, followed by a discussion and evaluation of the various approaches that have been used to measure exposure in studies of those occupationally and environmentally exposed to herbicides and TCDD. The next section describes exposure assessment in studies of Vietnam veterans and some of the problems of inaccurate exposure measurement in these studies.

Second, an additional task brought to this committee through its legislative charge was the determination of whether additional studies of Vietnam veterans are feasible. Drawing upon the committee's evaluation of the available literature and upon information on the military use of herbicides (see Chapter 3), this chapter summarizes what is known about exposure to herbicides in Vietnam in comparison to other populations with widely different types of exposure (e.g., in factories, of professional herbicide sprayers, from environmental accidents). Valid measures of exposure are critical to further epidemiologic studies, and this chapter proposes a method for developing such a measure for future studies of Vietnam veterans.

A third and related reason for the committee's concern about exposure assessment in epidemiologic studies is that these data are needed to draw inferences on the health effects of exposure in Vietnam veterans from studies of those occupationally and environmentally exposed. Studies of these other groups address the issue of whether herbicides are associated with particular health outcomes, but they have only an indirect bearing on the question of associations in veterans themselves. Exposure data in all groups are needed to translate the results of occupational and environmental studies to estimates of increased risk for Vietnam veterans.

AN OVERVIEW OF EXPOSURE ASSESSMENT FOR EPIDEMIOLOGY

When epidemiologists assess the potential health risks of exposure to a toxic chemical, they compare the disease experience of groups of people with different levels of exposure to the substance of interest. Accurate risk estimates depend on the ability to accurately identify those who are "exposed" and those who are not. When the concern is with low-level, possibly intermittent exposure to a chemical such as an herbicide, it becomes important not simply to assess exposure as its presence or absence, but to characterize the *degree* of exposure—its *intensity* and *duration*. At root there are three essential steps in epidemiology:

1. assembling a cohort of people with similar, well-defined exposures to some agent, and another cohort identical to the first but with members who lack exposure to the agent;
2. measuring and then comparing the disease experience of each of these cohorts; and,
3. drawing inferences from these comparisons about the risks that may derive from the exposure differences between cohorts.

Exposure assessment contributes to the epidemiologic study process in several ways. First, accurate measures of exposure are essential to a study's *validity* because if there is not a well-defined contrast in exposure among

the two (or more) groups being studied, then the results cannot be considered meaningful with respect to a judgment about the potential risk to an exposed individual or group. Second, very large groups must be studied in order to identify small risks, and conversely a relatively small study may be able to detect the effect of heavy or sustained exposure to a toxic substance. In this way, a study's *precision* is also linked to the extent of the exposure involved and the accuracy of its measurement.

The strength of an association between an exposure and a disease is just one of the criteria used in evaluating epidemiologic evidence, as described in Chapter 5. Another criterion often used in evaluating an association is whether or not there is evidence that as exposure increases, the risk of the disease also increases (Hill, 1971). This dose-response pattern can be detected only if there is some way to determine the degree of exposure among different cohorts or subcohorts of the study. Inaccurate assessment of exposure can obscure the existence of such a trend and thus make it less likely that a true risk will be identified as such. A recent review, for instance, has found evidence that studies of putative occupational carcinogens are more likely to identify these agents successfully when quantitative exposure data are used, instead of relying on qualitative categories or simply the duration of exposure (Blair and Stewart, 1992).

Once a given exposure-disease association has been established, it is often desirable to consider the implications of this risk for some exposed population other than the population with which the study was performed. In making this inference, it is critical to have accurate exposure assessments. If there is a risk of a certain disease in workers occupationally exposed to a herbicide, what would the risk be for a Vietnam veteran who was exposed only occasionally or for just a short period? The proper scale on which to compare these risks is the scale of quantitative exposure.

It is useful to view exposure to an environmental agent as a process involving a progression of events that links the chemical in the environment to the ultimate "target" tissue in the human body (Figure 6-1). It is particularly important to distinguish "exposure" from "dose"—words that are often used interchangeably but have quite different meanings. *Exposure* is the concentration of an agent in the environment in close proximity to a study subject. Depending on a variety of factors, some of this toxin may be taken up by the subject through inhalation, ingestion, or the skin. These *routes of exposure* then mediate uptake and, along with metabolic characteristics of the subject, determine the *dose* of the agent that reaches the *target tissues* in the body. With regard to Vietnam veterans, for instance, it has been suggested that troops who spent days in the field without the possibility of good personal hygiene may have received a larger effective dose from a given exposure. Although it is the toxic dose and not the exposure per se that causes a disease, this dose is rarely measurable, and often, exposure

must be used as a proxy for dose. The assumption that exposure approximates dose is not necessarily a bad one, but how strong this correlation actually is depends on the chemical and physical properties of the agent, the route of exposure, and the target tissue.

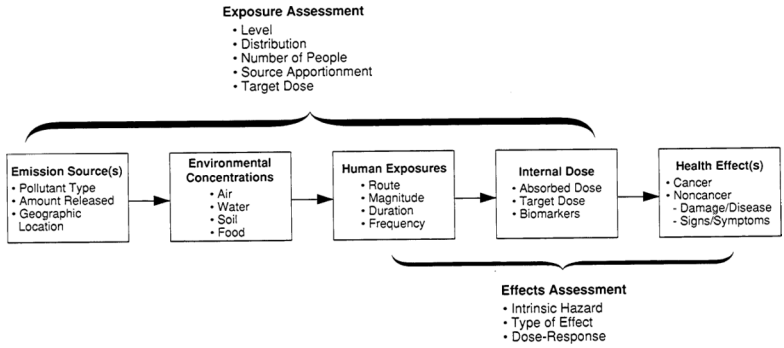

FIGURE 6-1 Progression of events from environmental exposure to health effects. Reprinted with permission from Heldref Publications.
SOURCE: Sexton et al., 1992.

Hierarchy of Exposure Assessment Strategies

Exposure has been characterized in many different ways in epidemiologic studies, depending on the availability of data and the hypothesis being tested. One can usefully distinguish a few basic approaches to exposure assessment (Checkoway, 1986; Smith, 1987). The simplest approach compares the members of a class presumably exposed to an agent with the general population or with an "unexposed" group. Occupational studies are often of this type, comparing for example, herbicide production workers to the general population. Vietnam veterans have also been compared to veterans who served during the Vietnam era but did not serve in Vietnam. The advantages of this approach are its simplicity and the ease of interpretation of the results. If there is something fundamental to class membership that increases risk—for example, all "woodworkers" are exposed to wood dust—then studies of this type can effectively identify the increased morbidity or mortality in the group. If, however, only a small fraction of class members are actually substantially exposed to a toxic agent (in all likelihood, as discussed in this chapter, only a fraction of the estimated 2.6 million to 3.8 million veterans who served in Vietnam were substantially exposed to herbicides), then any increased risk from exposure in this subgroup may be lost entirely when the disease risk of the full class (all Vietnam veterans) is assessed.

A somewhat more refined method of exposure assessment assigns to

each cohort member a qualitative degree or level of exposure. This may be done in several different ways. For example, a cohort of herbicide production workers could be divided into subgroups in such a way that those who were likely to have been heavily exposed through their job assignments are placed in one group (e.g., "high exposure"); a second group might be identified who had sustained exposures, but not in those jobs or departments in direct contact with the toxin ("moderate exposure"); and finally, a residual group might contain those with little or no exposure who were nonetheless employed at the production facility ("low exposure"). The disease risk may then be calculated separately for each of these groups compared to a referent or "unexposed" group. This method has several advantages over the simple exposed/unexposed comparison just described: it should (if the classification of exposure is done without serious errors, see below) yield less diluted risk estimates; provide support for a dose-response trend; and not necessarily require expensive and time-consuming measurements of the actual exposure of each cohort member.

Ideally, quantitative estimates should be available on the total exposure history of each subject in the study. When such data are available, it is possible to estimate quantitatively the relationship between a given level of exposure and the degree of risk that is expected to accrue. In occupational epidemiology studies, quantitative exposure data are sometimes developed through a process called *historic exposure reconstruction*, which is outlined in the following sections.

When quantitative estimates of the intensity of exposure are not available, it is sometimes possible to know the duration of exposure for each cohort member. Although less satisfactory, one may nevertheless assume that the intensity of exposure was relatively constant among cohort members so that the total exposure (sometimes called *cumulative exposure*) is proportional to its duration. Following these assumptions, one would hypothesize that a true risk would increase with the duration of exposure.

Epidemiologists generally think of these various exposure assessment strategies in a hierarchy of increasing accuracy: the exposed/unexposed approach being the least accurate, followed by the qualitative classification of level of exposure, and best of all, quantitative estimates of both the intensity and the duration of exposure. It is important to stress that all of these strategies may be *valid*, but they vary in their precision and in the degree to which they can contribute to the evidence for or against a particular exposure-disease association.

Exposure Assessment for Cohort Studies

In cohort studies it is sometimes possible to have other data sources available with which to estimate exposure for each study subject without

directly interviewing the subject. This is especially common in occupational cohort studies in which work records and industrial hygiene data may be available that cover the entire history of the factory being studied.

At minimum, it is often possible to know with considerable accuracy the length of time that each cohort member has spent in the industry. Somewhat more precise assessments may be possible if the cohort can be subdivided into those who were employed in one or more areas of the plant where the exposure of interest was heaviest.

A variety of approaches have been used to estimate the intensity of exposure in each job or department in an industry. Sometimes industrial hygiene measurements are available, in which statistical models can be used to "fill in the gaps" that inevitably occur in these data (Greife et al., 1988; Hornung, 1991). Often the potentially hazardous chemical is an essential component of an industry's production process, so the relative intensity of exposure can be estimated from data on production rates in each department of the factory being studied (Kalliokoski, 1990). Expert judgment has also been used to estimate relative intensity of exposure (Tankersley et al., 1991). Physical models of the workplace environment, the production process, and the location of workers may be used to estimate likely exposure levels (Schneider et al., 1991).

In recent years, considerable research has been done to develop systematic methods of historic reconstruction of exposure—the broad term covering all of these various strategies (Gamble and Spirtas, 1976; Smith, 1987; Bond et al., 1991; Rice, 1991; Smith et al., 1991; Stewart and Herrick, 1991; Tankersley et al., 1991). Three international symposia have been held on the subject (Rappaport and Smith, 1991; Stewart and Herrick, 1991; Axelson and Westberg, 1992). The essential features of historic exposure reconstruction applied to each member of a study cohort are (1) the use of surrogates of past exposure to toxic chemicals (if no measurements are available) to estimate the likely intensity of exposure an individual would have experienced in a particular location (often a job or industrial department) at a particular time; (2) the use of either work records, individual recollections, or a combination of the two to determine which locations or jobs the subject was in for which periods; this information can then be combined with the job/location exposure estimates to build up for each subject an estimated exposure history covering years to decades of past exposure; and (3) summation of individual exposure histories in parameters that can then be used in epidemiologic models to assess exposure-risk associations.

Historic exposure reconstruction is a lengthy and expensive process, and the field is still developing. There are however some recent examples of occupational epidemiologic studies in which exposure estimates derived from historic reconstruction have proven superior to those relying on simpler

measures such as the total duration of exposure (Dement et al., 1983; Stewart et al., 1986; Rinsky et al., 1987; Kriebel, 1988a,b; Seixas, 1990; Blair and Stewart, 1992).

Exposure Assessment for Case-Control Studies

Often case-control studies must rely on information provided by the study subjects—cases and controls—to estimate exposure histories. The problem of recall bias has already been mentioned, and it is a potentially serious one in case-control studies when subjects are asked directly to recall exposure to a potentially toxic chemical. Some case-control studies reduce the severity of this problem by gathering general data from study subjects, with which more specific exposures can be estimated, that are less likely to be recalled differentially by cases and controls. For example, in occupational case-control studies, it is common to collect from each subject a full occupational history including detailed job information, but not information on chemicals that may have been used in these jobs. Experts on industrial processes such as manufacturing engineers and industrial hygienists then independently develop a job exposure matrix (JEM), which estimates for each job the types of chemicals likely to have been used, and sometimes their likely intensity of use as well (Zahm et al., 1990; Gerin and Siemiatycki, 1991). There is doubtless a good deal of nondifferential misclassification that results from this approach because the JEM approximates the general use of chemicals in occupations and not the specifics of an individual subject's experience. However, recall bias is much less likely to occur because subjects are not asked directly about hazards.

An excellent example of this approach is provided by a large multicenter case-control study of hematolymphopoietic cancers being conducted in Italy and focusing on pesticides and solvents risk factors (Miligi and Masala, 1991). In the participating centers, which are located in different agricultural regions, detailed JEMs have been developed by agronomists with long experience in local pest control practices. Subjects who report employment in farming are asked detailed questions about the crops they grew and the pests they treated in the course of their working lives. Local agronomists who advise farmers on pesticide usage then can predict with some reliability which chemicals were used in that particular zone in a particular time period to combat the reported pest.

The committee recognized that case-control studies can only rarely provide quantitative estimates of exposure. Nevertheless, they can provide valid information on the existence of an association and the identification of a dose-response trend. An example of this comes from a recent study of non-Hodgkin's lymphoma (NHL) and the use of 2,4-dichlorophenoxyacetic acid (2,4-D) among Nebraska farmers (Zahm et al., 1990). Investigators

questioned cases and controls extensively about the use of all kinds of pesticides. One question asked how frequently the subjects changed clothes after spraying pesticides. The authors observed that for those reporting 2,4-D use, the risk of NHL was higher among those who tended to change clothes less often. There was a statistically significant trend in the odds ratio over three categories of clothes changing. Thus, although no conclusions can be drawn from this study about the amount of 2,4-D to which subjects were exposed, there are data that strengthen a finding of a statistical association.

It must be stressed that these methods of exposure assessment, like the historic reconstruction approach for cohort studies discussed previously, are rarely "confirmed" in a given study by comparison to some "true" measure, since such a measure generally does not exist. As explained below, however, it is unlikely that such methods, conducted in a systematic way, can produce false-positive associations (bias away from the null). On the other hand, it is entirely likely that estimates resulting from these methods may underestimate the true magnitude of risk.

Exposure Misclassification

Some degree of error is introduced into any exposure measurement. When a cohort member is incorrectly called "exposed," an individual is assigned to the "low-exposure" group when moderate exposure has actually occurred, or a quantitative estimate of exposure is highly inaccurate, then exposure misclassification occurs, which introduces bias into the study results. Epidemiologists distinguish two fundamental kinds of exposure misclassification.

Differential (or nonrandom) exposure misclassification occurs when errors in the assignment of exposure are unequally distributed between the diseased and nondiseased groups. A common example of this is *recall bias*, which may occur in case-control studies if cases and controls are asked directly about their exposure histories, and if cases recall their exposure differently than controls. It has been shown, for example, that mothers of newborns with birth defects (cases) are more apt to report exposure to many prescription and nonprescription drugs than are mothers of normal newborns (controls) (Werler et al., 1989). Differential misclassification is a particularly serious problem because the direction of the bias cannot be determined. That is, the effect of this kind of error in exposure assignment may be to increase artificially the magnitude of an association between exposure and disease (called a bias *away from the null*) or to reduce the apparent magnitude of association (a bias *toward the null*).

Nondifferential (or random) misclassification occurs when exposure is measured with error but the degree of error is the same for the diseased and

nondiseased groups. In well-designed epidemiologic studies, investigators are often able to reduce the likelihood that serious differential misclassification has occurred, but nondifferential misclassification is often inevitable because of the imprecision of any exposure measurement tool. It is often possible to determine with some confidence that the likely direction of the bias from nondifferential exposure misclassification will be toward the null (Fleiss and Shrout, 1977; Armstrong and Oakes, 1982; Kupper, 1984; Heedrik and Miller, 1988; Lagakos, 1988). That is, the magnitude of the association estimated with misclassified exposure data will be lower than that of the "true" exposure-disease association. One can think of this phenomenon as "diluting" the true effect, which occurs, for example, when all members of an occupational group are compared to nonmembers to estimate the risk of a toxin to which only some members are actually exposed.

For example, consider the relationship between Hodgkin's disease and exposure to Agent Orange, which was studied by the Centers for Disease Control (CDC) in the Selected Cancers Study (CDC, 1990b), but for which the primary exposure variable was Vietnam service, not something more closely related to herbicide exposure. The odds ratio relating Hodgkin's disease to Vietnam service was approximately 1.2, but it was not statistically significant. Suppose that (1) disease status is classified without error; (2) 100 percent of those truly exposed are classified as exposed; and (3) 95 percent of those truly not exposed are classified as not exposed, and this fraction is the same for Hodgkin's disease cases as for the controls in the Selected Cancers Study. Then, by using the method of Kleinbaum and colleagues (1982), the estimated relative risk adjusted for misclassification bias would be 1.6, which represents a tripling of the excess risk. If the percentages in assumptions (2) and (3) were further from 100, the adjusted relative risk would be even higher.

Several recent scientific papers have argued that the general principle of nondifferential misclassification producing bias toward the null may not always hold when ordered categorical exposure data (e.g., low, medium, high) are used; however, there is not yet agreement as to how frequent or how severe any bias away from the null may be (Dosemici et al., 1990; Myers and Ehrlich, 1990; Flegal et al., 1991; Wacholder et al., 1991; Birkett, 1992; Brenner, 1992; Delpizzo, 1992). There is general agreement that with continuous quantitative measures of exposure and with dichotomous measures (exposed/unexposed), nondifferential misclassification will bias toward the null.

A common method of exposure estimation in occupational cohort studies assigns exposure levels to each job in the factory; then each worker's lifetime exposure is estimated by multiplying the amount of time spent in each job by the exposure intensity for that job. The resulting lifetime cumulative exposure for each cohort member may not be seriously misclassified

even if there are substantial errors in the job exposure estimates on which the cumulative exposures are based. One reason that the practice of estimating exposure histories from data on the exposure levels in certain jobs and the amount of time spent in each job may be fairly robust against errors in exposure measurement is that if workers move from job to job over the course of their careers, the errors in each of the job exposure estimates tend to cancel one another. Often the process of reconstructing the exposure levels in jobs in the past is only approximate, but as long as the effect of the errors is to sometimes over- and sometimes underestimate the true exposure in the job, then a lifetime exposure estimate will not be strongly affected by these individual job errors.

One solution to the problem of misclassification of exposure is to conduct a small validation study in which elaborate and expensive measurements are performed alongside the quicker, more error-prone measurements that will be used in the full study. Analysis of the degree of correlation between the "true" and the approximate measures allows investigators to adjust the risk estimates from the full study for exposure misclassification (Fleiss and Shrout, 1977; Armstrong and Oakes, 1982; Marshall, 1990; Rosner et al., 1990).

Biomarkers

Biomarkers for TCDD

TCDD and other chlorinated dibenzo-*p*-dioxins and dibenzofurans are found in tissues of non-occupationally exposed humans at part-per-trillion (ppt; nanogram-per-kilogram) levels. Following absorption, TCDD is distributed to tissues with high lipid content. Adipose tissue appears to be the main site of accumulation, although TCDD has been found in all tissue samples that have been examined from autopsy (Ryan et al., 1986). On a whole-weight basis, adipose tissue contains the highest levels, followed by liver, muscle, and kidney; on a lipid basis, the concentrations vary less (Ryan et al., 1986). Normal exposure to TCDD leads to levels in the lipid stores of humans of about 5-6 ppt, as measured in the adipose tissue or the lipid portion of the blood (Needham et al., 1990).

Although exposure to TCDD from environmental sources, primarily food (Geyer et al., 1986; Byard, 1987), occurs on a continuing basis, both serum and fat biopsy samples taken from individuals with unusually high exposures indicate that TCDD may remain in the body for many years after exposure. For example, fat biopsy samples taken from three Vietnam veterans believed to have been exposed through herbicide use showed levels of TCDD up to 99 ppt approximately 10 years after exposure occurred. By comparison, veterans with no unusual herbicide exposure had TCDD levels

between 3 and 15 ppt (Gross et al., 1984). A number of other studies, including the Ranch Hand half-life study, the Missouri civilian study, and the National Institute for Occupational Safety and Health (NIOSH) study, suggest that current serum TCDD levels are useful for distinguishing between groups of individuals that were exposed to TCDD 15 to 20 years ago (CDC, 1989a). For example, Sweeney and colleagues (1990) measured TCDD levels in serum lipids of 143 workers at two chemical plants in New Jersey and Missouri. The median TCDD level in a group of 103 production workers at the New Jersey plant was 84 ppt compared to 11 ppt for a group of eight office workers.

The most reliable procedure for measuring serum lipid-associated TCDD was developed by the CDC in 1986 as a substitute for the considerably more difficult surgical procedure required to obtain fat biopsies (Patterson et al., 1987). This procedure involves an extremely sensitive mass spectroscopic technique that permits measurement of TCDD into the low part-perquadrillion levels. A study in which both serum and fat TCDD levels were compared in 50 individuals showed a very high correlation between the two, suggesting that TCDD levels in serum provide a valid measure of TCDD levels in the body (Patterson et al., 1988). The Pointman Project (Kahn et al., 1988), conducted by the New Jersey Agent Orange Commission, confirmed this result.

The pharmacokinetics of TCDD in humans—its distribution and passage through the body—are not fully understood, which makes individual serum TCDD levels difficult to interpret and also complicates the interpretation of epidemiologic studies relying on these measures of exposure. As described in Chapter 4, a complex, poorly understood process distributes dioxins among body tissues and slowly clears it from the body. There is evidence that this process is quite variable among humans, so it is difficult to model its behavior and thereby extrapolate backward to estimate the likely concentration of TCDD in fat or blood in the past. It is also assumed that TCDD is removed from the body according to first-order kinetics—that is, for a given period of time, a constant fraction of the TCDD body burden is eliminated—but some evidence suggests the process may be more complicated and may vary as conditions in the body change. Second, the metabolic processes governing this movement and disposition may not be relevant to the determination of the dose of TCDD to the brain or reproductive organs, for example. In the epidemiologist's view, there may be different "causal pathways" linking exposure to the biomarker and exposure to disease.

By measuring TCDD levels in 1982 and 1987 from serum samples of 36 Ranch Hand veterans, the median half-life of TCDD in humans was estimated to be 7.1 years, adjusted for background TCDD levels (with a 95 percent confidence interval of 5.8 to 9.6 years) (Pirkle et al., 1989). In this study, the background exposure level of TCDD in serum was taken to be 4

ppt on the basis of data from the Agent Orange Validation Study. Background levels appear to be log-normally distributed and to increase with age (Sielken, 1987). An expanded study of 337 Ranch Hand veterans, including the 36 from the previous study, estimated a median half-life between 11.5 and 12 years. The analytical measurement error (coefficient of variation) in these studies was about 22 percent (Pirkle et al., 1989). These data indicate that the half-life of TCDD is independent of its initial serum concentration, thereby supporting a first-order kinetics process for TCDD elimination. A follow-up study of this group collected a third set of serum samples in 1992, and a new estimate will be made when analyses are complete.

Variations in TCDD half-life estimates are likely to result from changes in weight and percentage of body fat. The normal aging process, as well as dietary changes, may play a role here. For example, an average 5 foot 10 inch male aged 20 to 24 years weighs approximately 70 kg, of which 15 to 18 percent is fat. If at age 40-49, this male weighs approximately 80 kg, fat content almost doubles. Thus, there may be an expected reduction in serum TCDD levels in Vietnam veterans just from the mechanisms of change in body composition with age (Albanese, 1991). If the percentage of body fat is found to significantly affect half-life estimates, with assumptions of a 6 to 12 year half-life and increased body fat characteristic of aging, it is possible that a small or moderate intake of dioxin among Vietnam veterans may no longer be detectable by using current serum TCDD level as a biomarker (Schlatter, 1991).

Still other confounding factors may complicate exposure assessment. In a study of 640 Vietnam veterans, researchers found that serum TCDD levels varied with several personal characteristics, including age, race, body mass, and region of residence (Devine et al., 1990). It has also been suggested that disease may affect serum TCDD levels (Michalek and Tripathi, 1992). Flanders and colleagues (1992) have shown, for instance, that reverse causality, in which health outcomes affect the measured serum TCDD level years after exposure, can better explain some relationships between serum TCDD and health outcomes in the Ranch Hand study than a direct model in which TCDD causes the outcome.

Based on these data, the committee concludes that serum TCDD measures are helpful in epidemiologic studies, but should not be taken as a "gold standard" of exposure. If there are *group* differences in serum TCDD levels, that probably does indicate a difference in exposure to TCDD between the two groups. However, even if there is a difference in TCDD exposure between two groups, it may disappear as subsequent serum levels fade to background levels with the passage of time between exposure and measurement. In particular, because of poorly understood variation among individuals in TCDD metabolism and relatively large measurement error, *individual* TCDD levels are usually not meaningful.

Although quantitative measures of exposure are highly desirable, it may still be quite valid to use approximate measures of exposures or even surrogates such as the amount of time spent in a job in which the toxin was likely to have been used. Thus a biomarker, especially one gathered years after exposure, is not necessarily better than qualitative exposure measures based on process records. Group differences in serum TCDD levels can be useful in confirming that occupational exposure measures reflect true differences in exposure, as has been done in the NIOSH study (Fingerhut et al., 1989, 1991; Sweeney et al., 1990) and other studies (see below).

Chloracne as a Biomarker

As discussed in Chapter 11, there is strong evidence indicating that chloracne develops in at least some individuals heavily exposed to TCDD. Some researchers have used chloracne as a clinical marker of TCDD exposure. As a biomarker, however, chloracne has several serious drawbacks.

Numerous studies of heavily exposed individuals have demonstrated that chloracne is neither a specific nor an exclusive indicator of TCDD exposure at the individual level (Suskind and Hertzberg, 1984; Bond et al., 1989; Zober et al., 1990; Mocarelli et al., 1991). Individual susceptibility to the activity of TCDD seems to vary, especially with respect to the seriousness of the manifestation (Del Corno et al., 1985). In Seveso, Italy, for instance, after the TCDD release in 1976, approximately half of the adults with the highest serum TCDD levels developed chloracne whereas the other half did not. Some of the individuals who did not develop chloracne had serum TCDD levels higher than those who did (Mocarelli et al., 1991). There are other causes of chloracne and chloracne-like conditions besides TCDD, so its presence does not indicate certain exposure (see Chapter 11). The etiology of chloracne can be attributed to a rather wide range of chemical compounds, and lesions mimicking chloracne, such as acne vulgaris, may also appear in subjects who have not been exposed to TCDD.

In summary, although chloracne has been used in epidemiologic studies as a biomarker for TCDD exposure, the data indicate that it is neither a sensitive nor exclusive indicator. It is usually not long lasting, is difficult to diagnose, and is not at all sensitive to exposure to herbicides that are not contaminated with TCDD.

EXPOSURE ASSESSMENT IN OCCUPATIONAL AND ENVIRONMENTAL STUDIES

The committee reviewed a large number of epidemiologic studies of occupationally or environmentally exposed groups for evidence of an association between health risks and exposure to TCDD and herbicides used in

Vietnam, especially the phenoxy herbicides 2,4-D and 2,4,5-T, chlorophenols, and other compounds. In reviewing these studies, two types of exposure were explicitly considered: exposure to TCDD per se and exposure to the various herbicides used, particularly 2,4-D and 2,4,5-T. This separate consideration is necessary because of the possibility that, for example, some health effects may be associated with exposure to 2,4-D in agriculture and forestry. The herbicide 2,4-D does not contain TCDD, although small quantities of other dioxins are present.

For TCDD-exposed populations, serum measures of TCDD concentrations can be made of a representative sample of those exposed. Serum biomarkers of TCDD exposure are sometimes used to estimate the degree of prior exposure of individuals; however, there are limitations to their use, as described in the previous section.

Occupational Studies

Problems exist in the estimation of exposures among nonveteran groups studied for health outcomes. In many of the studies reviewed, exposure to herbicides or TCDD was inferred simply by occupation as a surrogate measure. Types of occupations involving potential exposure include workers in herbicide or other chemical production plants, agricultural and forestry workers, herbicide and pesticide applicators, and paper and pulp mill workers. As noted in the beginning of this chapter, the problem with characterizing exposure based on occupation only is that misclassification can occur if those classified as exposed were actually unexposed, or vice versa. Any actual increased risk from exposure might not be detected in the entire group when exposure is classified simply by occupation. In studies of farmers, for example, outcome data may merely be related to the fact of being a farmer. A much more accurate measure would incorporate information on what specific herbicides the farmer had been exposed to, the number of days the farmer had been exposed to the herbicide in question, and the degree of direct contact the farmer experienced.

However, exposure measures in occupational studies depend on the nature of the available data. Data for occupational studies are obtained from a variety of sources with varying degrees of completeness and accuracy. Some studies have identified and followed cohorts of individuals based on company or union records, or government licensing registries. Others have relied upon detailed company records of duration of employment, or assignment to various jobs or to particular work locations within the plant, to construct exposure indices. In some cases, there has been additional qualitative information available regarding the type of herbicides used, the nature of the TCDD exposure, and whether other chemical exposures may have been involved. These studies, with supplemental information on herbicide

use, provide more refined measures of exposure than those based on membership in a particular occupational group.

In most studies, individual quantitative estimates of duration and intensity of exposure are unavailable and must be estimated from information on the number of years employed in a particular job, or through identification of areas or departments in the workplace that involved sustained or direct exposure to the herbicide or TCDD. Ideally, quantitative measures of both the intensity and the duration of exposure could be determined; although surrogate measures are less satisfactory, all of these strategies can contribute to the assessment of exposure-risk associations through the process of historic exposure reconstruction.

The process of historic exposure reconstruction has been applied to some occupational studies of industrial workers potentially exposed to herbicides or TCDD. The following examples illustrate some of the approaches used in the epidemiologic studies reviewed by the committee. Additional information on these studies is provided in Chapter 7.

Production Workers

One of the most extensive sets of data on workers engaged in the production of chemicals potentially contaminated with TCDD has been compiled by NIOSH. For 12 chemical companies, more than 5,000 workers were identified from personnel and payroll records indicating whether the worker had been involved in production or maintenance processes associated with TCDD contamination (Fingerhut et al., 1991). At each chemical plant, a review of operating conditions, job duties, and records of TCDD levels in industrial hygiene samples, intermediate reactants, products, and wastes was conducted. Exposure was estimated from job records according to duration of exposure in processes involving TCDD contamination and total length of employment at the plant; serum TCDD levels were measured in a sample of 253 workers. Data on current TCDD levels demonstrated a good correlation with duration of employment. Nonetheless, the authors note the possibility for misclassification of exposure. Workers were exposed concurrently to chlorophenols and phenoxy herbicides that were contaminated with TCDD, as well as numerous other chemicals during their employment.

A multisite study by the International Agency for Research on Cancer (IARC) involved 18,390 production workers and herbicide sprayers from 10 countries (Saracci et al., 1991). Exposure was estimated from a combination of factory records, work histories, spraying data, and questionnaires. The cohort included information according to whether individuals were exposed by spraying or during production, and by the type of chemical used or produced. Workers who sprayed chlorophenoxy herbicides or worked in

factory departments in contact with these chemicals were considered "exposed" (N = 13,482); workers "probably exposed" had no job title but were judged to have been exposed (N = 416). Workers with no exposure status information were treated as having "unknown" exposure (N = 541), and those who never worked in factory departments with exposure to chlorophenoxy herbicides, or who never sprayed these chemicals, were considered "nonexposed" (N = 3,951). Exposure to TCDD was assumed to be possible for those who worked producing or spraying 2,4,5-trichlorophenol (TCP) and 2,4,5-T or related products. The degree of exposure to TCDD, however, is more uncertain than that of the NIOSH study since some of the cohorts of individuals either sprayed or produced compounds such as 2-methyl-4-chlorophenoxy-acetic acid (MCPA) or mecoprop (MCPP), which are unlikely to contain significant quantities of TCDD.

Several other occupational studies of workers involved in chemical production plants have relied upon job titles as recorded on individual work histories and company personnel records to classify exposure (Ott et al., 1980; Zack and Gaffey, 1983; Coggon et al., 1986, 1991; Cook et al., 1986; Zober et al., 1990). Similarly, exposure in chemical plant workers has been characterized by worker involvement in various production processes (i.e., synthesis, packaging, waste removal, shipping, plant supervision; Manz et al., 1991; Bueno de Mesquita et al., 1993).

Chloracne has been observed in some chemical plant workers and used as an indicator of prior TCDD exposure (Cook et al., 1980; Moses et al., 1984; Bond et al., 1987, 1989; Collins et al., 1993). Chloracne, however, is neither a sensitive nor an exclusive indicator of prior TCDD exposure.

Agricultural/Forestry Workers

Occupational studies of agricultural workers have estimated exposure to herbicides or TCDD using a variety of methods. In the simplest method, data on an individual's occupation are derived from death certificates, cancer registries, or hospital records (Burmeister, 1981). Although this information is relatively easy to obtain, it is not possible to estimate duration or intensity of exposure, or to determine the specific type of herbicide or chemical a worker was exposed to. Some studies of agricultural workers have attempted to investigate differences in occupational practices to identify subsets of workers who were likely to have had higher levels of herbicide exposure (Vineis et al., 1986; Wiklund and Holm, 1986; Musicco et al., 1988; Wiklund et al., 1988a; Hansen et al., 1992; Ronco et al., 1992). Other studies have used county of residence as a surrogate of exposure, relying upon agricultural censuses of farm production and chemical use to characterize exposure in individual counties (Gordon and Shy, 1981; Cantor, 1982; Blair and White, 1985). Still other studies attempted to refine

exposure estimates by categorizing the exposure based on the number of years employed in a specific occupation as a surrogate for exposure duration, obtaining supplier records on the amount of herbicides purchased to estimate the level of exposure, or estimating acres sprayed to quantify the amount used (Wigle et al., 1990; Morrison et al., 1992). In some cases, self-reported information on exposure was obtained, including direct handling of the herbicide, whether it was applied by tractor or hand-held spray, and what type of protective equipment was worn or what safety precautions were exercised, if any (Hoar et al., 1986; Zahm et al., 1990). Some studies attempted to validate self-reported information, based on verification using written records, signed statements, or telephone contacts with coworkers or former employers (Carmelli et al., 1981; Woods and Polissar, 1989).

Forestry workers and other outdoor workers, such as highway maintenance workers, are likely to have been exposed to herbicides and other chemicals to varying degrees. Exposure has been classified in a manner similar to other studies, for example, by number of years employed, job category, and occupational title.

Herbicide/Pesticide Applicators

Studies of herbicide sprayers are relevant because it can be presumed that applicators had more sustained exposure to herbicides; however, applicators were also likely to be exposed to a multiplicity of chemicals, complicating the assessment of any individual or group exposure to specifically phenoxy herbicides or TCDD. Some studies have attempted to quantify exposure of applicators based on information from work records on the number of acres sprayed or the number of days of herbicide spraying. Employment records can also be used to extract information on the type of chemicals sprayed.

One surrogate indicator of herbicide exposure is receipt of a license to perform spraying. Several studies have specifically identified licensed or registered pesticide and herbicide applicators (Smith et al., 1981, 1982; Blair et al., 1983; Wiklund et al., 1988b, 1989; Swaen et al., 1992). Individual estimates of the intensity and frequency of exposure were rarely quantified in the studies the committee examined, however, and often applicators were known to have applied many different kinds of herbicides, pesticides, and other chemicals. In addition, herbicide spraying is generally a seasonal occupation, and information may not be available on possible exposure-related activities during the rest of the year.

One study provided information on serum TCDD concentrations in herbicide sprayers, that of Smith et al. (1992). Blood from nine professional spray applicators in New Zealand, who first sprayed before 1960 and were also spraying in 1984, was analyzed. The duration of actual spray work

varied from 80 to 370 months. The serum levels ranged from 3 to 131 ppt TCDD on a lipid basis, with a mean of 53 ppt. The corresponding values for age-matched controls ranged from 2 to 11 ppt; the mean was 6 ppt. Serum TCDD levels were positively correlated with the number of months of professional spray application.

Several studies have evaluated various herbicide exposures during spraying in terms of type of exposure, routes of entry, and routes of excretion: Kolmodin-Hedman and Erne (1980), Kolmodin-Hedman et al. (1983), Lavy et al. (1980a,b), Ferry et al. (1982), Libich et al. (1984), Frank et al. (1985). Based on these studies, it would appear that the major route of exposure is through dermal absorption, with 2 to 4 percent of that on the skin being absorbed into the body during a normal work day. Air concentrations were usually less than 0.2 mg/m^3. The absorbed phenoxy acid herbicides are virtually cleared within one day, primarily through urinary excretion. Typical measured excretion levels for ground crews ranged from 0.1 to 5 mg/day and less for air crews.

Paper and Pulp Mill Workers

Another occupational group likely to be exposed to TCDD and chlorinated phenols consists of paper and pulp mill workers. These workers are likely to have received varying degrees of exposure as part of the bleaching process in the production of paper and paper products. Pulp and paper production workers are also likely to be exposed to other chemicals in the workplace, which vary, for example, according to the type of paper mill or pulping operation, and the final product manufactured (Robinson et al., 1986; Henneberger et al., 1989; Solet et al., 1989; Jappinen and Pukkala, 1991).

Environmental Studies

Studies of environmental exposures related primarily to unintentional releases of TCDD into the environment at Seveso, Italy, and Times Beach, Missouri. In these cases, the simplest measure of exposure was classification according to place of residence. Intensity of exposure has been estimated by years of residence in a contaminated area; this measure does not take into account the concentration of TCDD or herbicide, or the frequency of individual contact with contaminated soil or water.

One of the largest industrial accidents involving environmental exposures to TCDD occurred in Seveso, in July 1976, as a result of an uncontrolled reaction during trichlorophenol production. A variety of indicators were used to estimate individual exposure; soil contamination by TCDD has been the most extensively used. On the basis of soil sampling, three

areas were defined about the release point. They were zone A, the most heavily contaminated, from which all residents were evacuated within 20 days; zone B, an area of lesser contamination that children and pregnant women in their first trimester were urged to avoid during daytime; and zone R, a region with some contamination, in which consumption of local crops was prohibited (Bertazzi et al., 1989). The samples so obtained are virtually unique in that they were numerous and were obtained prior to elimination and degradation of TCDD in the sample media.

Data on serum concentrations of zone A residents have been presented by Mocarelli et al. (1990, 1991) and earlier by the CDC (1988a). For those with severe chloracne ($N = 10$) the TCDD levels ranged from 828 to 56,000 ppt lipid weight. Those without chloracne ($N = 10$) had levels from 1,770 to 10,400 ppt. The levels in all controls but one were not detectable. The highest of these levels among TCDD-exposed workers exceeded any that had been estimated up to that time, based on backward extrapolation with a half-life of seven years. Data on nearby soil levels, number of days an individual stayed in zone A, and whether local food was consumed were considered in evaluating TCDD levels. None of these data correlated with serum TCDD levels, strongly suggesting that the exposure of importance was fallout on the day of the accident. The presence and degree of chloracne did correlate with TCDD levels; however, it appears that adults are much less likely to develop chloracne than children following an acute exposure, but surveillance bias may have played some role in this finding.

A number of reports have provided information on exposure to TCDD from environmental contamination in Missouri (Patterson et al., 1986; Andrews et al., 1989). In 1971, TCDD-contaminated sludge from a hexachlorophene production facility was mixed with waste oil and sprayed in various community areas for dust control. Soil contamination in some samples exceeded 100 parts per billion (ppb). One of the Missouri sites with the highest TCDD soil concentrations was the Quail Run mobile home park. Residents were considered exposed if they had lived in the park for at least six months during the time the contamination occurred (Hoffman et al., 1986). Other investigations of Times Beach have estimated exposure-risk based on residents' reported occupational and recreational activities in the sprayed area. Levels of exposure have been estimated from duration of residence and TCDD soil concentrations.

Andrews et al. (1989) provided the most extensive data on human adipose tissue levels for 51 persons who had ridden or cared for horses at arenas sprayed with TCDD-contaminated oil; persons exposed in residential areas where such oil had been sprayed; individuals involved in TCP production; persons exposed in TCP nonproduction activities, such as lab and maintenance workers; and 128 controls. Persons were considered exposed if they lived, worked, or had other contact for two years or more with

TCDD-contaminated soil at levels of 20-100 ppb or for six months or more with soil contaminated with TCDD at levels greater than 100 ppb. Of the exposed population samples, 87 percent of adipose tissue TCDD levels were less than 200 ppt; however, TCDD concentrations in 7 of the 51 exposed ranged from 250 to 750 ppt. For nonexposed persons, adipose tissue TCDD concentrations ranged from nondetectable to 20 ppt, with a median of 6 ppt. Based on a seven year half-life it is calculated that two of the study participants would have had adipose tissue TCDD levels near 3,000 ppt at the time of the last date of exposure.

Vietnamese Studies

Several studies have investigated exposure to herbicides among the residents of southern Vietnam (Constable and Hatch, 1985), comparing unexposed residents of the South to residents of the North. Other studies have attempted to identify veterans of northern Vietnam who served in the South during the Vietnam era. Records of herbicide sprays have been used to refine exposure measurements, comparing individuals who lived in sprayed villages in the South with those living in unsprayed villages. In some studies, residents of villages were considered exposed if a recorded herbicide mission passed within 10 km of the village center (Dai et al., 1990). Other criteria for classifying exposure included length of residence in a sprayed area and number of times the area had reportedly been sprayed.

A small number of studies provide information on TCDD concentrations in Vietnamese civilians exposed during the war. Schecter et al. (1986) detected TCDD in 12 of 15 samples of adipose tissue taken at surgery or autopsy in southern Vietnam during 1984. The concentrations in the positive samples were from 3 to 103 ppt. No detectable levels of TCDD were found in nine samples from residents of northern Vietnam who had never been in the South. The detection sensitivity was 2-3 ppt. Analysis of three breast milk samples collected in 1973 from Vietnamese women thought to have been exposed to Agent Orange varied from 77 to 230 ppt on a lipid basis.

Conclusions on Exposure Assessment in Occupational and Environmental Studies

In general, exposure assessment is the weakest aspect in most of the occupational and environmental studies that the committee has reviewed. The nature of the exposure is generally poorly characterized with regard to which of the herbicides or dioxins the subjects were exposed. Rarely is there any precise information on the intensity and duration of individual exposure; rather, surrogates such as the length of employment and job location

in the workplace are measured. Many studies use membership in a group to assign exposure, but some studies, such as the NIOSH and IARC studies of chemical production workers that evaluate subgroups with presumed higher, longer, or more relevant (to this study) exposure, are the exceptions to this pattern.

The range of occupational and environmental populations and the likely intensity and duration of their exposure to herbicides and TCDD are diverse. This provides an opportunity to compare results between studies to determine whether certain diseases are more common in populations likely to have higher exposures. Also, if one disease seems to be consistently associated with herbicides or TCDD among differently exposed groups, the evidence for an association is strengthened. Because of the complex pattern of exposures to the various herbicides and TCDD in the available epidemiologic studies, the committee was generally not able to differentiate among multiple chemical exposures to determine whether specific health effects were associated with any particular herbicide or TCDD.

EXPOSURE ASSESSMENT IN STUDIES OF VIETNAM VETERANS

Different approaches have been used in estimating exposure to herbicides in studies of Vietnam veterans. These studies generally rely on self-reported exposures, records-based exposure estimates, or biomarkers. Each of these approaches is limited in its ability to determine precisely the intensity and duration of individual exposure.

One of the simplest approaches to classifying Vietnam veterans' potential exposure to herbicides and TCDD assumes that service in Vietnam resulted in exposure to herbicides, so any adverse health effects associated with these substances would be discernible in veterans who served in Vietnam compared to Vietnam era veterans who did not serve there. In various studies, certain attributes of military service have been hypothesized to be associated with an increased likelihood of herbicide exposure. These include branch of service, combat experience, and military occupation. Another approach involves the reconstruction of herbicide exposure through a procedure of matching historical data on herbicide sprays with records of troop location and dates of service.

Self-Reports

Self-reported data are a common epidemiologic tool for assessing exposure, but they have a number of drawbacks when applied to studies of Vietnam veterans to determine herbicide and TCDD exposures. There include the general problem of recall and, in particular, the possible confusion of herbicide spraying with the spraying of insecticides, and underreporting

by individuals who unknowingly entered an area that had previously been sprayed and still contained residual herbicides or TCDD. On the other hand, some veterans who believe they were exposed to herbicides may not actually have been exposed. Veterans have been asked to recollect herbicide handling; or being in an area where there was ground or aerial spraying around base perimeters, or from boats, trucks, or helicopters; or being in an area that was previously sprayed. Self-reports of locations and dates of service have also been combined with a compilation of aerial spray data (Stellman and Stellman, 1986) to reconstruct an estimate of herbicide exposure. This approach, discussed in more detail below, is based on the assumption that Vietnam veterans recall the names and general time periods spent at various locations in Vietnam, but it is not clear how accurately this information can be reported many years later.

Records-Based Measures

Records-based classifications of the exposure of Vietnam veterans have been estimated by using information from military personnel records and historical documents on Vietnam service, branch of service, and military occupation. Levels of combat exposure have also been used as a surrogate measure of herbicide exposure. This military records-based approach is similar to reconstructing occupational exposure based on job classification. The inherent assumption is that specific jobs result in exposure or higher levels of exposure than other jobs.

Vietnam Service

As mentioned earlier, the advantage of using Vietnam service as an exposure measure is the simplicity of study design and analysis. The major limitation of Vietnam service as a measure of exposure is that not all men and women who served in Vietnam were actually exposed to herbicides or TCDD above global background levels, and therefore any epidemiologic study examining adverse health outcomes associated with herbicides or TCDD exposure would be diluted by the misclassification as exposed of individuals who served in Vietnam but were not exposed. Identifying such an outcome would therefore not be possible unless the exposure caused a very large excess in the incidence of disease.

Branch of Service

The rationale for using branch of service in classifying exposure is that the activities and locations of service of the Air Force, Army, Marines, and Navy varied, and therefore the potential for exposure to herbicides may also

vary. Among the factors that could differ among the four branches of service and potentially be related to the extent of exposure are troop mobility, the percentage of time spent outside of base camps, the availability of hygienic facilities, and the degree of reliance on local water and food. For example, it might be assumed that more mobile troops, spending more time in the field, having less access to hygienic facilities, and consuming more local food and water, would have greater potential exposures. However, those who operated primarily in the field cannot all be considered exposed because herbicide spraying was not distributed uniformly throughout the country. Also, support troops that operated from major bases might have been exposed because herbicides were used extensively around some base camps to remove tall grasses and heavy growth that obscured visibility (see Chapter 3). Since herbicides were used around base camps, military personnel could have received exposure in areas where no records of Operation Ranch Hand spraying exist. The violations of the simplifying assumptions would result in both false-positive and false-negative exposure assignments when "job classification" by branch of service is used. Classification of exposure by branch of service could be improved, for example, by stratifying Army troops by specialized duties or tactical operations and by including general geographical location information about individuals, units, or companies with estimates of the overall use of herbicides in those geographic areas.

Combat Experience

Classification using combat experience is based on the assumption that troops who had more frequent encounters with the enemy, and therefore engaged in combat more frequently, were more likely to enter areas that had been sprayed with herbicides. Since this was true only for some troops or regions in Vietnam, misclassification will occur. Military occupational specialties are sometimes used as a surrogate measure of combat. Individuals serving as riflemen or in artillery units are classified as having "combat-related" occupational specialties. Stratifying location of troops by geographic area, as well as by combat experience, as was suggested for the branch of service classification, would decrease exposure misclassification.

Military Occupation

Military occupation has been shown to be a valid exposure classification for two specific occupations that involved the direct handling and distribution of herbicides: first, the Air Force Ranch Hands, who were responsible for aerial spraying of herbicides and, second, the Army Chemical Corps, which performed ground and helicopter chemical operations. Biomarker

studies of the Ranch Hands are consistent with their exposure to TCDD as a group. When the Ranch Hand cohort was further classified by military occupation, a general increase in serum TCDD levels was detected with jobs that involved more frequent handling of herbicides. The median TCDD level for enlisted ground crew (24 ppt, range 0-618 ppt) was higher than the median level for enlisted flyers (18 ppt, range 0-196 ppt), and three times greater than the median level for officers (8 ppt, range 0-43 ppt) (AFHS, 1991).

The number of military personnel involved in these two units is small, however—approximately 1,000 for the Ranch Hands and, according to a recent abstract (Dalager and Kang, 1993), 2,954 for the Chemical Corps. Individuals serving in the Army Chemical Corps were also likely to be exposed to a number of chemicals other than herbicides, which can contribute to confounding in epidemiologic studies. Other classifications of general Vietnam veterans by military occupations are unlikely to improve the ability to predict exposure above what might be obtained from a category such as combat experience.

Reconstructing Estimated Exposure from Troop Location and Herbicide Spray Data

Another approach, the most detailed records-based classification scheme, matches troop location data with information on herbicide spray data from the HERBS and Services HERBS tapes to assign exposure categories based on the number of times a unit was within a defined time and distance from a documented herbicide spray. This strategy is similar to reconstructing occupational exposure based on the location of an employee within a plant during different time intervals and estimating the cumulative exposure associated with being in each location and performing specific job activities.

The available data on spraying missions have been compiled and stored on the HERBS and Services HERBS computer tapes. The HERBS tapes contain information on more than 6,500 herbicide missions and 17.6 million gallons of herbicides sprayed from August 1965 to February 1971. The tapes include information on the type of mission and the herbicide sprayed, the volume of herbicide used, the date of the herbicide mission, and the area sprayed. Data on helicopter defoliation missions are limited, and there is no information on ground herbicide sprays. Some of this information was compiled from various military documents and recorded on the Services HERBS tapes, prepared by the Department of Defense (DOD) Environmental Services Group (ESG). Because of the differences in reporting of Ranch Hand versus non-Ranch Hand sprays, it is likely that an unknown proportion of ground spray operations were not recorded. Further detail on the HERBS and Services HERBS tapes is provided in Chapter 3.

Data on herbicide sprays from the HERBS and Services HERBS tapes

can then be matched to information on troop location gathered either from self-reports of locations of military service or from military records. Military records, including morning reports, operational reports, and daily journals have been abstracted by the ESG for a subset of Army personnel to determine the location of their military units for a specified time period. Once individuals have been identified, information on their period of service can be verified from military personnel files archived at the National Personnel Records Center in St. Louis, Missouri.

Thus, at least in principle, one should be able to estimate the likelihood of herbicide exposure for a veteran or a military unit that served in Vietnam. This general approach has been tested, and different conclusions have been drawn as to the feasibility and utility of the estimates that result (see Appendix B). Like other exposure estimation strategies for retrospective studies, all of these strategies may be valid; however, they vary in their precision and in the degree to which they contribute to assessing a particular exposure-disease association.

The CDC Exposure Opportunity Index

The Centers for Disease Control (Erickson et al., 1984a,b) conducted a case-control study to determine if there was an increased risk of birth defects among the offspring of Vietnam veterans (see Chapter 9). The potential for an individual Vietnam veteran's exposure to Agent Orange ("exposure opportunity") was estimated by military records specialists of the Army Agent Orange Task Force without knowledge of case or control status. The exposure opportunity index (EOI) scores ranged from a value of 1 (minimum opportunities for exposure) to a value of 5 (most numerous opportunities for exposure). Higher values signify a greater likelihood of exposure but do not necessarily indicate a higher degree (duration or intensity) of exposure. An index score of 1, for example, was assigned to individuals who served in Vietnam prior to Agent Orange use or who were stationed offshore. Scores of 2 were assigned to men with noninfantry occupations stationed at selected bases with recorded perimeter spraying (e.g., a wireman serving in Chu Lai during 1968-1969). Individuals who served in selected noninfantry occupations at specified locations and times (e.g., an M.P. in Danang during 1968-1969) were allocated a score of 3. An index score of 4 was assigned to Army advisors serving during 1968-1969 and to infantry/combat arms serving in Tay Ninh in 1969 or 1970. The greatest opportunity for exposure, a score of 5, was assigned to Ranch Hand personnel and to individuals with service in specified areas at times of aborted Ranch Hand missions (during which herbicides may have been dumped on populated areas; for example, at Bien Hoa Air Force Base in July 1967 or November 1968) (see Table 6-1).

TABLE 6-1 Examples of Agent Orange Exposure Opportunity Index Scores [a]

Index Score = 1 (minimum opportunities for exposure)

1. Service in selected locations at specific times (any job description except handling Agent Orange), e.g., Cam Ranh Bay (1966), Qui Nhon (1968-1969), Nha Trang (1967-1968)
2. Non-Ranch Hand pilots and aircrew (1966-1967)
3. Specified controlled environments, e.g., battalion surgeon (1968)

Index Score = 2

1. Service in selected locations at specific times, e.g., Gia Le (1969-1970), Phan Rang (other than September-December 1968, March-September 1970), Qui Nhon (1968-1969)
2. Selected noninfantry occupations at specified places and times, e.g., company clerk—Duc Pho (1968-1969), radio repairman—Chu Lai (1966-1967), truck driver—Cu Lam Nam (1968)
3. Noninfantry stationed at selected bases with perimeter spraying, e.g., wireman —Chu Lai (1968-1969)

Index Score = 3

1. Service at bases with perimeter spray operations, specified times, e.g., Chu Lai (1968-1969), Camp Eagle (1968-1969), LZ English (1967-1968)
2. Selected noninfantry occupations at specified locations and times, e.g., salvage specialist—Danang (1969-1970), M.P.—Danang (1968-1969), wheeled vehicle mechanic—Long Binh (1966-1967)

Index Score = 4

1. Infantry/combat arms at specified locations and times, e.g., An Khe (1966-1967), Tam Ky (1967-1968), Tay Ninh (1969-1970)
2. Selected noninfantry at specified locations and times, e.g., Helicopter pilot— Cu Chi (1966-1967), M.P.—Long Binh (1967-1968)
3. Advisors of Army, Republic of Vietnam Divisions (1968-1969)
4. Special Forces Camps (field personnel), e.g., Nha Trang (1969-1970)

Index Score = 5 (most numerous opportunities for exposure)

1. Infantry/combat arms at specified locations and times, e.g., A Shau Valley (1969), Tay Ninh (1968), Phuoc Vinh (1967)
2. Service at specified locations and times with aborted Ranch Hand missions or other herbicide mishaps, e.g., Bien Hoa AFB (July 1967, November 1968), Long Binh Post (1967-1969), Phu Cat AFB (1969-70)

[a] See text for description.

SOURCE: Erickson et al., 1984a.

All individual veterans were given two index scores: one was derived from self-reported information on dates and location of service, and military duties, obtained during the interview; the second was developed based on a review of military records. The records-based EOI used unit location data determined from the Operational Report Lessons Learned (ORLL). The proximity of these general unit locations was compared to Agent Orange and other herbicide spray data by using the HERBS tapes and other data available on base perimeter sprays to construct the index scores.

Approximately 25 percent of interviewed Vietnam veterans reported that they had been exposed to Agent Orange. Fifty-two percent received the same score in both the index score and the self-reported Agent Orange exposure. A higher proportion of subjects who thought they had been exposed received scores of 4 or 5 than did subjects who thought they had not been exposed.

The CDC Agent Orange Study

In 1983, the Centers for Disease Control was assigned to conduct a study of the possible long-term health effects of Vietnam veterans' exposure to Agent Orange (see Chapter 2). The original design of the CDC Agent Orange Study (AOS) attempted to classify veterans' exposure to herbicides that occurred during military service. This was to be accomplished by determining the proximity of troops to Agent Orange spray using military records to track troop movement and the HERBS tapes to locate herbicide spraying patterns. Veterans' daily locations were to be abstracted from military records of company-sized units. The original study was to involve three cohorts, each containing approximately 8,500 men. Only Army units were to be studied. Two of the cohorts would consist of Army veterans serving at least 18 months in III Corps during 1967-1968. These cohorts would differ in their likelihood of herbicide exposure. The third cohort would be comprised of veterans from service support units stationed in other areas where there was evidence of little or no herbicide sprays. Participants were to have been draftees or single-term enlistees, had a rank of E1 through E5 at discharge, and served a single tour of duty in III Corps of Vietnam during 1967-1968. In addition, only individuals from infantry and artillery units were to be selected. These eligibility criteria and the exclusion of officers were intended to make the study groups as homogeneous as possible with respect to demographic characteristics and nonherbicide Vietnam-specific risk factors that could influence health.

The Environmental Support Group of DOD assisted CDC in the abstraction of military records on troop locations. According to the CDC protocol, 65 battalions were to be selected from III Corps. Herbicide exposure "scores" would be calculated at the company (about 250 men) level,

based on a reported unit location occurring within a specified time and distance from a known herbicide application. Four different time and distance criteria were proposed for an encounter, or "hit," with Agent Orange: an application within 59 days and 2 km, within 1 day and 5 km, within 3 days and 7 km, or within 5 days and 7 km. Based on these analyses, the CDC initially concluded that "many veterans were in close enough proximity to applications of Agent Orange to be classified as highly likely to have been exposed to the herbicide" (CDC, 1985).

The CDC also proposed three exposure scores—E1 (short exposure), E2 (intermediate exposure), and E3 (chronic exposure)—to estimate an individual's likelihood of exposure. These scores attempted to account for variations in TCDD half-life, dispersion of herbicides, error in the calculated distances from spray lines, and uncertainties regarding the time between spraying and possible exposure, as well as whether the exposure could be viewed as acute, chronic, or intermediate. Preliminary results indicated substantial variability in exposure scores among units and among individual veterans (CDC, 1985).

The abstraction of military records is very labor-intensive, and a number of potential problems may arise. Some of the difficulties encountered by the ESG during its efforts for the AOS involved filling in gaps in the daily records of a unit's location; assigning a location to an individual who was not with the unit at the time of the recording; and tracking geographical dispersion among troops within any individual unit. There continues to be disagreement regarding how and whether these problems can be resolved (see Appendix B). Some participants in this research estimate, based on a 30 month study period for 35 different Army companies, that the location of company-sized units is available 85-90 percent of the time (Christian, 1992).

In testimony before the committee, varying views on these important points were presented, and the committee could neither verify nor refute these positions. The committee believes that the eligibility criteria developed for the study may, however, have decreased the range of exposures in the study and exacerbated the problem of missing troop location information.

In January 1986, a Science Panel of the White House Agent Orange Working Group (AOWG) was convened to evaluate the feasibility of the AOS. A subpanel was formed to review the records and information related to exposure assessment. The subpanel concluded that misclassification of an individual's exposure status was serious enough to warrant cancellation of the study. Two issues were specifically noted as influencing the degree of misclassification:

1. Unit dispersion—On a substantial number of days, personnel in combat units eligible for the AOS were not located together as a unit; rather they were dispersed geographically up to 20 km on any given day.

2. Incomplete records—An unknown, but apparently large, proportion of fire base perimeter spray operations were never recorded. The degree to which these "unrecorded" operations may have influenced exposure is unknown.

The panel recommended that "any study of ground troops, which is dependent on military records for the assessment of exposure to herbicides, not be conducted *without an additional method to verify exposure*" (emphasis added; Young et al., 1986).

The Stellmans' Study

Drs. Steven and Jeanne Stellman (1986) developed and published a similar but independent method for assessing veterans' exposure to herbicides in 1986. Because they did not have access to military troop location data, they relied on self-reports of locations and dates of service, but used the same HERBS tapes to identify spray missions. Like the CDC, they used these data to derive a series of exposure scores based on geographic and temporal proximity of a soldier to known spray missions. Several probabilistic exposure indices were developed to estimate exposure to herbicides for individual veterans. Three indices, C_5, C_{10}, and C_{15}, provided counts of the number of times a veteran was located within a specified radius 5, 10, or 15 km from a recorded herbicide spray (there were no time restrictions). Three continuous exposure indices, E_1, E_2, and E_3, accounted for the actual distance from each spray and for concurrent exposures plus potential exposures from residual herbicides from all previous spray missions that occurred nearby (Stellman and Stellman, 1993). Self-reported Agent Orange exposure was also determined based on a number of questions regarding whether subjects had sprayed, loaded, or handled herbicides, or had entered a sprayed area. According to the Stellmans, these measures "are to be regarded as approximations to population exposures in the same sense that past exposures to dusts and chemicals are often estimated for workers in industries for which industrial hygiene measurements of past conditions are not available" (Stellman and Stellman, 1986).

To test the method, the Stellmans selected a sample of 478 veterans participating in an outreach program sponsored by the Veterans Education Project of American University (Stellman and Stellman, 1986). They concluded that the approach was feasible and yielded estimates of exposure likelihood that would be useful in epidemiologic studies. Once again, no direct validation of the estimates was possible, but the indices appeared consistent with other measures or proxies of exposure.

The indices were also applied to an epidemiologic study of various health effects among a sample of 6,810 American Legionnaires (Stellman et

al., 1988). Those in the American Legion survey reporting a military occupation likely to involve heavy exposure (e.g., spraying herbicides from a helicopter, or loading or handling herbicides) had a higher mean exposure score than those reporting no direct exposure. Those who reported heavy combat experience were also more likely to have a higher estimated herbicide exposure. Because the exposure score was based on veterans' recall of dates and locations of service, rather than on recall of exposure per se, recall error was probably reduced.

Ranch Hand Exposure Index

The exposure index initially proposed in the Air Force Ranch Hand study relied upon military records of TCDD-containing herbicides (Agents Orange, Purple, Pink, and Green) sprayed as reported in the HERBS tapes for the period after July 1965, and military procurement records and dissemination information for the period prior to July 1965. A TCDD weighting factor (based on the concentration of TCDD in the herbicide and the time of spraying) was applied to the number of gallons of herbicides sprayed during each subject's tour of duty in Vietnam. The dates of each subject's tour(s) in Vietnam were determined by a manual review of military records. The HERBS tapes were used with quarterly operations reports to construct a table of gallons of TCDD-containing herbicides sprayed for each month during the Ranch Hand operation.

The exposure index for a Ranch Hand was defined as the product of the TCDD weighting factor and the number of gallons of TCDD herbicides sprayed during his tour of duty, divided by the number of Ranch Hands sharing such duties during his tour. The weighting factor served to separate from the total those who served in Vietnam prior to July 1965, a period in which TCDD levels in herbicides were known to be higher. Exposure levels were defined for five occupational categories: (1) officer-pilot, (2) officer-navigator, (3) officer-nonflying, (4) enlisted flyer, and (5) enlisted ground crew. Each Ranch Hand was placed in an exposure category (high, medium, or low) based on the value of the individual's exposure index.

This index was intended to distinguish between groups of individuals who served in Operation Ranch Hands units rather than as a measure of individual exposure. The measure did not account for individual variation in work habits or job duties—all enlisted flyers, for example, serving a similar tour of duty, received the same exposure classification. As noted in Chapter 3, concentrations of TCDD varied across herbicide lots—this index assumed that the relative concentration of TCDD was 2 ppm in Agent Orange, 33 ppm in Agent Purple, and 66 ppm in Agents Pink and Green. The index includes exposure from recorded Ranch Hand sprays only—the measure did not allow for other unrecorded herbicide exposures, such as chemical dumps

or perimeter sprays, or other non-Ranch Hand herbicide applications. In 1991, the exposure index was compared to the results of the Ranch Hand serum TCDD analysis. The exposure index and the TCDD body burden were weakly correlated.

Biomarkers of Exposure in Vietnam

Gross and colleagues (1984) reported on the results of a preliminary study designed to determine whether elevated levels of TCDD could be detected in adipose tissue of 20 Vietnam veterans who reported being exposed to Agent Orange. Investigators used gas chromatography and high-resolution mass spectrometry to measure dioxin levels in adipose tissue surgically removed from the abdominal area of volunteers. Three veterans who reported heaviest exposure had the highest levels of TCDD, ranging from 20 to 173 ppt. Veterans reporting less exposure did not differ appreciably in TCDD levels from a control group of veterans who had not served in Vietnam; mean TCDD levels in these groups were 5 to 6 ppt.

Several other studies to measure dioxin levels in adipose tissue of Vietnam veterans have been conducted. Some have found elevated TCDD levels among veterans thought to be exposed to herbicides (Schecter et al., 1987; Kahn et al., 1988), whereas others have not (Kang et al., 1991).

In the Pointman I Project (Kahn et al., 1988), conducted by the New Jersey Agent Orange Commission, 10 Vietnam veterans reported to be heavily exposed to Agent Orange were compared to matched controls in both blood and adipose tissue levels of TCDD. Nine of the ten veterans had handled herbicides regularly—five were members of Operation Ranch Hand, one was an Air Force freight handler, two were Army Chemical Corps specialists, and one was an Army helicopter crew chief who flew herbicide spray missions. Ten nonexposed Vietnam veterans and seven Vietnam-era veterans who did not serve in Vietnam were selected as controls. The mean TCDD level in the exposed men was reported to be about tenfold higher than the 4-5 ppt for controls. The levels of TCDD in the exposed men exceeded those in the pooled control subjects in both blood (p 8 .01) and adipose tissue (p 8 .001). Furthermore, blood TCDD levels were highly correlated with adipose tissue levels (r = +.89).

In an extension of the Pointman I study, Pointman II, researchers measured serum TCDD levels in 55 Vietnam veterans who did not handle herbicides directly but were assigned to areas known to be heavily sprayed and in 15 unexposed Vietnam veteran controls. The exposed veterans were representatives of an Army cavalry unit that served in III Corps, a Marine unit from I Corps, and a brown water Navy riverboat crew from IV Corps. The Marines had a mean serum TCDD level of 5 ppt, and the Navy riverine subjects had a mean level of 4 ppt, compared with a mean level of 3 ppt for

the controls. Army personnel had a mean level similar to the controls. Although some observers have argued that all of these measured TCDD levels are within a normal "background" level, project scientists maintain that the results suggest apparent group differences in military service characteristics that are important (Kahn et al., 1992; Lewis, 1993).

The Air Force Ranch Hand study examined serum TCDD levels in 866 Ranch Hands compared to 804 controls, who were Air Force veterans involved in C-130 aircraft missions in Southeast Asia (AFHS, 1991, vol. 1). Ranch Hands as a group had higher serum levels (median = 13 ppt, range 0-618 ppt) than comparison subjects (median = 4 ppt, range 0-55 ppt). Among Ranch Hands, there was a gradient for TCDD level: enlisted ground crew had the highest levels (median = 24 ppt, range 0-618 ppt), followed by flying enlisted personnel (median = 18 ppt, range 0-196 ppt) and then by officers (median = 8 ppt, range 0-43 ppt). All medians in the comparison group similarly stratified by rank and job were less than 5 ppt.

CDC Agent Orange Validation Study (AOVS)

To test the validity of several indirect methods for estimating exposure of ground troops to Agent Orange in Vietnam, in 1987 the CDC measured serum TCDD levels in a nonrandom sample of Vietnam veterans and Vietnam era veterans who did not serve in Vietnam (CDC, 1988b). Participants were chosen from a pool of 65 Army battalions that operated 18 months or longer in III Corps, a heavily sprayed region around Saigon, during 1967 and 1968. From this pool, veterans who served in combat companies, were discharged at pay grade E5 or lower, and had served a single term of enlistment, were included. Vietnam era veterans were selected from among those previously interviewed for the CDC's Vietnam Experience Study (but were not chosen for the physical examination). Men in the Vietnam cohort were chosen from among those who had served in a unit for which the ESG had locational data (as determined for the Agent Orange Study). Vietnam veterans were selected for further study based on their estimated number of Agent Orange hits, derived from the number of days for which at least one company location was within 2 km and 6 days of a recorded Agent Orange spray; the "low" exposure group included 298 veterans, the "medium" exposure group included 157 veterans, and 191 were included in the "high" exposure group. Blood samples were obtained from 66 percent of Vietnam veterans (N = 646) and 49 percent of the eligible comparison group of veterans (N = 97). More than 94 percent of those whose serum was obtained had served in one of five battalions.

Five indirect exposure scores based on military records and two scores based on self-reports were used to rank veterans according to their likelihood of exposure to Agent Orange. The five indirect scores incorporated a

variety of assumptions concerning possible sources of TCDD exposure, the estimated half-life of TCDD in the environment, and the completeness of data on troop and spray location. Two Agent Orange exposure scores were calculated based on proximity to recorded Agent Orange sprays. Two similar scores were computed for recorded sprays of "unknown" agents. The fifth score, an area score, depended less on precise military unit location data than the other four scores. It was computed based on the number of days a company was in one of five heavily sprayed areas in III Corps during 1967 and 1968. Two self-assessed exposure scores were determined based on the number of days an individual reported direct and indirect exposure to herbicides during military service (CDC, 1988b).

The median TCDD level in Vietnam veterans was 4 ppt, with a range from less than 1 to 45 ppt and two having levels greater than 20 ppt; the distributions of these measurements were nearly identical to those for the control group of 97 Vietnam era veterans (Figure 6-2). None of the records-derived estimates of exposure, and neither type of self-reported exposure to herbicides, identified Vietnam veterans who were likely to have currently elevated serum TCDD levels (CDC, 1988b). The study concluded it is unlikely that military records can be used to identify a large number of U.S. Army veterans who might have been heavily exposed to TCDD in Vietnam.

When restricting the AOVS analysis to veterans with TCDD levels in the upper quartile and decile, the Stellmans (1993) reported a correlation between serum TCDD levels and military records-based scores. This finding results from one veteran whose serum TCDD concentration was 45 ppt. This veteran had an exposure score of 115 (1 Agent Orange hit and 114 unknown agent hits).

A draft of the AOVS was reviewed by an Institute of Medicine advisory committee in 1987 (IOM, 1987). The committee raised the concern that current serum levels (for this study, obtained in 1987) may not be related to an actual exposure that occurred some 20 years previously. Four possible scenarios for observing "background" levels in three hypothetical groups of Vietnam veterans (high, medium, and low exposure) who served in Vietnam in 1967 were suggested (see Figure 6-3): (1) All three veteran groups in the study could have been similarly exposed during the war, but their levels were not sufficiently high in the late 1960s to prevent them from decaying to background level in 1987. (2) All three exposed veteran groups could have had significantly higher blood levels than background before they were potentially exposed in Vietnam, but with the passage of time, all TCDD concentrations had decayed to similar levels. (3) The three groups could have been differentially exposed in Vietnam, and if their serum had been sampled then, variation in TCDD levels would have been detected. However, differences in decay rates and initial concentrations could result in all

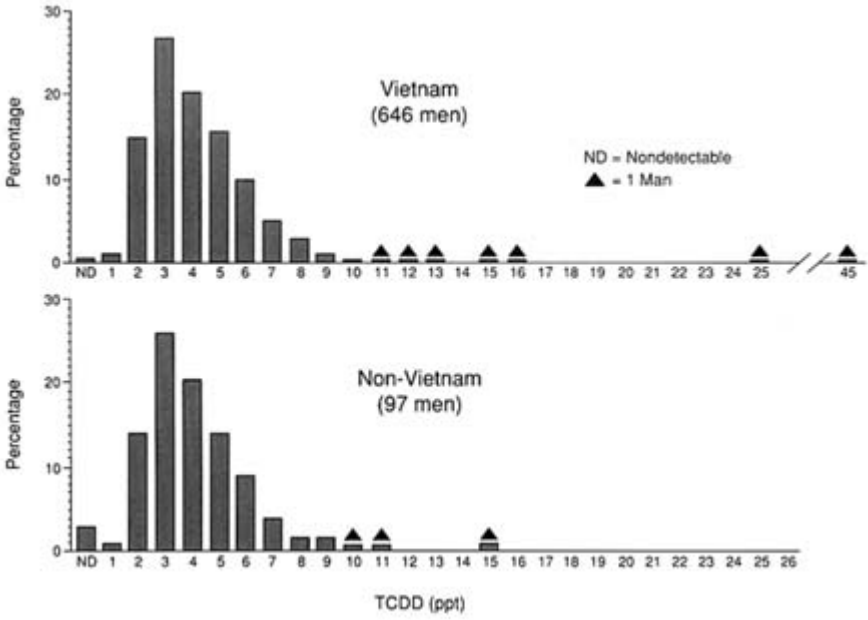

FIGURE 6-2 Distribution of serum dioxin levels in Vietnam veterans and non-Vietnam (Vietnam era) veterans, CDC Validation Study. Reprinted with permission from CDC, 1989a.

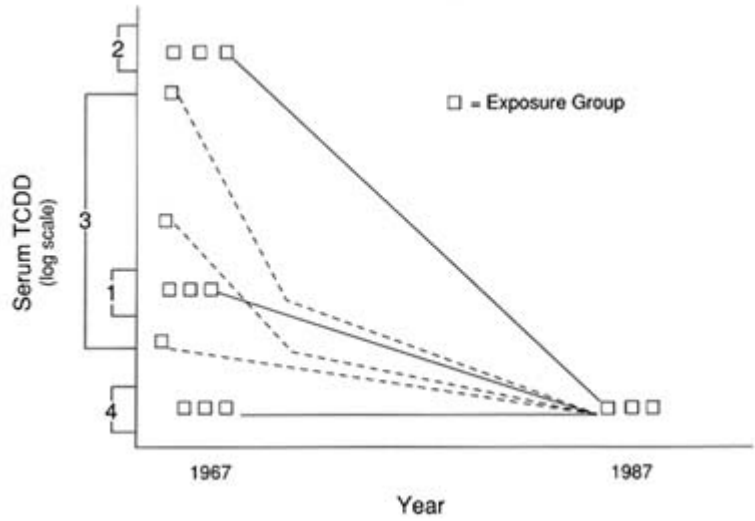

FIGURE 6-3 Possible scenarios for describing the relationship between current and prior serum TCDD levels, 1967-1987.
SOURCE: Institute of Medicine, 1987.

demonstrating current levels at background. (4) None of the veterans ever had serum TCDD concentrations above background.

Without adequate knowledge of the decay rate and pharmacokinetics of TCDD in humans, it is not possible to distinguish among the alternative explanations. If initial concentrations of TCDD differed but then over the years fell to background, estimates based on reconstruction of troop locations and herbicide spraying activities might be more reliable indicators of exposure than current serum TCDD levels.

ESTIMATES OF EXPOSURE TO HERBICIDES AND TCDD DURING VIETNAM SERVICE

As the committee's review of the literature indicates, exposure assessment has been a weak aspect of epidemiologic studies of Vietnam veterans. Different approaches have been used in estimating the exposure of Vietnam veterans, and these studies generally rely on self-reported exposures, records-based exposure estimates, or biomarkers of TCDD exposure. Each of these approaches is limited in its ability to determine precisely the degree of individual exposure. Some studies rely on gross markers such as service in Vietnam, perhaps enhanced by branch of service, military region, military specialty, or exposure to combat as proxies for exposure to herbicides. The CDC Vietnam Experience Study and Selected Cancers Study, the Department of Veterans Affairs (DVA; formerly the Veterans Administration) mortality studies, and most state veterans studies are of this sort. This approach almost surely dilutes whatever health effects of herbicides exist. At the other extreme, some studies rely on fine details of military records on troop movements and herbicide spraying, perhaps combined with self-reported retrospective data, for individuals or small units on a daily basis. The Stellmans' study and the proposed but not completed CDC AOS are examples of this sort. Even though measures of this type may be accurate for many individuals, such fine detail *may* exceed the accuracy of a record system not designed for this purpose, and the accuracy of the resulting exposure measure cannot be guaranteed for all potential subjects.

Serum TCDD measurements are difficult to interpret with current knowledge, yet they may provide valuable information in some situations. They are most useful in defining differences in exposure levels among groups rather than among individuals, and particularly between more highly exposed groups and less exposed or unexposed groups. The groups must be of sufficient size to provide the statistical power to make meaningful assessments. In general, group differences in serum levels are likely to be reflective of differences in true past exposure to TCDD, but the failure to detect mean differences does not necessarily indicate that there were no differences in past exposure.

Current serum TCDD levels in Vietnam veterans, with the exception of measured levels in Ranch Hand veterans, suggest that exposure to TCDD in Vietnam was substantially less, on average, than that of occupationally exposed workers or of persons exposed as a result of the industrial explosion in Seveso, Italy (see Figure 6-4). The CDC's Validation Study found that study subjects could not be distinguished from controls based on serum levels. The median TCDD levels for Vietnam veterans and Vietnam era veterans were both 4 ppt. Serum TCDD levels obtained from Ranch Hand veterans in 1987, however, were able to distinguish among groups of Ranch Hand veterans (based on their military duties) with different prior exposures to TCDD, although the median levels for the Ranch Hand groups were lower than those of several occupational cohorts and Seveso residents. The NIOSH study of 253 workers measured serum TCDD 23 years (on average) after their occupational exposure. Therefore, although definitive data are lacking, the available evidence concerning herbicide exposure suggests that as a group, Vietnam veterans had lower exposure to TCDD than the subjects exposed occupationally or in Seveso.

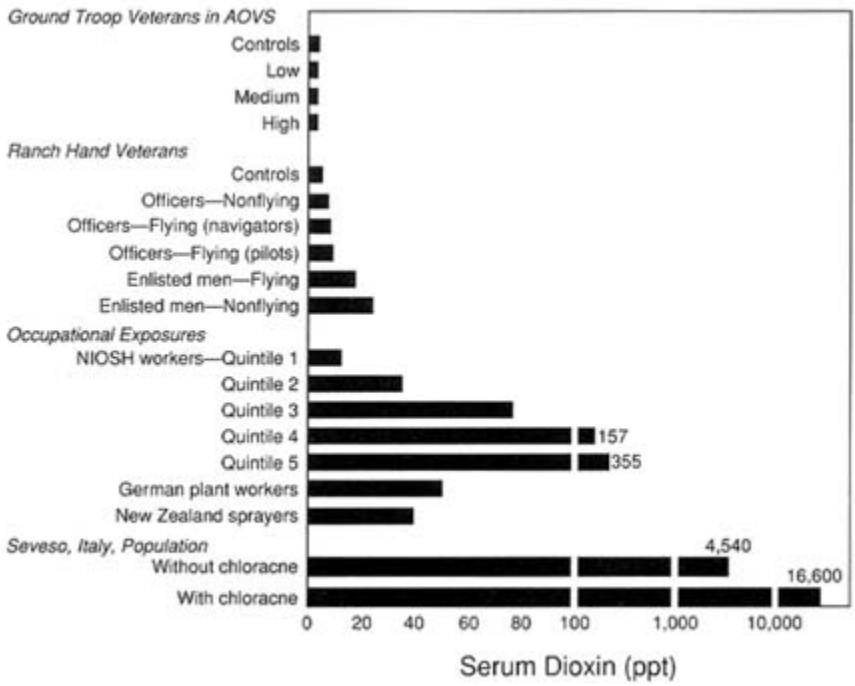

FIGURE 6-4 Median serum dioxin levels in selected populations. Reprinted with permission from Pirkle, 1993.

Historical data, personal testimony, and other information made available to the committee suggest that there was substantial variability in an individual's wartime experience, and that opportunities for exposure are also likely to have varied (see Chapter 3). Approximately 1.5 million U.S. military personnel served in South Vietnam from 1967 through 1969, the period of heaviest herbicide use; according to Air Force operations data, nearly 14 million gallons of herbicide were sprayed over Vietnam during this period. In addition to Ranch Hands, an unknown number of veterans were likely to have received varying degrees of exposure to herbicides. Localized spraying, while conducted on a smaller-scale than Ranch Hand spraying, may have resulted in higher degrees of exposure since it was done in close proximity to ground troops. Most military bases had vehicle-mounted and backpack spray units available for use in routine vegetation control programs. Approximately 750 personnel have been followed who were assigned to the Army Chemical Corps. They were responsible for the storage, preparation, and application of chemicals around the perimeters of base camps and, as a group, were likely to have been more highly exposed. U.S. Army Special Forces camps were often located in enemy territory throughout Vietnam and may have been in the target area of a Ranch Hand mission. Formation of Special Forces camps or fire support bases often required defoliation, so soldiers in these units may have been exposed to high levels of herbicides. Navy riverine units operating along the river and canals of the Mekong delta reportedly sprayed base perimeters with herbicides (Zumwalt, 1993).

Oral and written testimony presented before the committee also suggests that substantial numbers of veterans were exposed to Agent Orange and other herbicides. Results from a survey of U.S. military officers in Vietnam, across all branches of service (Army battalion commanders and advisors, Navy personnel and advisors involved in riverine operations, Air Force Ranch Hand personnel, chemical officers, Marine ground and air personnel, and advisors) indicated the utility of herbicides to their military operations (U.S. Army, 1972). In addition, surveys of veterans indicate that 25 to 55 percent believe they were exposed to herbicides while serving in Vietnam (Erickson et al., 1984a,b; Stellman and Stellman, 1986; CDC, 1989b). It appears that groups of veterans, other than those involved in Operation Ranch Hand, were likely to have been exposed to herbicides during their service in Vietnam.

It is clear that the military use of herbicides in Vietnam was not uniform either spatially or temporally, and that the movement and behavior of troops also varied, so one cannot assume that all troops were equally exposed to herbicides. In the committee's judgment, a sufficiently large range of exposures may exist among Vietnam veterans to conduct a valid epidemiological study for certain health outcomes. The difficulty (from the perspective

of epidemiological studies) is that the available data do not precisely quantify individual exposure. None of the measures that the committee has reviewed would be free of nondifferential misclassification bias. The effect of this bias on risk estimates would likely be to underestimate true effects if they existed, possibly to such an extent that these effects could be missed entirely by future studies. Thus, the committee recommends (see Chapter 12) the following set of activities.

EXPOSURE RECONSTRUCTION

The committee believes that it may be possible to develop a valid exposure reconstruction model for epidemiological studies based on existing records and structured interview data. Such a model would estimate the likelihood that each individual veteran was exposed to herbicides in Vietnam and could possibly quantify the likely degree of exposure. This model would incorporate information in existing military records about herbicide spraying (the HERBS and Services HERBS tapes) and troop movements. It would also include less formal sources of information on ground and perimeter spraying from records of herbicide shipments to various military bases, and would consider the type of terrain, typical foliage of the locations, and military mission of the bases and troops located there. Surveys and interviews of Vietnam veterans, stratified by location and period of service, might also provide useful information on situations in which herbicide spraying was prevalent and, if validated, may be incorporated into the exposure reconstruction model.

Development of the Exposure Reconstruction Model

This new effort would model the conditions under which herbicides were used in Vietnam and should consider the following information:

1. *troop location*—based on all available military records, including morning reports, daily journals, situation reports, intelligence summaries, ORLL, and combat operations after action reports;
2. *aerial spray mission data*—from the HERBS and Services HERBS tapes;
3. *estimated ground spraying activity*—not based solely on existing records (discussed in more detail below);
4. *estimated exposure opportunity factors*—including the identification of occupations involving the handling of herbicides, and considerations of how likely troops were to have heavier exposure through eating local food, bathing in or drinking local water, contact with contaminated soil, etc.;
5. *military indications for herbicide use*—systematic, historical reviews

of the conditions under which military use of herbicides was warranted, including information on typical use patterns in those situations such as the Army survey of military commanders conducted in 1971 (U.S. Army, 1972); and,

6. *considerations of the composition and environmental fate of herbicides* —including changes in the TCDD content of herbicides over time, the persistence of TCDD and herbicides in the environment, and the degree of likely penetration of the herbicides into the ground.

Once an exposure reconstruction model based on these data has been developed, it should be possible to estimate an exposure score for the large numbers of veterans needed for epidemiologic studies.

Considerable research has also been conducted on the environmental fate and persistence of TCDD and this information could be incorporated into the exposure model. TCDD deposited on foliage and the surface of soil could be photodegraded within a matter of days. Volatization could also be a significant removal mechanism. Once TCDD penetrates the surface, it could remain stationary for an indefinite period. It could persist essentially immobile in the soil for years. There is little translocation of TCDD within plants, although plant roots in contact with TCDD contaminated soil would, in turn, be contaminated.

Ground spraying, although probably representing a smaller quantity of herbicide than aerial spraying, may actually have resulted in heavier human exposures since it probably was done in closer proximity to ground troops, at higher application rates (i.e., number of gallons per acre), and potentially by less trained individuals than Ranch Hand spraying. This spraying was often performed around camp perimeters and along communication routes in response to enemy attack or during relocation to a new fire base. Thus, troops may often have remained in the area during spraying or passed through it soon afterward. Ground spraying could reportedly be approved by unit commanders at the Corps level, and the spraying does not appear to have been documented as carefully as the aerial missions of Air Force Ranch Hands. To incorporate estimates of exposure from ground spraying, the committee suggests the development of a subsidiary estimation model for this source of exposure. Because ground spraying data are incomplete, this model would be based on a series of factors likely to determine the extent of ground spraying in Vietnam. From testimony presented to the committee, it appears that the following factors might have been important determinants of this activity: region and date of military service, terrain, type of vegetation, intensity of enemy activity, any existing data on shipment of herbicides to different regions, and size of military base. Through structured interviews with military personnel who served in various regions and various military units, data would be gathered to permit the development of

a model predicting the likelihood and intensity of ground spraying around base perimeters, fire camps, and along roads for different areas and times. A partial check on the utility of this model could be accomplished by comparing its predictions to the limited ground spraying data that do exist in the Services HERBS tapes. This proposal incorporates ideas for exposure reconstruction previously described by others (Bricker, 1981; Erickson et al., 1984a; Stanton, 1989; Lewis, 1993).

Evaluation of the Exposure Reconstruction Model

The overall exposure reconstruction model can be evaluated in several ways. First, model developers should determine whether the data used in the exposure reconstruction model are internally consistent. This involves checking whether existing spraying records indicate more spraying in areas where it was likely to have been militarily useful from the point of view of terrain, foliage, and military mission. It would also be possible to cross-check the estimated spraying intensity data with a systematic survey of the recollections of veterans who served in particular areas.

In a second method of evaluation, exposure estimates based on the reconstruction model should be compared with serum TCDD measurements for a random sample of veterans, stratified according to records-based measures. Although the committee concludes that group differences can be useful in confirming that exposure measures reflect the differences in prior exposure, the absence of group differences cannot be interpreted to indicate that groups were not exposed earlier. Serum TCDD measurements should not, however, be regarded as a gold standard—a perfect measure of herbicide exposure. In addition to the problems with interpreting serum TCDD measures discussed above, some of the herbicides used in Vietnam such as Agent White did not contain TCDD, so it is possible for a veteran to have been exposed to a large amount of Agent White without having an elevated serum TCDD level at any time.

A third evaluation of the exposure estimation strategy would be to assess the association between the exposure reconstruction estimates and the incidence of health outcomes that are truly associated with herbicides. One would expect a positive association between the exposure reconstruction measure and those outcomes found in this report to have sufficient evidence for a statistical association. For instance, the non-Hodgkin's lymphoma data from the CDC Selected Cancers Study (CDC, 1990a) and the DVA case-control study (Dalager et al., 1991) can be combined and exposure measures calculated on the basis of the new exposure reconstruction model. Because there are sufficient data from occupational studies to suggest an association between herbicides and/or TCDD and NHL (see Chapter 8), one would expect to see a positive association between this cancer and the new

exposure reconstruction model data in the combined cases from the CDC and DVA studies. If such an association were found, it could be interpreted as positive evidence for the validity of the new exposure model. If no association were found, it would not be clear whether this was due to problems in the new exposure measure, to small sample sizes or low average herbicide exposure even in those exposed, or to the lack of a real association between herbicides and NHL.

Exposure reconstruction models for herbicides in Vietnam must be thoroughly evaluated before epidemiologic studies based upon them proceed. Chapter 12 contains recommendations about how this evaluation should be carried out by an independent, nongovernmental scientific panel with expertise in historic exposure reconstruction and epidemiology, and how the resulting exposure measures should be used in a program of epidemiologic studies of Vietnam veterans.

SUMMARY

The existing epidemiologic data base reviewed by the committee is severely lacking in quantitative measures of individual exposure to herbicides and TCDD. Assessment of the intensity and duration of individual exposures is a key component in determining whether specific health outcomes are associated with exposure to TCDD or other chemicals found in the herbicides used in Vietnam. Although different approaches have been used to estimate exposure in Vietnam veterans and in others exposed occupationally or environmentally, each of the approaches is limited in its ability to determine precisely the degree and level of individual exposure. New biochemical techniques that can detect small amounts of TCDD in the blood many years after exposure have some merit, especially for detecting *group* differences. However, because of common background exposures to TCDD, poorly understood variations among individuals in TCDD metabolism, and relatively large measurement errors, *individual* TCDD serum levels are usually not meaningful. Furthermore, because not all of the herbicides used in Vietnam contained TCDD, serum TCDD levels are not good indicators of overall exposure to herbicides.

Although definitive data are lacking, the available quantitative and qualitative evidence about herbicide exposure suggests that Vietnam veterans as a group had substantially lower exposure to herbicides and TCDD than the subjects in many occupational studies. The participants in Operation Ranch Hand are an exception to this pattern, and it is likely that others among the approximately 3 million men and woman who served in Vietnam were exposed to herbicides at levels associated with health effects. It is clear that the military use of herbicides in Vietnam was not uniform either geographically or temporally, and that the movement and behavior of troops also

varied, so one cannot assume that all troops were equally exposed to herbicides. Thus, in the committee's judgment, a sufficiently large range of exposures may exist among Vietnam veterans to conduct a valid epidemiologic study for certain health outcomes. The difficulty (from the perspective of epidemiologic studies) is that the available data do not precisely quantify individual exposure. None of the measures that the committee has reviewed would be free of nondifferential misclassification bias. The effect of this bias on risk estimates would likely be to underestimate true effects if they existed, possibly to such an extent that these effects could be missed entirely by future studies.

The committee believes that it may be possible to develop a valid exposure reconstruction model for epidemiologic studies based on existing records and structured interview data, using principles of historic exposure reconstruction developed by industrial hygienists. Such a model would estimate the likelihood that each individual veteran was exposed to herbicides in Vietnam, and could possibly quantify the likely degree of exposure. This model would incorporate information in existing military records about herbicide spraying (the HERBS and Services HERBS tapes) and troop movements. It would also include less formal sources of information on ground and perimeter spraying from records of herbicide shipments to various military bases, and would consider the type of terrain, typical foliage of the locations, and the military mission of the bases and troops located there. Surveys and interviews of Vietnam veterans, stratified by location and period of service, might also provide useful information on situations in which herbicide spraying was prevalent and, if validated, may be incorporated into the exposure reconstruction model.

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7

Epidemiologic Studies

In seeking evidence for associations between health outcomes to be considered in subsequent chapters and exposure to herbicides and TCDD, a variety of different kinds of epidemiologic studies must be considered. Each study has varying degrees of strengths and weaknesses, and contributes evidence to an association with the health outcomes considered in Chapters 8 through 11 in accord with a balance of these factors. The historical basis for the variety of groups studied with respect to herbicides and TCDD has been discussed in Chapter 2. How the actual articles reviewed were selected from the literature for this study is described in Appendix A.

In this chapter, the epidemiologic studies and reports that were reviewed by the committee are summarized to present the study methods used, including, where available in the articles, how the study subjects were ascertained; how the data were collected; the inclusion criteria; and how the exposure, including 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), chlorophenols, and the TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) contaminant, was determined. Additionally, the numbers in the study and comparison populations, when available, are given along with a brief description of the study in Tables 7-1, 7-2, and 7-3. No results are presented here; rather the chapter provides a methodologic framework for the health outcome chapters that follow. Qualitative critique of the study design, population size, methods of data collection, case and control ascertainment, or exposure quality has been reserved for the individual health outcome chapters in which the results of these studies are discussed. To allow for cross-references, Appendix E provides an index

that lists where each study is discussed in this and the health outcome chapters.

The organizational structure of the chapter is indicative of the exposure groupings subsequently used in considering the evidence for individual health outcomes. The chapter text and tables are organized in three basic sections, occupational studies, environmental studies, and studies in Vietnam veterans, with subsections included under each major heading. Figure 7-1 provides a simplified basic scheme of how the chapter is organized. Each of these three major sections is organized, for the most part, with studies having the most specific or intense exposures discussed first, moving to less specific, and in many cases, group determination of exposure as compared to individual exposure determination. Studies have been reviewed that include exposures to herbicides, TCDD, and sometimes other chemicals; in several instances, it has not been indicated by the study authors to which specific chemicals study participants were exposed, or to how much. Where available in the papers reviewed, details are given with regard to exposure assessment and how exposure was subsequently used in the analysis. In order to aid interpretation, Figure 7-2 indicates the relationships among the groups of chemicals to which study subjects may have been exposed, and also indicates which are contaminated with TCDD. Among the herbicides or chemicals of special interest to this review are those which were sprayed in Vietnam, or were potentially contaminated with TCDD, including: 2,4-D, 2,4,5-T, MCPA, picloram, hexachlorophene, and chlorophenols including trichlorophenol.

The subsections in the occupational studies section include production workers, agricultural/forestry workers (including herbicide/pesticide applicators), paper/pulp workers, and case-control studies of specific cancers and the association with exposures to herbicides and related compounds in many of these occupations.

The environmental section includes studies of populations exposed to excessive herbicides in the environment, including the residents of Seveso, Times Beach and Quail Run, Vietnam, and other areas. The Vietnam veteran studies include those conducted in the United States by the Air Force, Centers for Disease Control (CDC), Department of Veterans Affairs (DVA, formerly the Veterans Administration), American Legion, and several individual states, as well as other groups. Vietnam veteran studies were also conducted in Australia and are presented here.

For each section of the chapter, there is an extensive table (Tables 7-1 through 7-3) that lists and briefly describes each of the epidemiologic studies that was brought to the attention of the committee. The studies are presented in the same general order as in the text. Studies that were published with only minor modifications in multiple publications are cited in the text with the earliest publication date, although multiple citations appear in the table.

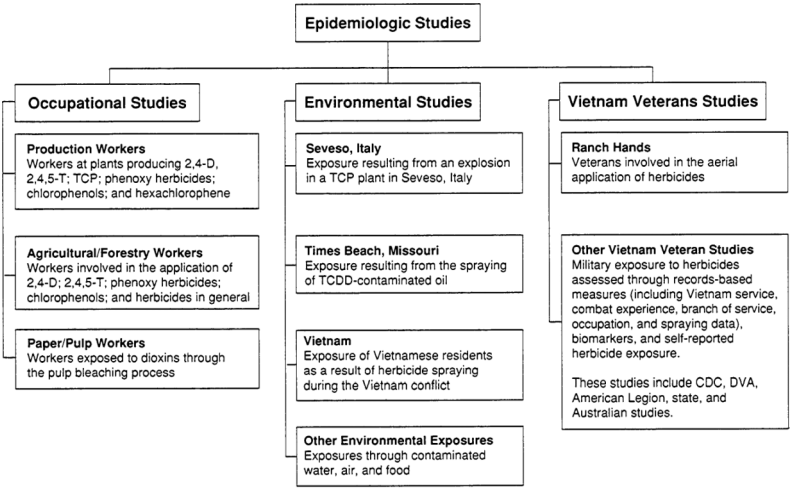

FIGURE 7-1 Organization of the epidemiologic studies.

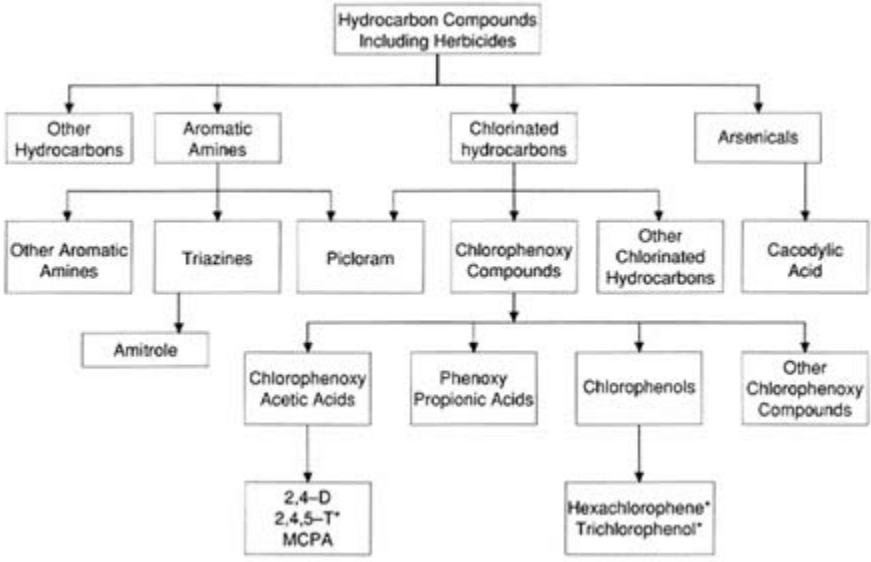

FIGURE 7-2 Hydrocarbon compounds including herbicides.
NOTE: * = compounds contaminated by TCDD.

OCCUPATIONAL STUDIES

Several occupational groups in the United States and elsewhere have been exposed to the herbicides of concern in this review (i.e., the ones used in Vietnam) and, more specifically, to TCDD, a contaminant of some herbicides and other products (see Table 7-1). Occupational groups exposed to chemicals in question, including herbicides, are farmers, agricultural/forestry workers, herbicide sprayers, workers in chemical production plants, and workers involved in paper/pulp manufacturing. In addition, studies linking job titles as broad surrogates of exposure and disease registry data have been conducted. Exposure measures vary widely in these occupational studies, in terms of measurement, quantification, level of detail, confounding by other exposures, and individual versus surrogate or group (ecological) measures.

The National Institute for Occupational Safety and Health (NIOSH) study by Fingerhut and colleagues (1991) described below includes in the cohort analyzed many of the individual study cohorts discussed and presented in this section of the chapter (Ott et al., 1980, 1987; Zack and Suskind, 1980; Zack and Gaffey, 1983; Moses et al., 1984; Suskind and Hertzberg, 1984; Cook et al., 1980, 1986, 1987; Townsend et al., 1982; Bond et al., 1983, 1987, 1989a,b; Sobel et al., 1987; Thomas, 1987; Calvert et al., 1991, 1992; Alderfer et al., 1992; Collins et al., 1993; Sweeney et al., in press). In the cancer health outcome chapter (Chapter 8), only the NIOSH study is referenced, because, except for one Dow cohort of 2,4-D production workers, the individual cohorts are subsumed into the larger cohort. As described below, a subset of the NIOSH cohort was selected to have serum TCDD measurements, and the NIOSH research team carried out studies of these subcohorts for outcomes other than cancer. The results of these subcohort studies are discussed, where appropriate, in Chapters 9, 10, and 11.

There are numerous studies of farmers and their mortality and morbidity experience to be found in the literature. The committee has included studies of farmers in which keywords in literature searches indicated herbicide exposure, or in which the authors of the articles state in their introduction that such exposures are hypothesized to be associated with the disease outcome, or which were identified through searches of the secondary literature.

Production Workers

National Institute for Occupational Safety and Health

In 1978, NIOSH undertook to identify all U.S. workers potentially exposed to TCDD in the manufacture of contaminated products between 1942 and 1984 (Fingerhut et al., 1991). For 12 chemical companies, 5,000 workers

were identified from personnel and payroll records indicating that the workers had been involved in production or maintenance processes associated with TCDD contamination. Derivatives in the process of producing 2,4,5-trichlorophenol and in which TCDD was a contaminant included 2,4,5-trichlorophenoxyacetic acid, Silvex, Erbon, Ronnel, and hexachlorophene. An additional 172 workers previously identified by their employers as being exposed to TCDD were also included in the study cohort. TCDD was also measured in serum from a sample of 253 workers. Vital status as of December 31, 1987, was determined; death certificates were used to establish numbers of deaths from each cause. Person-years were calculated from the first documented assignment to a process involving TCDD contamination until date of death or December 31, 1987. Vital status was determined for all but 77 members (2 percent) of the cohort. Those with unknown vital status were assumed to be alive. General U.S. population rates were used for calculation of expected deaths. The 12 plants involved were large manufacturing sites of major chemical companies. Thus, many of the study subjects had exposures to many other chemicals, some of which could be carcinogenic. Data were analyzed for mortality according to duration of exposure in processes involving TCDD contamination (determined from personnel records) and latency; total years of employment at the plant was also considered.

A cross-sectional study that included a comprehensive medical history, medical examination, and measurement of pulmonary function was conducted on workers employed more than 15 years earlier in the manufacture of chemicals with TCDD contamination at chemical plants in Newark, New Jersey (1951-1969) and Verona, Missouri (1968-1969, 1970-1972) (Sweeney et al., 1989, in press; Calvert et al., 1991, 1992; Alderfer et al., 1992). The plant in New Jersey manufactured TCP and 2,4,5-T (N = 490 eligible); the Missouri plant manufactured TCP, 2,4,5-T, and hexachlorophene (N = 96 eligible). Production of these chemicals produces TCDD in the process as a contaminant, which was the actual exposure of interest. Information on health status, occupational history, time in Vietnam, time in agriculture, residential history, hospitalizations, medications, demographics, and life-style variables was collected through interview. Health outcomes of interest included peripheral neuropathies, neurobehavioral effects, chloracne, pigmentary changes, skin cancer, hepatic enzyme changes, porphyria, angina, myocardial infarction, ulcers, lipid changes, diabetes, lymphocyte cell types and function, and adverse reproductive outcomes including fetal loss, reduced fertility, and major malformations (Sweeney et al., 1989). Physical examination included (1) clinical assessment of respiratory function and adverse health outcomes, including chronic bronchitis, chronic obstructive pulmonary disease (COPD), ventilatory function, and thorax and lung abnormalities (Calvert et al., 1991); (2) assessment of hepatic and gastric

systems including determination of laboratory tests associated with liver function, hepatitis, cirrhosis, fatty liver, gastritis, gastrointestinal hemorrhage, and ulcer disease (Calvert et al., 1992); (3) psychological testing to determine presence of depression (Alderfer et al., 1992); (4) assessment of peripheral neuropathy through examination, electrophysiologic and quantitative sensory tests, and symptoms (Sweeney et al., in press). Serum levels of TCDD were determined as indicating exposure and were adjusted for lipids. The matched comparison group consisted of individuals with no occupational exposure to phenoxy herbicides who lived in the same communities as the workers and were within five years of age, and of the same sex and race as the exposed worker; comparison subjects underwent the same series of medical examinations and interviews as workers exposed to TCDD (Sweeney et al., in press). A total of 281 workers and 260 unexposed referents participated in the medical examination; 360 exposed worker interviews and 325 neighborhood interviews were completed. Data on important confounders, including cigarette and alcohol consumption, were collected and adjusted for in the analyses.

Monsanto

Cohort members of studies of Monsanto workers described in this section are included in the assembled cohort by Fingerhut et al. (1991) described above. On March 8, 1949, a violent reaction occurred in the trichlorophenol (TCP) production process at the Nitro, West Virginia, plant of Monsanto (Zack and Suskind, 1980). Fumes and tarry residues were discharged into the atmosphere and building interior when a relief valve opened. One hundred and twenty-one male workers who developed chloracne following this accident were identified for inclusion in the study cohort from plant safety records at the time of the accident, plant medical records, and workers' compensation records. Chloracne was used as an indicator of TCDD exposure. Vital status of all cohort members was determined as of December 31, 1978; death certificate-coded causes of death were compared with expected deaths, based on U.S. population rates. Information is not provided about the magnitude of the exposures any individual might have experienced. Each person was assumed to have entered the study on March 8, 1949, the date of the accident. The group was selected for exposure to TCDD, and potential exposure to other chemicals is not discussed. No information is provided on the total number of individuals in the process area at the time of explosion or on the number involved in the cleanup.

An additional Monsanto study evaluated health outcomes among a group of active and retired workers involved in any aspect of producing 2,4,5-T, including maintenance, and employed between 1948 and 1969 at the Nitro, West Virginia, plant (Suskind and Hertzberg, 1984). The exposed group

included men who had been exposed to the accident described above, as well as workers potentially exposed during manufacturing processes; comparison subjects were male workers at the same plant who, according to interview and work history, had never been exposed to 2,4,5-T processing. A total of 436 individuals who worked at the plant volunteered to be interviewed and examined during the week of July 11-18, 1979. The participation rate among the exposed group, those who worked in the 2,4,5-T process, was 61 percent and among the unexposed group 46 percent. Of the 436 examined, 69 were excluded because of demographic considerations or because their exposure history could not be adequately documented. Of the remaining 367 participants, 204 had a history of exposure and 163 had never worked in 2,4,5-T production or maintenance. Data on health outcomes were determined from laboratory results, physical examination, and medical history; smoking and alcohol use histories were also obtained. All comparisons were between these two cohorts.

All workers, current and retired, identified by union records as occupationally exposed to 2,4,5-T production were invited to participate in a health survey in April 1979 in Nitro, West Virginia; workers without known exposure were invited to participate in a control group (Moses et al., 1984). The exposure source included a factory explosion in 1949 and daily exposure through work from 1948 to 1969; the comparison group was factory workers from the same plant. Of the 425 workers invited to participate, who were potentially exposed and unexposed, 235 actually participated; after exclusions, 226 subjects were available for analysis. The final cohorts consisted of 117 men with current or reported history of chloracne and 109 men with no chloracne. Of interest to investigators was the difference in health outcomes reported among those indicating the presence of chloracne as a surrogate of exposure, compared to those who reported no chloracne. It was recognized that those without chloracne may have been exposed to TCDD and were not "unexposed controls." Participants were interviewed on lifetime occupational history, current symptoms, past medical history, reproductive history, medication, and tobacco and alcohol use; examination included dermatologic, neurologic, and laboratory tests. Worker recall was originally used to determine exposure to 2,4,5-T, but this classification was not useful for analyses, which motivated development of a different mechanism for classifying exposed versus unexposed: the result was classification of exposure based on chloracne as a surrogate.

All workers at the Nitro, West Virginia, Monsanto plant who worked for at least one day between March 8, 1949 (date of accident), and November 22, 1949 (date last chloracne case from cleanup was reported), were identified from work records and Internal Revenue Service Form 941 and followed for mortality through December 31, 1987 (Collins et al., 1993). Vital status for the cohort of 754 workers was determined from Monsanto

files, the Social Security Administration (SSA), the National Death Index (NDI), state motor vehicle records, and credit bureaus; death certificates were obtained for 99 percent of the 363 deceased cohort members. Exposure to TCDD was of interest in the study and was determined from company records of chloracne or chloracne-like events and review of company processes for additional cases exposed. A comparison with local population rates was conducted for mortality differences with the exposed group experiencing chloracne and the group presumed to be unexposed because they did not have chloracne. To examine the effects of exposure to 4-aminobiphenyl, a potential carcinogen to which workers may have been exposed, subcohorts of the original study population were determined.

A cohort mortality study was undertaken to evaluate the mortality experience of the entire white, male Monsanto hourly work force with one or more years of employment on or after January 1, 1955, and prior to December 31, 1977, in Nitro, West Virginia (Zack and Gaffey, 1983). A total of 884 men were followed for mortality and compared to mortality rates in the U.S. population. For those deceased, an additional proportionate mortality analysis was conducted among those potentially exposed to 2,4,5-T and those unexposed; exposure was based on assignment to a 2,4,5-T operation in the company as indicated in work history records. Government earnings reports were used to identify eligible cohort members on company file between 1951 and 1977; mortality was ascertained for the cohort through December 31, 1977.

Dow

Several studies of workers in the Dow chemical plant have been published and are described in this section. It should be noted, however, that the population discussed here, except for one article by Bond and colleagues (1988) is included in the NIOSH study by Fingerhut and colleagues (1991), and results of the subsumed studies are addressed in the individual health outcome chapters as described in the introduction. The first publication examining the Dow Chemical Company's work force engaged in the production of 2,4,5-T was a study of the mortality experience of 204 persons exposed to the chemical during manufacturing (Ott et al., 1980). Ott and colleagues reported on a cohort that manufactured 2,4,5-T from March 1950 until May 1971, along with several other chemicals to which workers were potentially exposed. 2,4,5-T manufacturing was done by a crew of four, including a reactor operator, a salt wheel operator, an acid wheel operator, and a dryer operator; the 2,4,5-T dust in the plant was suspected to be a result of the final drying process. Identification of study subjects involved determining, from census lists for 1951-1971, who was in the departments where the process was organized; secondly, the work histories

of each person were reviewed to determined experience in any of the four jobs listed above. This constituted the exposed cohort of 204 members, with exposure determined by length of time on the four jobs. Vital status of the cohort was determined through the SSA and verified by study subject or relative; comparisons in outcome were to the mortality rates of the U.S. white male population by five year intervals. Follow-up of the population extended from first exposure to 2,4,5-T until December 31, 1976.

An outbreak of chloracne among employees from a trichlorophenol manufacturing area of the Dow Chemical Company, with possible exposure to TCDD, occurred in 1964 (Cook et al., 1980). All 61 employees who worked in the particular production area during 1964 constituted the cohort, as identified from company census lists. Study subjects were assigned by an industrial hygienist to high (*N* - 39, including workers involved in production, sampling, and maintenance of the immediate area) and low (*N* = 22, including workers who worked away from the most highly contaminated areas) potential exposure to TCDD by job classification. Acne-like lesions were observed in 49 of the 61 employees; 34 of 39 highly exposed and 15 of 22 low-exposed workers had chloracne. Exposure groups were further divided into those employed in the process area before June 1964 or during July to December 1964. Workers were followed for ascertainment of vital status through December 31, 1978, and comparisons were to expected numbers of death according to cause-specific and age-specific rates for the U.S. white male population. One worker with fibrosarcoma who died in 1975 appears in later studies (Bond et al., 1987; Ott et al., 1987; Fingerhut et al., 1991) as the individual with a death certificate diagnosis of soft tissue sarcoma (STS) that was later determined to be a renal clear cell carcinoma based on review of tissue specimens.

In an extension of the earlier study (Cook et al., 1980), Dow employees with chloracne, established on the basis of past diagnosis or clinical description, were enrolled in a prospective mortality study (Bond et al., 1987). The cohort consisted of 322 chemical workers with chloracne, out of 2,192 Dow employees with medical records in the NIOSH Dioxin Registry. Chloracne was graded as "definite," "probable based on recorded diagnosis," "probable based on clinical description," "possible," and "none." Only those diagnosed as definite or probable constituted the chloracne group. Follow-up for chloracne cases extended from the year of diagnosis and continued until they were lost, died, or January 1, 1983. Mortality was compared to the U.S. white male population, adjusted for age and calendar year, as well as to a group of employees who did not have chloracne.

Follow-up of Dow employees, using cohorts previously studied for potential manufacturing exposure to TCDD (Cook et al., 1980; Ott et al., 1980), compared medical examination and morbidity surveillance from 1976 to 1978 with those of matched unexposed employees (Bond et al.,

1983). From the original cohorts of 204 (Ott et al., 1980) and 61 participants (Cook et al., 1980), 135 and 48, respectively, were included for this study based on whether they were eligible to participate in the medical examination surveillance available at the company between 1976 and 1978. Controls were selected from others who had participated in the surveillance, but who were not exposed to high levels of chemicals potentially containing TCDD. Four controls were matched to each exposed worker by year of birth within five years, salary or hourly payment, smoking habits, and year and month of most recent health examination. Outcomes of interest were results of medical laboratory tests and prevalence of disease.

With growing concern about health effects of TCDD exposure, Dow Chemical Company assembled a cohort of 2,189 men identified from company census lists and personnel records at the Midland, Michigan, plant. Workers identified were involved in TCP production, neutralization, and distillation; 2,4,5-T and Silvex production and support; 2,4,5-T formulation; Ronnel production and support; Erbon production; and chlorophenol production and finishing. These processes potentially involved contamination with TCDD. Workers were followed to determine patterns of mortality between 1940 and 1979 (Cook et al., 1986). A detailed exposure profile was developed by Dow industrial hygienists, which included job and process descriptions, analyses of process and product streams, and all available industrial hygiene records. Intensity of exposure to TCDD was measured on a scale of 0 to 4 for every relevant job; each increase in score represented approximately a tenfold relative increase in exposure. Additionally, duration of exposure was calculated to complete the cumulative exposure index used for analyses. The U.S. national mortality rates for white males were used for comparisons. Vital status was obtained for all but six of the 2,189 men who comprised the cohort, and death certificates were obtained and reviewed by a nosologist for all 298 known deaths. A cause-specific dose-response analysis was undertaken according to five categories of the TCDD exposure index.

In an extension of the cohort mortality study described above, an additional three years of follow-up were included (through 1982), and more detailed analyses by exposure categories were undertaken among 2,192 Dow chemical workers (including 5 women who were subsequently excluded from the analysis) who had potential exposure to chlorinated dioxins (Cook et al., 1987; Ott et al., 1987). Comparisons, as done previously, were with U.S. white male mortality rates. Eligible employees were included as previously described, for the period 1940-1980, with an exposure profile developed for each employee. The assessment included (1) description of activities or tasks of job classifications; (2) development of process flow diagrams and potential changes; (3) review of analytic and bioassay data

related to dioxin in process streams and end products; (4) compilation of available industrial hygiene data; (5) assessment of TCDD and hexa- to octachlorodibenzodioxin (H/OCDD) exposure intensity score; and (6) development of an exposure profile using computer algorithm (Ott et al., 1987). No air exposure data were available, but some data on TCDD content of wipe tests and contamination of process streams or product content were given. Average concentrations for all samples of TCDD in each of four herbicides ranged from 0.3 to 5.1 parts per million (ppm) over various periods from 1964 to 1979. Higher average concentrations, up to 1,818 ppm, were present in process streams. The same TCDD exposure intensity index was used as previously described (Cook et al., 1986). Additionally, scoring of 0 to 2 was made for H/OCDD. Latency analysis was done using the serially added expected dose model, which compares the cumulative dose of individuals exposed to TCDD with others not so exposed over years of employment. A later study provided two additional years of follow-up through 1984 for this group of 2,192 employees identified as having potential exposure to chlorinated dioxins (Bond et al., 1989b).

Among this Dow cohort of 2,192, company medical charts were reviewed to determine whether they were ever diagnosed as having chloracne (Bond et al., 1989a). A study of risk factors for chloracne among these cases, compared to those without chloracne, was conducted. Chloracne cases were considered as definite or probable, according to clinical description in the company medical records. Logistic regression analysis was used to adjust for factors including demographics, work history, and exposure data, previously described.

Dow also undertook a cohort mortality study of workers exposed to 2,4-dichlorophenoxyacetic acid (Bond et al., 1988). The herbicide was manufactured in several Dow plants; in some plants it was the only chemical produced, and in others, formulation of 2,4,5-T and other herbicides containing TCDD also took place. It was estimated that 77 percent of the group had opportunity for exposure to TCDD or H/OCDD, due to the proximity to 2,4-D manufacturing. Prior to 1950, levels of 2,4-D ranged from 0.5 to 3.0 mg/m^3. Thereafter concentrations decreased to 0.2-0.8 mg/m^3, depending on the job classification. After 1978, 2,4-D concentrates were below the detection limit of 0.01 mg/m^3. The cohort was comprised of 878 workers from four production areas and was followed from 1945 until employee death, loss to follow-up, or December 31, 1982. Analysis was conducted according to cumulative dose of 2,4-D, as determined from information including job history lists, industrial hygiene data, and years on the job. Allowance for latent period was made by lagging exposures by an interval of 15 years. Expected numbers were calculated for two comparison groups. The first comparison group consisted of U.S. white men, adjusted for age and calendar year, and the second comparison group was with all other male

employees of the company. This comparison was adjusted for age, interval since entry into follow-up, and pay status.

In the only case-control study of the Dow Chemical Company, in Midland, Michigan, STS cases were identified among more than 37,000 chemical workers who had been employed for at least one year between 1940 and 1979 (Sobel et al., 1987). Cases were identified from death certificates listing diagnosis of STS as the underlying or contributing cause; where possible, medical records, pathology reports, and tissue specimens were obtained and reviewed. A combination of the best available information from death certificates, medical records, and histopathology reports was used to determine case definition. Controls were matched from among other Dow employees who had been employed for at least one year and had not died from an STS-related disease. Nine controls were individually matched to cases on sex, race, birth, and year of hire within one year; survival of controls had to have been at least as long as that of cases. As described for other studies, exposure history was determined from a number of sources. Information on potential confounders was obtained from medical record review. Analyses were based on 14 cases (4 women, 10 men) and 126 matched controls.

As part of Dow Chemical Company's surveillance program, reproductive outcomes among employees were evaluated for possible association with paternal exposure to TCDD or other polychlorinated dioxins (Townsend et al., 1982). Wives whose husbands had been potentially exposed for at least one month to dioxins during 1939-1975 were identified through company census lists. There were 930 male employees qualified according to potential exposure to dioxin, and 586 had potentially eligible wives; 370 agreed to be interviewed. A control group of equal size was established from among lists of male employees who had worked for Dow for at least one month, but not in potentially exposed areas; of 559 wives potentially eligible as controls, 345 were interviewed. Tracing was accomplished by using company benefit records. Estimation of father's exposure was through methods similar to those described for earlier Dow studies. Women were interviewed in person where possible, and questionnaire responses included information on demographics, marital and familial history, and relevant pregnancy outcomes. Congenital malformations were coded as to whether diagnosis had occurred prior to one year after birth or whether the time of diagnosis was uncertain. If conditions were diagnosed after the first year, the information was reviewed by a physician blind to the exposure group, to determine if the malformation could be diagnosed in the first year of life. If conception occurred before the father's exposure to dioxin, the conceptus was assigned to a "no-exposure" group, although the father had been exposed. Therefore, because of these regroupings, the final analysis was based on whether the conceptus was potentially exposed to dioxin through

paternal job exposure, rather than whether the employee was exposed. Outcomes of interest were live births, miscarriages, and stillbirths; birth defects were coded only among live births.

BASF

In Germany, an uncontrolled reaction during a trichlorophenol process at BASF Aktiengesellschaft on November 17, 1953, resulted in the exposure to TCDD of workers in the plant, who were identified and followed for mortality, along with additional workers who were potentially exposed in the building following the accident. The BASF study cohort originally identified by Thiess and colleagues (1982), described below, is later subsumed by Zober and colleagues (1990) for extended follow-up; therefore, in the health outcome chapters, the results of Zober et al. are discussed.

Investigators reported results of a mortality study undertaken 27 years after persons were exposed to TCDD in the explosion during trichlorophenol processing at BASF (Thiess et al., 1982). There were 70 persons initially exposed and 4 persons involved in cleanup operations for a cohort of 74; no data on direct exposure to TCDD were available because no TCDD measurements had been taken at the time of the accident. Follow-up of the cohort was maintained by the company and was complete; death certificates were obtained from public offices. Comparisons were made with external population control groups (town of Ludwigshafen, governmental district of Rhinehessia-Palatinate, and country of former West Germany) and two internal comparison groups selected from participants in other studies who were unexposed to TCDD; all were matched to the exposed subjects by age and date of entry to the factory.

Additional mortality of BASF employees was evaluated by Zober and colleagues (1990). Of the 247 employees followed, three study cohorts, a basic cohort and two additional cohorts, were assembled to establish all those exposed during the accident at the BASF plant as well as during cleanup operations, for follow-up over 34 years. The potential amount and reliability of exposure information were the defining factors in compiling the cohorts. The basic cohort consisted of those workers who were on the May 5, 1954, list as being exposed during the accident (N = 69); of the 69 in this cohort, 66 were included in the cohort of Thiess et al. (1982). The first additional cohort identified by the BASF Occupational Safety and Employee Protection Department consisted of those workers who had been potentially exposed by August 31, 1983, since more people were reporting potential exposures to the company medical department (N = 84); the degree of exposure for this group was less clear, according to the authors, than the basic cohort. The second additional cohort was assembled (1984 through December 1987) through the "Dioxin Investigation Programme," which informed

employees of potential hazards from exposure and identified, through a variety of methods, other employees, investigators, and demolition workers potentially exposed (N = 94). Occupational descriptions of jobs held by these employees were investigated, and for those included in the "Dioxin Investigation Programme," medical examinations were conducted. Vital status for the final cohort of 247 persons was established as of December 31, 1987. The cohort mortality was compared with the national mortality rates in the Federal Republic of Germany for different periods of time since the first exposure. Analysis included a subcohort of members of the three cohorts described above with chloracne or erythema for cancer outcomes.

Other Chemical Plants

All workers in a Hamburg-Moorfleet, German herbicide plant contracted to Boehringer for at least three months between 1952 and 1984 were identified from company and union records for follow-up (1,184 men, 399 women) through 1989 (Manz et al., 1991). Community registries were used to identify deaths, and medical records were also reviewed for cause of death. Comparisons were made to the national West German mortality experience as well as, for men, to a control group of men employed at a Hamburg gas supply company (N = 3,120). This control cohort had been used for a previous study and had been followed only through 1985; therefore, comparisons to this population were limited to follow-up through 1985 for the study group, as well. This control group was required to have been employed in the gas company for at least 10 years, whereas inclusion in the Boehringer cohort required 3 months of employment. The gas company control group was selected to account for potential bias from the healthy worker effect. Exposure to TCDD was determined by production processes in which employees were involved; exposed workers were classified as having high, medium, or low exposure according to work department. Duration of employment and year employment began were also considered in relation to TCDD exposure. A sample of workers (N = 48) was also tested for TCDD levels in adipose tissue for comparison to the categorizations listed.

A study involving numerous cohorts from different countries was conducted by the International Agency for Research on Cancer (IARC) (Saracci et al., 1991). Independently conducted studies of various cohorts exposed to phenoxy herbicides and chlorophenols were combined for this study cohort. Several of the individual studies are also described in this section. Although several studies in this section have been included in the overall cohort, they are of sufficiently different potential exposure that some of these individual study results are discussed in the health outcome chapters.

The cohort of international workers, the "International Register of Workers

Exposed to Phenoxy Herbicides and Their Contaminants," included information on mortality and exposures of 18,390 workers, which includes 16,863 men and 1,527 women. In an effort to avoid the problems of small studies with insufficient power to detect increased cancer risks, Saracci and colleagues (1991) at the IARC created a multinational registry of phenoxy herbicide- and chlorophenol-exposed workers. The Danish production worker cohort studied by Lynge (1985) is included in this larger study, as are the cohorts of Green (1991), Coggon et al. (1986, 1991), and Bueno de Mesquita and colleagues (1993). The cohort of Lynge (1985) contributes a very large fraction of all the person-years in the IARC study, and all four of the deaths counted as STS [International Classification of Disease (ICD) 171]. Workers are included from 20 cohorts who had ever been involved in herbicide production or spraying, except for the Australian, Canadian, and New Zealand cohorts, which required a minimum employment of one year, six months, and one month, respectively; follow-up for all cohorts was either through the computerized systems for that country or from medical records and cancer registries.

Questionnaires were sent to factories producing chlorophenoxy herbicides or chlorinated phenols and for spraying cohorts; job histories were examined if available. The cohort was subdivided according to whether members were exposed, whether they were producers or sprayers, and the group of chemicals produced. Workers who sprayed chlorophenoxy herbicides or worked in factory departments in contact with these chemicals were considered "exposed" (N = 13,482); workers "probably exposed" had no job title but were judged to have been exposed (N = 416). Workers with no exposure status information were considered "unknown" exposure (N = 541), and those who never worked in factory departments with exposure to chlorophenoxy herbicides, or who never sprayed these chemicals, were considered "nonexposed" (N = 3,951). There were 12,492 workers categorized as producers and 5,898 as sprayers. Exposed and probably exposed workers were additionally classified by chemical produced or sprayed (9,377 chlorophenoxy herbicides, 408 chlorinated phenols, 4,133 both) and by department within manufacturing cohort (3,034 main production; 1,522 maintenance and cleaning; 1,665 other; 1,907 unclassifiable). For the analysis, results are presented for the potential categories of exposure, with the "exposed" category combining production workers and sprayers into one category. Comparison mortality rates were calculated from the World Health Organization Mortality Data Bank, standardized for sex, age, and calendar year period; mortality coding was done nationally, with a conversion table developed to allow pooling over ICD revisions. Determination of vital status began in 1955 or the date of first exposure thereafter and continued for an average of 17 years. Exposure to TCDD was assumed to be possible for those who worked producing or spraying 2,4,5-TCP and 2,4,5-T or

products. Because certain factories produced no or very little 2,4,5-T, it was possible to differentiate workers by probable TCDD exposure. Exposure was not exclusively focused on TCDD as in the NIOSH-assembled cohort (Fingerhut et al., 1991), and workers assembled by Saracci et al. may have been exposed to multiple chemicals.

In Britain, a cohort of 5,754 males employed between 1947 and 1975 by a company that manufactured, formulated, and sprayed 2-methyl-4-chlorophenoxyacetic acid (MCPA) and other phenoxy acid herbicides was followed for mortality through 1983 (Coggon et al., 1986). The exposed men were employed at the factory or spray depots, and were identified from personnel records. The recorded job title allowed for classification of potential exposure to phenoxy acids into high (chemical process workers, spray operatives, some lab occupations), low (maintenance staff, chemical stores, transport workers), and background categories (clerical, sales staff, managers). The National Health Service Central Register at Southport was the source for tracing the cohort, with the Office of Population Censuses and Surveys providing copies of death certificates. Expected numbers of deaths for age and calendar period were calculated using England and Wales national death rates; a second comparison group of rates was formed for rural areas of England and Wales for 1968-1978.

Six British cohorts contributed members to the IARC study described above (Saracci et al., 1991); results of mortality from one of those cohorts is reported above (Coggon et al., 1986), and mortality of four of the six cohorts was assessed through December 31, 1987 (Coggon et al., 1991). The four cohorts represent workers at four factories manufacturing a range of chemicals through similar manufacturing processes. A total of 2,239 men employed were included according to the following definitions: (1) factory A (N = 1,104), all manual employees during April 1975-October 1985; (2) factory B (N = 271), all weekly paid employees during March 1969-November 1985; (3) factory C (N = 345), all process workers in phenoxy plants during January 1963-December 1984, and all formulators and packers during January 1982-December 1984; (4) factory D (N = 519), all weekly paid employees during April 1969-December 1985. Personnel or wage files were used to identify workers at factories A, B, and D; workers at factory C were identified from bound registers kept by shift foremen and from personnel records. Potential exposure to phenoxy herbicides and chlorophenol was determined from job history information; those who worked only in nonphenoxy plants were considered as "background" exposed. The National Health Service Central Register and National Insurance Index were used to trace the cohorts; cancers among living workers were obtained from registered notification. Two comparisons were done, one with the national death rates for England and Wales and one using expected numbers of deaths from 1974 to 1985 for the local authority area in which the factory was located.

Eighteen workers in the United Kingdom who were exposed to TCDD as a result of an industrial accident on April 24, 1968, underwent a complete clinical assessment and laboratory series to determine immunological abnormalities 17 years later (Jennings et al., 1988). Workers may have been exposed in the building when the accident occurred, during cleanup after the explosion, or while washing and repainting the building. Fifteen controls were selected from the portering and estate management at the plant, and were matched for age, sex, percentage of ideal body weight, social class, alcohol consumption, and smoking habits.

Workers exposed and possibly exposed to the above accident were also examined 10 years later, and compared to a control group for differences in health outcomes, including reproductive outcomes and biochemical and hematological tests (May, 1982). Workers selected for examination included those reporting chloracne after the accident, those who worked regularly at the TCP plant (including laboratory workers and management personnel), and a control group (recruited from remaining laboratory staff and management). Of those with chloracne who were considered to be definitely exposed to dioxin, 41 participated; 54 employees with possible exposure were included, along with 31 having no dioxin exposure. Along with the medical examination, a complete work history was obtained; contact with TCP and related chemicals was confirmed from company records.

In the Netherlands, the National Institute of Public Health and Environmental Protection contributed a cohort to the IARC registry described above, with workers from two companies that produced several chlorophenoxy herbicides; this cohort was also evaluated apart from the IARC registry for cancer mortality (Bueno de Mesquita et al., 1993). Factory A produced primarily 2,4,5-T, which can result in exposure to TCDD contamination; in March 1963, an uncontrolled reaction in the factory resulted in an explosion in which polychlorinated dibenzodioxins (PCDDs) including TCDD were released. Anyone employed at this factory between 1955 and June 30, 1985, was eligible for study inclusion; workers contracted to clean up after the accident were also included in the cohort. Factory B produced primarily MCPA and MCPP [2-(4-chloro-2-methylphenoxy)propanoic acid], with 2,4-D produced in smaller amounts; all persons employed between 1965 and June 30, 1986, were included in the cohort. The total cohort included 2,310 workers, and follow-up was 97 percent complete; analysis was presented for the 2,074 male workers who were exposed and unexposed in the factories. The causes of death were provided from the Netherlands Central Bureau of Statistics. The important steps for phenoxy herbicide exposure, which might occur in a number of different departments, included synthesis of the chemical, formulation of the herbicide, and packaging. Since individual measures of exposure were not available, occupational history including working in the above departments and exposure to the accident was used to define exposure.

Workers were considered exposed if they worked in synthesis, finishing, formulation, packing, maintenance/repair, laboratory, chemical effluent/waste, cleaning, shipping/transport, or plant supervision; if they were exposed to the accident; or if they were exposed by proximity to the above departments. Comparisons were made to total and cancer-specific mortality using expected numbers standardized for the Netherlands; exposed and unexposed workers were also compared by selected mortality causes.

In the former USSR, 292 workers involved in the production of 2,4-D were studied through examinations for the frequency of negative health outcomes (Bashirov, 1969). A more comprehensive examination was conducted on 50 exposed individuals and 20 controls, presumed unexposed to toxic substances, to specifically study the effects of exposure on the cardiovascular system and digestive organs. Details of exposure assessment are not given.

A cohort study of cancer incidence was conducted among employees of manufacturing facilities in Denmark that produced phenoxy herbicides, including 2,4-D, 2,4,5-T, MCPA, and MCPP (Lynge, 1985). All workers in the manufacture of phenoxy herbicides in Denmark before 1982 were eligible for inclusion in the exposed study cohort; two factories were the source of identifying 4,459 workers (3,390 men and 1,069 women) who were followed for vital status and cancer incidence through 1982. Vital status was ascertained by using the Central Population Register, and cancer incidence was determined by using the Danish Cancer Registry. The incidence of cancer in the cohort was compared to the expected incidence in the entire Danish population, by sex, five year age group, and calendar period. Individual exposure was not indicated; however, department worked in the factory was used as a means of classifying those exposed.

A descriptive study followed 55 men in Prague, Czechoslovakia, for symptoms of TCDD intoxication 10 years after occupational exposure during the production of 2,4,5-T (Pazderova-Vejlupkova et al., 1981). There were originally 400 workers involved in production, 80 of whom became ill with a variety of symptoms; the 55 men described were from this original 80 and were available for long-term follow-up. TCDD was not measured in individuals; however, its presence was noted in the company product and the building structure. Medical lesions, neurological lesions, skin lesions, and psychological changes were evaluated as indicating TCDD intoxication.

Physical examination and medical histories in 1969 of volunteers who had worked in a factory producing 2,4-D and 2,4,5-T are reported (Poland et al., 1971). Workers who volunteered for physical exam ($N = 73$) were divided into groups based on present work location: administrators, lab technicians, and janitors ($N = 20$), production supervisors ($N = 11$), production workers ($N = 28$), and maintenance workers ($N = 14$). Comparisons between these groups were made for various portions of the report. In

addition to physical exam and laboratory tests for presence of porphyria cutanea tarda (PCT), chloracne, hepatotoxicity, and neuropsychiatric symptoms, medical history included occupational history, smoking, drinking, and medication history; physical exams were conducted by three separate physicians, and diagnoses were considered final. The Minnesota Multiphasic Personality Inventory was also administered.

A cohort of 1,412 white male workers employed for at least one day between 1945 and 1965 at a chemical plant manufacturing flavors and fragrances was followed to determine potential excess overall mortality and mortality from cancer (Thomas, 1987). Men were exposed to multiple chemicals in the manufacture of fragrances, flavors, aroma chemicals, and other organic substances; traces of TCDD had been found in and around plant buildings that used trichlorophenol in the production of hexachlorophene. Workers were identified from company records, and information concerning work history, as indicated by job title, was abstracted. No information was available on buildings in which the employees worked, and not all buildings were involved in the production of hexachlorophene; therefore, there was no way to identify those workers specifically exposed to TCDD. Jobs were categorized into chemical operator, maintenance worker, compounder/weigher/bulker/labeler, shipping and receiving, laboratory worker, plant chemist, engineer/draftsman, research and development, clerk/administrator, warehouse worker, and safety worker. Multiple chemical exposures were possible. Vital status as of January 1, 1981, was established, using company, SSA, credit bureau, and state motor vehicle records; person-years were accrued from January 1, 1945, or date of hire (whichever was later) through date last known alive. Deaths reported after January 1, 1981, were considered to be alive for the analysis. Cause-specific standardized mortality ratios (SMRs) were calculated for the entire study population and for several subsets by likelihood of exposure to chemicals, duration of employment, and year of hire. Rates for U.S. white men, adjusted for sex, race, age, and calendar time were used to calculate cause-specific mortality; local rates for the state in which the factory was located were used to obtain expected numbers of cancers.

Agricultural/Forestry Workers

Cohort Studies

Agricultural Workers By using both proportionate mortality and standardized mortality analyses, the mortality experience of white, male, Iowan farmers was compared to that projected from the population of white men, employed, more than 20 years old, and living in Iowa in January 1975 (Burmeister, 1981). White male death certificates for the years 1971-1978

were examined to establish coded causes of death and usual occupation. Proportionate mortality ratios (PMRs) were computed separately for all men, for men under age 65, and for men 65 and over. Exposure was "farming," with categorization of type of farming, where available. This cohort study formed the basis for further case-control studies of tumors for which the PMR was significantly elevated.

In Canada, the Mortality Study of Canadian Male Farm Operators covering the years 1971-1985 was undertaken to investigate the relationship of farm work practices, especially herbicide spraying, to the risk of all causes of mortality (Wigle et al., 1990; Morrison et al., 1992, 1993). The cohort was established by linking records from the 1971 Census of Agriculture, the 1971 Census of Population, and the 1971 Central Farm Register, which combined agricultural, population, and personal identifying information on the cohort. A second step linked the 1971 Central Farm Register to the 1981 Farm Register as a follow-up of the cohort; a third step linked the cohort to the Mortality Data Base (1971-1985) for the mortality experience. The agriculture census included information on individual number of acres sprayed with herbicides (types not specified) and insecticides, total acreage of land operated, and surrogate measures for pesticide exposure. In order to determine duration of exposure, indices for individual farmers were developed from 1971 and 1981 data; farmers appearing in both censuses on the same farm had a continuing exposure for those years. Farmers not appearing in both censuses had more uncertain continual exposure to herbicides. Also, subgroups of farmers who did not employ workers or who did not hire outside workers to do work on the farm were assumed to be more likely to be exposed, as the sole and primary worker on the farm. Other than these assumptions, no information on individual exposures was available. The Farm Register, established as a mailing list for agricultural questionnaires, contained all farm operators in the 1971 agriculture census, in addition to agricultural variables and personal identifiers.

In Saskatchewan, Canada, a total of 69,513 men over 35 were identified in this manner for study. A detailed multivariate analysis of the risk of death from non-Hodgkin's lymphoma (NHL) was undertaken (Wigle et al., 1990).

Also from the Mortality Study of Canadian Male Farm Operators, farmers in Manitoba, Saskatchewan, and Alberta, Canada, were evaluated for prostate cancer mortality (Morrison et al., 1993). Mortality records between June 1, 1971, and 1987 were linked with these names to identify all those who were at least 45 years at census or at some time in the follow-up period and who had died. Additionally, information from the 1971 registry compilation was linked with 1966, 1976, 1981, and 1986 Central Farm Registers and corresponding censuses of agriculture to examine farming practices and exposures reported, which included herbicide information except

for 1966 or 1976. Thus, individual exposure to herbicides was not determined, except as these methods allow for assumption of exposure.

A total of 145,383 farmers were eligible according to the age criterion of 45 years at some point in the follow-up period for inclusion and contribution of person-years data. Expected numbers of deaths were obtained from the Canadian prairie mortality rates, which include the three provinces from which the farmers were obtained. Potential confounders included calendar period and age, and were included in all statistical models; other variables, although not known to be associated with prostate cancer, were considered as potential confounders.

The Canadian farmer cohort was also studied further for brain cancer mortality using methods described above (Morrison et al., 1992). A total of 155,547 male farm operators from Alberta, Saskatchewan, and Manitoba provinces who were at least 35 years old at some point in the study period were followed between June 1971 and December 1987. Brain cancers and histologic information were obtained through the National Cancer Incidence Reporting System of Statistics Canada.

Two cohorts of farmers, one in Denmark and one in Italy, were evaluated for cancer experience through linkage of occupational census data with incidence of cancer in Denmark and with cancer mortality in Italy (Ronco et al., 1992). In Denmark, male and female farmers between 15 and 74 years were identified from the Danish Occupational Cancer Register for a 10 year cancer follow-up in the 1970 census population and the Danish Cancer Registry. Categories of male farmers in Denmark were "self-employed" and "employees"; for women, an additional category of "family workers" was included. In Italy, the cohort of male and female farmers was 18-74 years, identified from the 1981 census; cancer mortality was established by linking the death certificates with the census after 6 months of follow-up from the time of the census. In the Italian cohort, the two categories "self-employed" and "employees" were considered. For each of these cohorts, the respective Danish or Italian population base provided comparison rates for expected numbers. The exposure of interest was farming, with type of farming available to a limited degree. More detailed occupational groups were considered in evaluating associations with lymphatic cancers in Denmark.

In the southern Piedmont area of Italy, the use of chlorophenoxyacetic herbicides was higher than the national average, and this was therefore the site of a cohort study of cancer risk in farmers licensed to spray pesticides (Corrao et al., 1989). An original cohort of male farmers licensed between 1970 and 1974 to buy and use pesticides was established. Given the design of Italian law obligating licensure, this cohort also included agricultural workers with an exposure. Additionally, a computer file of records of all admissions to public hospitals and private clinics between January 1976 and

December 1983 in the Piedmont area was available. A linkage of the hospital admissions for malignant neoplasms (N = 44,494) with the licensed pesticide users (N = 25,945) resulted in a final exposed cohort of 642 hospitalized users of pesticides and an unexposed cohort of 18,839 hospitalized nonusers. Diagnoses were verified for birth of the study subject before 1952, and a diagnosis of bone, connective tissue, or skin tumor; tumor of the brain or other part of the nervous system; and lymphatic or hematopoietic tissue tumors. Exposure, as represented by having a license, was classified in clusters, according to type of agricultural activity in the villages; the clusters represented arable farming, woodland, and mixed areas.

Farmers who were considered to be exposed to 2,4-D (N = 32) as measured by gas chromatography testing of the urine, were compared to a group of men unexposed to herbicides (N = 25) for abnormalities in their sperm (Lerda and Rizzi, 1991). How the study groups were obtained was not detailed in the report. Exposure was possible through either oral, inhalation, or dermal contact. The study was conducted over the period of March through July 1989, with exposure periods defined as (1) exposure from August to September; (2) effect in March; and (3) possible recovery in July. Laboratory testing was done for sperm collected four days after abstaining from intercourse, and included sperm volume measured within two hours, necrospermia, sperm count, sperm motility, and morphologic sperm abnormalities.

The cancer incidence among a cohort of male and female Danish gardeners was investigated, to assess the risk of cancer among these workers highly exposed to pesticides and other chemicals (Hansen et al., 1992). The cohort was established to include all members on May 1, 1975, of a gardeners trade union, associated with the Danish Union of General Workers; 859 women and 3,156 men were identified. All identified cohort members were traced for 10 years with respect to emigration and vital status through the Danish Central Population Registre, accumulating person-years at risk from identification date through date of death, emigration, or January 1, 1985. The Danish Cancer Registry provided information on cancer incidence; additional surrogate exposure data for cases of STS, hematopoietic, or lymphatic tumors were assessed through contact with the local union, which maintained data on type of work area and duration of union membership. Exposure as a gardener included workers in greenhouses, nurseries, public parks, gardens, and cemeteries; greenhouse workers were primarily exposed to fungicides and insecticides, including chlorinated compounds, whereas outdoor gardeners were primarily exposed to herbicides, including phenoxyacetic acids (2,4-D, 2,4,5-T, and MCPA) and amitrole. Outdoor gardeners were also reported to have been exposed to insecticides and fungicides. Comparison rates were based on national incidence rates for five year age-, sex-, and period-specific groups.

Several studies involving pesticide, forestry, agricultural, and railroad workers have been conducted in Sweden. A cohort study was undertaken, based on data from the Swedish Cancer Environment Register (CER), which links population census data, including occupation, with the Swedish Cancer Registry (Wiklund, 1983). Both men and women were included in the study. A comparison was made to expected numbers of cases based on five year birth cohort and gender-specific rates of the general Swedish population. The CER is formed for a decade of census data; for example, the CER 60 links the 1961-1973 cancer data with the 1960 census. Agriculture was the primary economic activity of 19,490 of the 331,767 people with cancers listed in the CER, which defined the study cohort. Cancers observed among this cohort of 19,490 were compared to the number expected based on the 1960 Swedish census. Exposure was agricultural economic activity as indicated in the data. This study formed the basis for subsequent studies discussed.

The risk of STS among male agricultural and forestry workers with possible exposure to phenoxy acid herbicides (MCPA; 2,4-D; 2,4,5-T and some of their esters) was also studied in the cohort identified above, with additional years of diagnosis (1974-1979) available for supplementing the CER (Wiklund and Holm, 1986). The cohort consisted of 354,620 men born between 1891 and 1940, who had indicated in the 1960 census that they were agricultural and forestry workers. The reference cohort was 1,725,845 men born in the same years who had not indicated these occupations as primary economic activity. A total of 331 cases of STS were observed in the study cohort and 1,508 cases in the reference group. The greatly increased use of phenoxy acid herbicides from 1947 to 1970 was the exposure of interest, and assumptions were made as to the types of possible exposures that occurred based on subcohorts in the agriculture and forestry industries. As there was heterogeneity in exposures among the subcohorts, the categories described below were considered rough estimates of exposure in each group. These subcohorts included land and/or animal husbandry (MCPA; 2,4-D; 2,4,5-T), horticulture (herbicides other than phenoxy acids), other agricultural occupations (heterogeneous herbicide exposure), silviculture (planting and thinning of young trees; 2,4-D; 2,4,5-T), timber cutting (passive 2,4,5-T and 2,4-D), and other forestry occupations (heterogeneous herbicide exposure). The most commonly used phenoxy acid in Sweden was MCPA (also called 4-chloro-2-methylphenoxyacetic acid; Chemical Abstracts Service No. 94-74-6). Also examined in further detail among this cohort of agricultural and forestry workers was the risk of malignant lymphoma (Wiklund et al., 1988a).

A later cohort study was undertaken, based on data from the Swedish Cancer Environment Register (Eriksson et al., 1992). Similarly, the CER 70 links 1970 census data with the cancer incidence from 1971 to 1984.

Using the CER 70, associations between occupations (farming, farming-related activities, forestry, horticulture, electrical and electronics workers, sawmill workers, carpenters, pulp mill workers, paper mill workers, and health care employees) and non-Hodgkin's lymphoma, Hodgkin's disease (HD), and multiple myeloma were evaluated. Trends over time were considered by using both the CER 60 and the CER 70. Occupational group was the surrogate for exposure to phenoxy acids and chlorinated phenols.

Forestry Workers A cohort mortality study was conducted to evaluate the mortality experience for 1,222 men employed six months or more in forestry work at a Canadian public utility during the period 1950-1982 (Green, 1987, 1991). Mortality was determined for the follow-up period (1950-1982) from internal company records, drivers' license records, and the Canadian Mortality Data Base. Death certificate data were used for cause of death for the population comparison. The occupational cohort consisted of 1,222 males who were assumed to have been exposed to herbicides, including, 2,4-D; 2,4,5-T; and picloram, based on herbicide use data reported within the utility during the period of interest. Prior to 1974 it is estimated that the 2,4,5-T may have been contaminated by 60-120 ppm of TCDD; in 1975 the levels were required to be less than 0.1 ppm. The mortality experience of the workers was compared to the total male population of Ontario.

A short account of the suicide experience of this cohort was reported, as well as the number of years subjects worked as a forestry worker. This was done as a surrogate for possible years of exposure to the phenoxy herbicides (Green, 1987).

A briefly outlined study of a selected sample of 54 forestry workers exposed to 2,4,5-T compared to 54 workers not exposed presents comparisons for prevalence of acne and liver dysfunction (van Houdt et al., 1983). A high prevalence of tumors in a small village in the Netherlands prompted this investigation from among 400 persons who had used 2,4,5-T. Very few details on sampling, exposure, or disease assessment, and very little discussion, are provided.

Herbicide/Pesticide Applicators Mortality (1957-1972), specifically from cancer, among railroad workers spraying herbicides in Sweden was evaluated, compared to the mortality expected in the general population (Axelson and Sundell, 1974). Four exposure cohorts were established based on a minimum total exposure to herbicides of 45 days, which was approximately the length of a spraying season: (1) total herbicide exposure > 45 days (N = 348); (2) phenoxy acids and combinations exposure (2,4-D and 2,4,5-T) > 45 days (N = 207); (3) amitrole and combinations exposure > 45 days (N = 152); and (4) other herbicides and combinations exposure > 45 days (N = 28). These were not mutually exclusive cohorts. Information on

persons involved in herbicide spraying and on duration of spraying was obtained for the state-owned Swedish railways. Mortality was determined from the National Central Bureau of Statistics, and cause of death was obtained from death certificates. Mortality from additional person-years of follow-up through 1978 was reported (Axelson et al., 1980).

A cohort of 3,827 white men who were licensed with the Florida Department of Health and Rehabilitative Services to apply pesticides in Florida during 1965-1966 were traced through January 1, 1977, and their mortality was compared with U. S. and Florida general population rates (Blair et al., 1983). Among the pesticides used were 2,4-D; 2,4,5-T; and Silvex. With the exception of those licensed only to control lawn and ornamental pests, all other categories were not exclusive but included applicators with multiple-category licenses. Workers were exposed to a multiplicity of chemicals; the individual or group exposure to phenoxy herbicides or to any TCDD is unknown. Multivariate analyses based on the Cox proportional hazards model were undertaken.

In Finland, 1,971 male herbicide applicators were identified from the personnel records of the four main Finnish employers involved with chemical brushwood control, who had been exposed for at least two weeks during 1955-1971 to 2,4-D and 2,4,5-T (Riihimaki et al., 1982). After excluding 45 individuals who had died before 1971, the final cohort of 1,926 persons was followed prospectively from 1972 to 1980 for mortality, by checking the names with the population register of the Social Insurance Institution. Underlying cause of death was determined from death certificates. Expected numbers of deaths were determined by using age- and cause-specific death rates for the nation in 1975. Since data on exposure were collected from personnel records, files did not always contain assignment information, and in some cases, recall of exposures was based on the memory of clerks or foremen. Cancer morbidity and mortality in this cohort were reported separately (Riihimaki et al., 1983).

A study of chemical applicators in New Zealand, as well as a follow-up to the original study, examined the reproductive outcomes of births to families where the husband was potentially exposed as compared to outcomes of births where the husband was unexposed (Smith et al., 1981, 1982). All chemical applicators registered (N = 652) at any time between 1973 and 1979 with the Agricultural Chemicals Board in New Zealand were identified, along with a control group of agricultural contractors (N = 532) for inclusion in the study. A detailed questionnaire assessed demographic data, duration of chemical spray use, and number of months of spraying 2,4,5-T during 1969-1979; for those currently married, information was completed by wives of births from that marriage about any defects, miscarriages, or stillbirths. The overall response yielded 459 married chemical applicators and 422 married agricultural contractors. Rates of congenital

defects, stillbirths, and miscarriages among the 1,172 chemical applicator births were compared with the rates among 1,122 agricultural contractor births.

These study results (Smith et al., 1981) indicated that among the chemical sprayers, there were some who had not been exposed to chemicals, and among the agricultural contractors, there were those who had been exposed. Therefore, the combined group of 989 respondents was categorized by exposure to chemicals: no chemical exposure; chemicals sprayed, but not 2,4,5-T; and 2,4,5-T exposure (Smith et al., 1982). The numbers of births, congenital defects, and miscarriages from 1969 to 1980 were identified by a postal questionnaire, and each pregnancy outcome was classified according to whether or not the father sprayed 2,4,5-T during the year of the pregnancy outcome or the previous year.

A cohort of 20,245 pesticide/herbicide applicators in Sweden, licensed between 1965 and 1976, was followed, with results reported after the 1982 and 1984 follow-ups (Wiklund et al., 1987, 1988b, 1989a,b). For each follow-up period, cancers were ascertained from the Swedish Cancer Register, from date of license through 1982 or 1984. A questionnaire was mailed to a sample of the cohort to assess the use of pesticides, protective clothing, tobacco, and occupations from the 1950s through the 1970s. Expected numbers of cases were determined using the 5 year age-specific incidence for the entire Swedish population. Although the study methods did not utilize individual exposure estimates, helpful qualitative data on the use of phenoxy herbicides by pesticide applicators were provided. The principal chemicals used were MCPA, mecoprop (MCPP), and dichloroprop; 2,4-D and 2,4,5-T were also used, but to a lesser extent. Since 1965, all of the above herbicides have been applied by licensed applicators. Their exposure, as a group, exceeds that of other agriculture or forestry workers.

Risk for all cancers in this cohort through 1982 has been reported (Wiklund et al., 1989a). Results for HD and NHL through 1982 (Wiklund et al., 1987) and STS through 1984 (Wiklund et al., 1988b) have been reported. Additional follow-up through 1984 evaluated the risk for STS, HD, and NHL by number of years since license, birth cohort, and whether protective clothing was used (Wiklund et al., 1989b).

A cohort study of male licensed herbicide applicators in the Netherlands evaluated the mortality experience, particularly from cancer, among the cohort, compared to the population of Dutch men (Swaen et al., 1992). The cohort was identified from a central agency of herbicide applicators who were certified before 1980; follow-up was until January 1, 1988. When fact of mortality was determined, cause of death was requested from the Central Bureau of Statistics. Subclassification by type of job was possible based on information obtained at the time of licensing. Data on municipality use of herbicides were obtained and are presented to convey an idea of

the kinds of chemicals sprayed, although data on individual exposures could not be obtained.

The cancer mortality experience of Minnesota highway maintenance workers was evaluated compared to the numbers expected for white men in Minnesota (Bender et al., 1989). Highway workers are exposed to a number of potentially harmful substances, and herbicides were among the list of potential exposures, although no assessment of actual exposures was made. Personnel records were abstracted to accumulate employment and allow for discontinuous years of employment. Men who worked one or more years as highway maintenance workers in Minnesota for the Department of Transportation, with at least one day after January 1, 1945, were eligible for inclusion; vital status was determined as of December 31, 1984. Cause of death was determined from the death certificate. A total of 4,849 men were included in the follow-up; comparisons were to expected numbers of cancers in Minnesota white men.

Lung cancer morbidity was investigated among 1,658 male subjects who had been employed as agricultural plant protection workers for at least 5 years in 14 districts of the former German Democratic Republic during 1948-1972 (Barthel, 1981). The cohort was identified from lists compiled by plant production offices, personnel records, and colleagues of the subjects; extensive exposure to a variety of pesticides, fungicides, and herbicides (including 2,4-D and MCPA) was possible. On average, 130 days per year were spent applying pesticides (May through September). During the rest of the year, subjects repaired and cleaned equipment and stored or moved pesticides, resulting in potential year-round exposure. The cancer deaths during the years 1970-1978 were obtained from county tumor reference centers, with cause of death from death certificates obtained from county medical officers or the Central Statistics Administration: autopsy reports were obtained from pathology institutes and patient records. The expected deaths were calculated for lung cancer, based on 1973 incidence data for the population. An investigation of the smoking patterns of plant protection workers indicated that they are similar to the general population.

Case-Control Studies

Sweden In 1977, a case series report in Sweden (Hardell, 1977, 1979) of a potential connection between STS and exposure to phenoxyacetic acids prompted several case-control studies throughout Sweden to further investigate this potential association These studies are presented together in a separate section, because many of the methods used are similar between studies, and the discussion is facilitated.

The first case-control study (Hardell and Sandstrom, 1979) identified 52 male cases of STS (21 living and 31 deceased) between the ages of 26

and 80 years who were admitted to the Department of Oncology, University Hospital, Umea, between 1970 and 1977. Four controls were matched to each case by vital status. Living controls were selected from the National Population Registry and matched on sex, residence in the same municipality as the case at the time of admission to the hospital, and age within five years. Deceased controls were selected from the National Registry for Causes of Death and matched on the same variables in addition to year of death; deaths from tumors and suicides were excluded. Controls were required to have been working until two years before retirement of the case or, if the case was not retired, until two years before death; this was done to allow for the probability of controls having opportunity for comparable time for occupational exposure.

A questionnaire was mailed to study subjects or next of kin for deceased subjects, following a telephone call regarding the upcoming mailing. After the form was returned, information in the questionnaire was supplemented by telephone by an investigator blind to case or control status. The questionnaire included items about past and present occupations, chemical and other exposures in the workplace, and smoking. Those with low levels of exposure (less than one day) or late exposure (i.e., less than five years before tumor diagnosis) were not considered exposed to the chemical groups of interest, phenoxyacetic acids and chlorophenols. A questionnaire was also sent to employers of study subjects, who indicated forest work or sawmill/pulp industry work, to verify exposures to phenoxyacetic acids and chlorophenols, respectively. Chlorophenols can be contaminated by polychlorinated dibenzodioxins (PCDDs; including TCDD) and dibenzofurans (PCDFs). Exposures occur from contact with cutting oils or wood-protection agents and in shoe or leather industries. Response rates from employers in the forest industry were low, and not based on records, whereas responses from sawmill/pulp industries were at 97 percent, with good agreement with statements from the interview.

To verify the findings of the first case-control study, a case-control study of STS was conducted in southern Sweden, where MCPA, 2,4-D, and phenoxypropionic acids are used in agricultural areas (Eriksson et al., 1979, 1981). Pathologically confirmed cases of STS diagnosed between 1974 and 1988 among residents in the five most southern counties of Sweden and reported to the National Social Welfare Board Cancer Registry were eligible. Two controls were selected for each living and deceased case using methods described above. The total number of subjects included 110 cases and 219 controls for analysis. Questionnaire assessment of exposure was the same as described above.

A later study repeats the study methods described, and evaluates the association between STS and phenoxyacetic acids without overlapping the previous series of cases ($N = 55$), using cases diagnosed between 1978 and

1983, and reported to the Regional Cancer Registry in Umea, Sweden (Hardell and Eriksson, 1988); the three most northern counties in Sweden were included in this reporting. Two control groups were used: the first was population-based and selected as previously described; the second was drawn at random from the Regional Cancer Registry, excluding lymphomas and nasopharyngeal cancers.

The Regional Cancer Registry at the University Hospital in Linkoping, Sweden, was the source of STS cases in southeastern Sweden diagnosed between 1975 and 1982 (Wingren et al., 1990). Cases were men between the ages of 25 and 80 years at diagnosis. Two control groups were chosen, one selected randomly from the population and one selected from among those with other cancers residing in the same three counties in southeast Sweden as the cases. A total of 71 cases, 164 cancer controls, and 315 population-based controls or their next of kin responded to the study questionnaire. Exposure was evaluated by a mail questionnaire for the occupations that had potential exposure to phenoxy herbicides and chlorophenols. Telephone interviews followed for some occupations, and detailed questions about the pesticides were asked at that time.

The last case-control study, chronologically, of STS in Sweden included cases diagnosed in central Sweden and reported to the Regional Cancer Registry in Uppsala between 1978 and 1986 (Eriksson et al., 1990). A population-based control series was used, and responses for analysis were available from 218 cases and 212 controls or their next of kin. Study methods and exposures of interest were the same as for the other Swedish case-control studies of STS.

A matched case-control study of patients with lymphomas including HD ($N = 60$) and NHL ($N = 109$) was conducted in northern Sweden (Hardell et al., 1980, 1981); four unclassifiable lymphomas were included in the NHL case series. Methods for sample identification and exposure were as described previously (Hardell and Sandstrom, 1979). Cases were males 25-85 years old with histologically verified malignant lymphoma, admitted to the hospital between 1974 and 1978. Two controls ($N = 338$) were matched to each case. Analyses were done both for phenoxy acids/chlorophenols and for organic solvents. A later study examined the HD cases in a separate analysis (Hardell and Bengtsson, 1983).

In an attempt to address criticism regarding potential observer bias in the Swedish case-control series, another case-control study was done using colon cancer ($N = 154$), both as an index case and as controls in a comparison with the original STS and malignant lymphoma cases (Hardell, 1981). The referent groups from the two earlier studies (Hardell and Sandstrom, 1979; Hardell et al., 1981) were used as the referent group for the colon cancer case evaluation. Similar study methods were employed, and the questionnaire was similar; however, results were compared for those who

responded by questionnaire to those responding with a combination of questionnaire and telephone follow-up answers, to evaluate potential bias.

By using a study design and methods similar to those described in this section, males aged 25-85 were identified from the northern Swedish Cancer Registry having nasal (N = 44) and nasopharyngeal (N = 27) carcinomas reported between 1970 and 1979 (Hardell et al., 1982); controls were the same 541 referents combined from the STS and malignant lymphoma studies (Hardell and Sandstrom, 1979; Hardell et al., 1981). Cases of primary or unspecified liver cancer (N = 98) diagnosed between 1974 and 1981 were also studied (Hardell et al., 1984). The controls were from the national population register (N = 200) and the cases were mailed questionnaires identical to those sent for earlier studies.

In Sweden, potential risk factors for HD and for NHL were evaluated in a case-control study; phenoxy acids were included in the occupational exposures potentially associated with disease (Persson et al., 1989). Cases diagnosed between 1964 and 1986 who were still alive and listed in the Department of Oncology at Orebro Medical Center Hospital registry, at least 20 years old at diagnosis and less than 80 years old at interview, were included. A total of 175 Swedish-born men and women were eligible; a total of 160 were in the final interviewed sample. Controls were 275 subjects enrolled in other studies who met eligibility set for cases. Information about various occupational exposures including solvents, welding, wood preservatives, phenoxy acids, and fresh wood (sawmill workers, lumberjacks, paper/pulp workers) was obtained by questionnaires mailed to the subjects; a minimum exposure of one year was required.

In a case-control study of NHL, 167 cases were evaluated for association with exposure to organic solvents, phenoxy acids, and chlorophenol from information obtained by interview, compared to two groups of healthy men (Olsson and Brandt, 1988). Cases were identified from the Department of Oncology, University Hospital, Lund, Sweden, between 1978 and 1981; all were men between 20 and 81 years of age. Controls were identified from control series used for other studies; 50 were included from the same geographic area as the cases, and 80 were identified from other parts of Sweden. Cases and controls were interviewed concerning occupational history and exposure to various chemicals, including phenoxy acids and cholorophenol. Exposure was considered to be at least one day minimum for phenoxy acids and chlorophenol used occupationally. Analysis was also conducted by duration of herbicide and chlorophenol exposure.

New Zealand Prompted by the Swedish studies of STS and exposure to phenoxy herbicides, a case-control study was undertaken in New Zealand including all male cases of STS reported to the New Zealand Cancer Registry by public hospitals between 1976 and 1980, and preliminary results

were reported (Smith et al., 1983). Ninety-eight eligible male cases and 111 controls were identified; 80 cases and 92 controls actually participated in the interview. Controls were obtained by a random selection of other cancer patients listed on the cancer registry, matched to the case for year of registration and age of birth within 2 years. Each patient or next of kin was interviewed; questions included whether the subject had sprayed phenoxyacetic acids or had contact with phenoxy herbicides and were classified for analysis as definitely, probably, or possibly exposed. When the specific compounds used were known, 2,4,5-T and 2,4-D were the predominating exposures reported. Another classification included individuals who had sprayed agricultural chemicals of unknown type. If the specific chemical was unknown but vegetation including gorse and blackberry was sprayed, the subject was classified as potentially exposed, as 2,4,5-T was the compound most widely used on these plants. For those who reported having done spraying, their primary occupation when exposed to the herbicides was also noted. Analyses were also undertaken excluding those who were exposed during the 5 or 10 years prior to cancer registration.

Results of further investigation of phenoxy herbicides as well as chlorophenols for association with STS incidence and mortality were reported by Smith and colleagues (Smith et al., 1984). The incidence data were obtained from 1955 to 1979 from the New Zealand National Cancer Registry, and mortality data were obtained from the National Health Statistics Center; trends showed increases in STS over time. Therefore, further study of this increase as related to occupation was done for the STS cases. The cases and controls described above (Smith et al., 1983), plus two additional cases that had next-of-kin interviews, were evaluated for associations with phenoxy herbicides and chlorophenols. Interviews were conducted by telephone by a single interviewer; for 50 subjects who were dead or too ill, the next of kin were interviewed. The questionnaire included items on occupational history and chemical exposure, including use of phenoxy herbicides and chlorophenols, in a number of occupations. Questions on use of herbicides or involvement in spraying activities were used as prompts to ask further questions about specific chemicals involved.

An updated extension of this study was conducted with cases identified through 1982 from the New Zealand Cancer Registry (Smith and Pearce, 1986). A control series interviewed for another study (of multiple myeloma and lymphoma) was used for this study, rather than the cancer controls described above. The updated series included an additional 51 cases and 315 controls; combined with the previous study, 133 cases and 407 controls were available for study. Questions regarding phenoxy herbicide use were the same as those used in the earlier study. A portion of the analysis for exposure to phenoxy herbicides included a definition of probable or definite, for more than 1 day, but not in the 5 years before cancer registration.

Additional studies were conducted of malignant lymphoma and multiple myeloma for association with agricultural occupations and phenoxy herbicides (Pearce et al., 1985, 1986a,b). The New Zealand Cancer Registry was used to identify all males 20 years or older at registration, between 1977 and 1981, with HD, NHL, and multiple myeloma. Four controls per case were selected from other cancer patients (excluding NHL, HD, STS, and multiple myeloma) registered in the same year who were within 2 years of age. Death and cancer registry data were utilized to obtain the study subject's most recent or current occupation. A total of 734 cases of malignant lymphoma and multiple myeloma, along with 2,936 controls, constituted the study population (Pearce et al., 1985). The vast majority of agricultural workers were classified as general or unspecified farm workers. Exposure to herbicides was not indicated as being of primary interest in this study; however, it provides a basis for subsequent studies, particularly with respect to study methods.

A second phase involved a series of case-control studies that investigated farming and phenoxy herbicide association with NHL and multiple myeloma (Pearce et al., 1986a,b, 1987). From the study described above, 106 cases of NHL (ICD 202) were identified (1977-1981) and 83 were ultimately included in the sample; similarly, 168 of the 212 other cancer controls were included (Pearce et al., 1986b). A population control sample of 300 male registrants on the New Zealand electoral roll was identified for comparison to living, interviewed cases; 228 were in the final sample. A second control group of 168 males with other cancers was also used for comparison in the analysis. Cases and controls were interviewed by telephone and questioned about their occupation, specifically about potential spraying exposure to phenoxy herbicides and treatment exposures to chlorophenols (Pearce et al., 1986b). The questionnaire used was similar to that used in the study of STS described above (Smith et al., 1983, 1984).

This study was expanded to include additional NHL cases diagnosed under ICD 200, and additional controls were included from the same years (1977-1981) (Pearce et al., 1987). The expanded case series was combined with the original NHL series (Pearce et al., 1986b), and exposures of interest were reexamined, with some additional details in the questionnaire including type of farming, contact with animals, and medical conditions and allergies. Questionnaire information related to phenoxy herbicides and chlorophenol exposure was obtained as described above.

A separate study of the 76 cases of multiple myeloma and 315 controls with other types of cancer, all of whom had been included in the earlier study (Pearce et al., 1985), were interviewed concerning their occupation, with particular interest in potential exposure to phenoxy herbicides and chlorophenol, as previously described (Pearce et al., 1986a).

United States An early study in Nebraska was to become the basis for later studies of farmers and mortality. Geographic mortality patterns for white males indicated elevated leukemia mortality in the central part of the United States, which prompted a study of leukemia mortality of Nebraska farmers (Blair and Thomas, 1979). Computerized mortality listings in Nebraska between 1957-1974 were the source of white male deaths 30 years or older from leukemia as the underlying cause of death (N = 1,084). Two deaths from other causes (N = 2,168) were matched to leukemia cases by sex, race, county of usual residence, age at death (±2 years), and exact calendar year of death. Occupation was as noted on death certificates, with farm owners, tenants, and laborers classified as farmers. Risk associated with being a farmer was not established on an individual basis but according to demographics and agricultural practices in the usual county of residence. Counties (N = 30) with the highest levels of different agricultural products as well as fertilizer, herbicide, and pesticide use were identified; cases of leukemia and matched controls in the county groupings (considered "highest" or other) were compared, with farming as the exposure of interest.

To further evaluate farming as a potential exposure for risk of leukemia, a case-control study was conducted of 1,084 white men 30 years or older, with cause of death listed as leukemia, in Nebraska from 1957 to 1974 (Blair and White, 1985). Controls (N = 2,168) were nonleukemia deaths, matched by sex, race, county of usual residence, and age and calendar year at death within two years. Analyses were made of the risks of acute lymphatic, chronic lymphatic, acute myeloid, and chronic myeloid leukemia across birth cohorts and Nebraska agricultural regions distinguished by predominant type of farming as surrogate for various exposures. Agricultural region surrogate measures included multiple possible exposures. Occupation as a farmer was determined from the death certificate with farm owners, tenants, and laborers classified together.

In response to these early studies indicating possible associations between farming and leukemia, a large case-control study of leukemia was conducted in Iowa and Minnesota (Brown et al., 1990). Cases of leukemia newly diagnosed in white men aged 30 years or older were identified from tumor registries and hospital records for one year prior to the study and for two years prospectively. In Iowa, cases were identified through the Iowa Tumor Registry from March 1981 to October 1983; in Minnesota, cases were identified between October 1980 and September 1982 through a special network established by the state in hospitals and laboratories. Major city residents were excluded from the study, because farming exposures were of interest. Controls were identified from the population of white men, without leukemia or NHL, based on a stratified sample, in five year age groups, and considering state of residence and vital status. Cases included 578 men from both states combined, who were interviewed or for

whom a surrogate was interviewed; 1,245 controls were in the final population. An extensive questionnaire obtained information on a variety of farming exposures, including insecticides, herbicides, and fungicides.

A population-based case-control study of NHL in men from Iowa and Minnesota was conducted (Cantor et al., 1992). Cases of NHL newly diagnosed between March 1981 and October 1983 in men 30 years or older were identified in Iowa from the Iowa State Health Registry records; in Minnesota, men diagnosed between October 1980 and September 1982 were identified from a special surveillance of Minnesota hospital and pathology laboratory records. Cases from the entire state of Iowa were eligible; cases residing outside of the major cities of Minneapolis, St. Paul, Duluth, or Rochester were eligible in Minnesota. Case histologic specimens were reviewed by a panel of pathologists, and confirmed from diagnosis (N = 622) as well as subtype: follicular, diffuse, small lymphocytic, and "other NHL." Controls (N = 1,245) were randomly selected from the population of white men without hematopoietic or lymphatic cancer, and frequency matched to cases by 5 year age group, vital statistics at interview, and state of residence using random-digit dialing for living subjects under 65 at diagnosis; a 1 percent random Medicare files listing from HCFA for living subjects 65 or older; and death certificates for deceased subjects. Interviewers obtained comparable data on sociodemographic characteristics, medical diagnosis, and other known and suspected risk factors from subjects, next of kin, or friend for deceased or incompetent subjects. For those who had lived on a farm at least 6 months since age 18, detailed farming and pesticide use history were obtained as well as information on 38 specific herbicides (including phenoxy herbicides, 2,4-D, 2,4,5-T). Information on ever use, first/last year use, and method of application (aerial, surface, incorporated in soil, other), whether subject had personally applied, mixed or handled and use of protective equipment. Analysis obtained ORs adjusted for confounders and other possible risk factors.

Additional case-control studies have evaluated other cancers as well as leukemia for association with farming. A case-control study of white men 21 years or older residing in 66 counties of eastern Nebraska and diagnosed between July 1, 1983, and June 30, 1986, with NHL (Zahm et al., 1990) was conducted. Cases in eastern Nebraska were identified through the Nebraska Lymphoma Study Group and area hospitals. Histologically confirmed cases were eligible for inclusion (N = 220); interviews were successfully completed with 201 cases or their next of kin. Residents of the same 66-county area were eligible as controls, and were matched 3:1 by race, sex, vital status, and age (±2 years). Living controls less than 65 years of age were selected by random-digit dialing; controls were selected from Medicare records to be matched to living cases 65 years or older. Controls were selected from the Nebraska mortality files by matching for year of death

with deceased cases. Deaths due to NHL, HD, multiple myeloma, leukemia, unknown malignancy site, aplastic anemia, suicide, or homicide were excluded as controls. A total of 725 white male controls or their next of kin were interviewed. The interview was designed to obtain information on all agricultural exposures, including 2,4-D, to determine potential association with NHL. Herbicide use, application method, use of protective equipment, years of use for each herbicide and insecticide, average annual number of days of use on the farm, duration of time wearing work clothes after handling pesticides, and personal handling were among the questions included in the interview.

Incident and prevalent cases of multiple myeloma identified from death certificates obtained in a follow-up study of cancer mortality were compared to a control series for association with several occupational exposures (Boffetta et al., 1989). A volunteer cohort of 508,637 men and 676,613 women who had been enrolled by the American Cancer Society (ACS) in a prospective study of mortality in 1982 completed a questionnaire which obtained information on medical history including cancer, medication and vitamin use, menstrual and reproductive history, diet, drinking, smoking, and occupational history and exposures. Exposures in or outside of the workplace to 12 groups of substances, including pesticides and herbicides, and duration of exposure were ascertained. Follow-up of the cohort occurred in 1984 and 1986 for mortality, with volunteers checking on vital status and recording date and place of death. Nosologists coded cause of death from death certificates. From this mortality study, all cases of MM reported on death certificates as underlying or contributing cause of death were the cases for the nested case-control study. Controls (4 per case) were randomly selected from the cohort and matched to the cases on sex, ACS division, year of birth, and ethnic group. Cases were determined to be incident or prevalent based on whether the cancer had been mentioned in completing the questionnaires for the cohort mortality follow-up. There were a total of 282 cases (128 incident, 154 prevalent) and 1,128 controls (512 matched to incident and 616 matched to prevalent) studied. Exposure of interest for analysis was considered to be "pesticides and herbicides." Subanalysis examined these exposures among those who were reported to be farmers and among nonfarmers.

As an extension of a previous cohort study of cancer associated with farm employment in Iowa (Burmeister, 1981), a case-control study of leukemia mortality in white males in Iowa (Burmeister et al., 1982) was conducted. Deaths of white males who were at least 30 years old at the time of death from leukemia (N = 1,675) were obtained from computer listings in Iowa (1964-1978). Two controls (N = 3,350) were selected from among white male deaths other than leukemia, matched to cases on county of usual residence, age at death within two years, and calendar year of death. The

death certificate was the source of usual occupation for the years 1964-1970 as coded by the 1960 U.S. Census, and according to the U.S. Bureau of Labor for 1971-1978. "Farmers" were defined as farm owners, tenants, and laborers and were evaluated separately in the analysis for associations between exposures, including herbicide use, and leukemia. Agricultural data were provided by the Iowa Crop and Livestock Reporting Service, and pesticide use was provided by the 1964 agricultural census; herbicide use was also available by county distribution for those classified as farmers. No individual exposures were ascertained.

In a later study, the relationship of cancers of the stomach, prostate, NHL, and multiple myeloma to agricultural practices and farm products was examined (Burmeister et al., 1983). Deaths of white male Iowa residents, 30 years of age or older, occurring between 1964 and 1978 from any of the four cited cancers were abstracted from computer listings. Two deaths of white men, from any other cause, including other cancers, were selected as controls, matched by county of residence, age of death within two years, and calendar year of death. The usual occupation indicated on the certificate of death was coded according to census criteria; farm owners, tenants, and laborers were all categorized as "farmers." The geographic distribution by county of selected farm commodities was reviewed, and counties were classified as to their status in the top third or otherwise for production of each selected commodity. Odds ratios were computed according to birth cohort and production levels for the following: egg-laying chickens, milk products sold, number of cattle and hogs, and amount of soybeans or corn per acre, as well as herbicide or insecticide use and amount of fertilizer applied. As with the study above, no individual measure of exposure was available.

In an attempt to replicate the Swedish studies of Hardell and colleagues, a population-based case-control study of STS, HD, and NHL in relation to farm use of herbicides was conducted in Kansas (Hoar et al., 1986). Use of 2,4-D and 2,4,5-T is heavy in Kansas, with minimal use of insecticides, compared to other states with high phenoxy herbicide use. White, male residents of Kansas, 21 years of age or older, were identified by using a population-based, statewide cancer registry for the years 1976-1982, with STS, HD, and NHL for study inclusion as cases; pathological review confirmed diagnosis. Controls were obtained by using a random-digit dialing technique for controls under age 65 and Medicare records for controls 65 or over. Study participants or their next of kin were interviewed by telephone regarding employment and specific types of farm work, including crops raised and herbicide or insecticide use. Herbicide and pesticide use was confirmed for each case and control by contact with suppliers; duration and frequency of use were also examined. 2,4-D was the most commonly used herbicide, although 2,4,5-T and many other herbicides

were also used. The interviewed cases totaled 133 for STS, 121 for HD, and 170 for NHL.

A total of 774 cases of NHL in white male residents of Wisconsin counties other than Milwaukee were identified from computerized mortality records of Wisconsin deaths, 1968-1976 (Cantor, 1982). Cases were 30-89 years of age at time of death. Controls dying from other causes were frequency matched in a 4:1 ratio, within strata based on county size, by year of death, five year age group, and residence county population group; smoking-related cancers among controls were excluded. Because an individual's farming activity was unknown, county data were used as a surrogate for farm-related exposures, including data on measures of agriculture activity, such as total acreage, type of crops, tons of fertilizer applied, areas sprayed for weed control or with insecticides, and inventory of hogs, cattle, and chickens. Trends with age and calendar year were examined.

A case-control study was conducted of NHL (N = 61) and HD (N = 15), with cases identified from 1958 to 1983 using death certificates of white male residents of Hancock County, Ohio, which is an area of reported heavy herbicide use (Dubrow et al., 1988). Controls were selected as a stratified (by age at death and year of death) random sample from other causes of mortality (N = 304). Cases and controls were compared for usual occupation and industry, as listed on the death certificates, and adjusted for age at death and year of death. Exposures of interest were farming as an occupation or agriculture as the industry in which subject worked.

A population-based case-control study of all persons under 80 years of age with multiple myeloma diagnosed between July 1, 1977, and June 30, 1981, was conducted through cancer registries serving four Surveillance, Epidemiology, and End Results (SEER) areas (Detroit, Washington State, Atlanta, Utah) (Morris et al., 1986). Controls were individuals selected at random from the same geographic areas as the cases; the method of control selection varied according to study area. Three of the areas used random-digit dialing, while one (Washington) used standard area sampling methods. Controls were matched by age (W5 years), sex, and race. Interviews were conducted with study subjects or next of kin if the subject was unable to be interviewed. Information on risk factors hypothesized to be associated with multiple myeloma was ascertained through interview questions on specific occupational and chemical exposures, particularly from herbicide use. A total of 698 cases or their next of kin were interviewed; 1,683 controls or next of kin completed the interview. Of cases, 69 percent were interviewed themselves, whereas 99 percent of the controls interviewed were the study subjects.

A case-control study of spontaneous abortions compared to live births was undertaken in Oregon and Washington to evaluate the association with father's occupational exposure to 2,4-D (Carmelli et al., 1981). Employers

in the Northwest (Oregon and Washington) were contacted to sample, from the worker population, married men 35 years or younger, living in these states. Employers included farm groups, forest industry, forest service, licensed applicators, transportation, utilities, formulators, and manufacturers; of a total of 14,747 questionnaires sent, 8,287 responded. From those responses, a total of 3,787 were considered eligible, with both husband and wife under 35 years of age. For a subsample of 1,098 couples reporting either late, heavy period, or pregnancy within the past two years, a telephone interview was conducted. This interview included questions on reproductive history; work history; birth control; smoking, marijuana, and medication use; and exposure to herbicides. The final study sample included 134 cases of miscarriage and 311 control live births. Validation of a reported miscarriage was attempted through the review of physician or hospital records; records were obtained for 75 (56 percent) of the cases, and 69 miscarriages were confirmed. The critical period of exposure was taken to be the month of the last menstrual period plus the month of the first missed period. Questions were asked about exposure to herbicides around the home, as well, Exposure was classified as "high" exposure (directly exposed in the manufacture, formulation, mixing, or application of 2,4-D), "medium" exposure (indirectly exposed by being in an area in which 2,4-D was sprayed or being in the area after it had been sprayed), and "low" exposure (not exposed at work). Attempts were made to validate exposures, including verification using written records of exposure dates, signed statements from coworkers and supervisors, and other sources.

A population-based case-control study was conducted in western Washington where phenoxyacetic acid herbicides and chlorophenol are widely used by agricultural, forestry, and wood product industries (Woods et al., 1987; Woods and Polissar, 1989). Cases of STS and NHL were identified between 1983 and 1985 from a population-based tumor registry covering 13 counties of western Washington. Eligible cases were men diagnosed between the ages of 20 and 79 years, during 1981 to 1984, according to International Classification of Diseases for Oncology (ICD-O) coding; pathologic review was also obtained for cases. Live controls aged 20 to 64 were selected from the population by random-digit dialing; living controls aged 65 to 79 were randomly selected from the files of the Health Care Financing Administration. Death certificates were used to identify deceased controls with date of death during the study period and residence within the study area, for noncancer deaths, excluding homicides and suicides, of persons aged 20 to 79. The final study population included 97 living and 31 dead STS cases, 402 living and 174 dead NHL cases, and 475 living and 219 dead controls. Study subjects or their proxies were interviewed in person; the interview included residential, military, and medical history, as well as a detailed occupational history. Information about specific activities involving

the manufacture or use of phenoxy herbicides or chlorophenol was obtained in the interview. Confirmation of the self-reported occupational exposure to phenoxy herbicides, chlorophenol, and other chemicals was sought by telephone contact with former employers or coworkers. Generally there was good agreement with the self-reports. A later study using this case series evaluated the associations between NHL and the exposures of interest among farmers only (Woods and Polissar, 1989).

In a two-part study the mortality experience of all U.S. Department of Agriculture (USDA), white, male employees who died between 1970 and 1979 and who had ever been employed as extension agents (Alavanja et al., 1988) was evaluated (N = 1,495). PMR analysis examined causes of death, with detailed cancer causes noted. Excess mortality at specific organ sites identified from the PMR analysis resulted in a case-control study for the following: colon, prostate, kidney, brain, HD, NHL, multiple myeloma, and leukemia. Four controls were selected for each case from the mortality analysis who died from any cause of death different from the case, and matched on date of birth (±2 years) and race. Exposure of interest for the case-control analysis included extension agent status, duration, time period, and location of employment as an extension agent.

The mortality experience of males employed as forest or soil conservationists (N = 1,411) was evaluated for the same period and utilized the two-part study methods described above (Alavanja et al., 1989). Case-control studies of NHL, pancreas, kidney, colon, and prostate cancers were conducted. Trends with duration of employment as either a forest or a soil conservationist were also examined.

Under the hypothesis that exposure to dioxins may result in immunosuppression and subsequently decrease host resistance to infection, the association between exposure to TCDD and Kaposi's sarcoma in AIDS patients was evaluated (Hardell et al., 1987). AIDS patients with Kaposi's sarcoma (N = 50) and 50 homosexual controls matched to cases by age were identified at outpatient clinics and interviewed. Interviews included questions on occupational exposure to pesticides, service in Vietnam, exposure to Agent Orange, home use of pesticides, and smoking.

Other Case-Control Studies In the Piedmont region of Italy, the association between occupational exposure to herbicides used in the rural areas and ovarian neoplasms arising from the serosal surfaces of the ovary, which are of mesothelial or mesodermic origin, was investigated (Donna et al., 1984). Subjects for this case-control study consisted of 66 newly diagnosed cases of histologically confirmed primary mesothelial ovarian tumors diagnosed between January 1, 1974, and June 30, 1980, in the Department of Pathology of the City Hospital in Alessandria. Sixty women or their next of kin were successfully interviewed in 1981 regarding their occupational history

to assess herbicide exposure. Controls consisted of newly diagnosed cases of cancer in sites other than the ovary, selected from the same file in the Pathology Department, matched to the cases by year of diagnosis, age (±2.5 years), and district of residence. One hundred and thirty-five controls were obtained, with a variable matching ratio from 1:4 per case. Of these, 127 were successfully interviewed in 1982. Many of these controls were breast cancer cases, whereas the others were a variable mix of other types of malignancies in females. Exposure was defined by three categories: definite exposure (subject or next of kin described personal herbicide use and could name brands), probable exposure (subject was farmer after 1960 when herbicides were in heavy use, or resided in areas of known herbicide use), and no exposure (denied herbicide use).

Incident brain gliomas (N = 240) from two hospitals in Milan, Italy, were evaluated in comparison to patients with nonglioma nervous system tumors (N = 465) and patients with other neurologic diseases (N = 277) for association with chemical exposures among farmers (Musicco et al., 1988). Two of the major centers in Milan for treatment and diagnosis of brain tumors were the source of cases for this Italian study; all newly hospitalized cases were enrolled between January 1983 and December 1984 who were 20-74 years old. Cases were patients with gliomas; tumor controls had nonglioma tumors, and neurologic controls had nonneoplastic neurologic diagnoses. Subjects were interviewed concerning all occupations for six months or more, with additional information collected on agricultural occupations, including the use of fertilizers, herbicides, insecticides, and fungicides.

Incidence of STS was investigated in the highly agricultural area of northern Italy, where exposure to phenoxy herbicides by rice weeders was quite common (Vineis et al., 1986). All persons diagnosed with STS between 1981 and 1983 who were at least 20 years old were identified from the pathology departments of the three northern province hospitals. After histologic confirmation and other exclusions, 68 of the 135 potential cases were interviewed: 31 cases were diagnosed among women, and 37 were diagnosed in men. A total of 158 controls were interviewed of the 208 originally identified. Living controls were randomly sampled from population electoral rosters; deceased controls were identified from the demographic offices of the same municipality as the deceased case. Interviews were obtained with subjects or their next of kin and included demographic information, smoking history, occupational history, radiological therapy treatment, and history of residence changes, including periods of time in rice-growing areas. For those who had worked in agriculture, including rice-growing, additional information was obtained concerning the handling, transportation, distribution, and spraying of herbicides. Exposure was categorized by potential for phenoxy herbicide exposure, based partially on the

assumption that certain occupational groups would be exposed to those chemicals during certain time periods.

Data from 15 regional cancer registries, which constitute the National Cancer Register in England, 1968-1976, were used to identify cases of STS and other cancers as controls to evaluate association with farming, agricultural, and forestry occupations as surrogates for exposure to phenoxy herbicides (Balarajan and Acheson, 1984). Cases were men at least 15 years old, for whom occupational information was available in the registry data base; similarly, controls were registrants with other cancers and occupational data, matched to cases within five years of age and by region of residence (N = 1,961 pairs).

A case-control study of STS and malignant lymphoma was also conducted in Australia to evaluate potential association between the cancer and exposure to phenoxy herbicides and chlorophenol in a number of occupations (Smith and Christophers, 1992). Living cases of STS and malignant lymphoma, and other cancer controls, registered in the Victoria Cancer Registry after January 1, 1982, who were male, 30 years or older at registration, and patients at any of six major Melbourne hospitals, were selected. The study progressed until 30 patients with STS and 52 patients with malignant lymphomas had been interviewed; diagnoses were confirmed through review of hospital records. One control with another type of cancer was randomly selected from the cancer registry, and matched for sex, age within three years, and area of current residence; patients with leukemia, multiple myeloma, or bone sarcomas were excluded. An additional control group was selected at random from the population by using the electoral register and matching on the same criteria as the cancer controls. Interviews regarding occupational herbicide and chemical exposure were conducted either in person or by telephone and asked about comprehensive occupational history, leisure activity, and alcohol and tobacco use; details on the nature and duration of exposure were determined by the interviewer when the subject reported any occupation or activity that might have involved use of phenoxy herbicides or chlorophenol. Additional questions included work or living on a farm, work with asbestos, use of pesticides, herbicides, or wood preservatives, with further details obtained by interviewer probing. Exposures within five years prior to diagnosis were ignored for both the case and the matched control; lifetime exposure of less than one day was counted as not exposed. In the analysis, exposure was assessed with two indicator variables, one for possible exposure and one for definite exposure, such that the risk for one was adjusted for the other. Exposure to phenoxy herbicides or chlorophenols was classified as none, possible, or definite/probable for analysis.

As part of an ongoing study of neoplasms in Milan, Italy, incident cases between 1983 and October 1988, aged 15-74 and histologically confirmed,

were identified along with hospital controls to evaluate occupational exposures for association with lymphoid cancers (LaVecchia et al., 1989). Cases of HD (*N* = 69), NHL (*N* = 153), and multiple myeloma (*N* = 110) were included for study along with 396 patients admitted for acute conditions to the same hospitals from which cases were identified. Diagnoses for controls included traumatic conditions, nontraumatic orthopedic conditions, acute surgical conditions, and miscellaneous ailments. The questionnaire included items on demographic characteristics, life-style habits, selected diet, medical history, menstrual and reproductive history, start and stop date for 16 industries or occupations, role in the industry, and exposure to occupational agents including herbicides.

Paper/Pulp Workers

Four studies of paper and pulp workers are included, which hypothesize and evaluate potential exposures to dioxins in the processing of wood to form pulp, and patterns of mortality among workers (Robinson et al., 1986; Henneberger et al., 1989; Solet et al., 1989; Jappinen and Pukkala, 1991).

A cohort of 3,572 white, male, pulp and paper workers employed for at least one year between 1945 and 1955 was identified from 5 mills selected from a possible 37 paper and pulp mills in the states of Washington, Oregon, and California, to be followed through March 1977 for mortality experience (Robinson et al., 1986). Three mills produced sulfate pulp and two produced sulfite pulp; four of the five mills used different bleaching processes as well. Men were identified from personnel records, which also contained demographic data and detailed work histories; vital status was determined through the SSA, Internal Revenue Service (IRS), state motor vehicle departments, telephone directories, and other sources. Those of unknown vital status on March 31, 1977, were assumed to be living for analyses. Comparisons were made with expected numbers of cause-specific mortality from U.S. standardized mortality rates. Analyses were not specific for individuals involved in processes with potential dioxins exposure.

Participants in a cohort study to evaluate respiratory health in Berlin, New Hampshire, in 1961 were identified for inclusion in a cohort to evaluate mortality (Henneberger et al., 1989). Occupational histories were available for those in the 1961 cohort, which were used to determine employment in the paper and pulp mill in Berlin, New Hampshire, as well as exposures. Those who participated in the 1961 study who had worked in the mill for at least one year, and who had worked in another paper and pulp mill for less than five years, were eligible as the cohort for the mortality study; a total of 883 white men were included. Exposure groupings were defined for the cohort as (1) pulp mill work for at least one year; (2) paper mill work for at least one year; and (3) other paper company work for at

TABLE 7-1 Epidemiologic Studies—Occupational Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Production Workers				
NIOSH				
Fingerhut et al., 1991	Cohort	Cancer mortality in male workers from 12 plants producing TCDD-contaminated chemicals (1942-1984), compared to U.S. population	5,172	—
Calvert et al., 1991	Cohort	Study of workers employed at one of two plants manufacturing substances contaminated with TCDD 15 years or more prior to assessment of chronic bronchitis, COPD, ventilatory function, thorax, and lung abnormalities, compared to neighborhood controls without exposure to TCDD	281	260
Calvert et al., 1992	Cohort	Assessment of liver and gastrointestinal systems in same group as Calvert et al. (1991)	281	260
Alderfer et al., 1992	Cohort	Assessment of psychological variables to determine depression in same group as Calvert et al. (1991)	281	260
Sweeney et al., In press	Cohort	Peripheral neuropathy in same group as Calvert et al. (1991)	281	260

Monsanto				
Zack and Suskind, 1980	Cohort	Evaluation of mortality experience among employees with chloracne exposed to TCP process accident in 1949 at Monsanto, compared to U.S. male population standard	121	—
Zack and Gaffey, 1983	Cohort	Study of mortality experience of all white male workers (1955-1977) employed at a Monsanto plant through Dec. 31, 1977, compared to mortality of standardized U.S. population rates	884	—
Suskind and Hertzberg, 1984	Cohort	Evaluation of health outcomes (1979) at clinical examination among workers exposed to 2,4,5-T (1948-1969) compared to non-exposed workers at same Monsanto plant	204	163
Moses et al., 1984	Cohort	Study of health outcomes in Monsanto workers (1948-1969) with chloracne reported as a surrogate to 2,4,5-T exposure compared to health outcomes in workers without chloracne as surrogate for no exposure	117	109
Collins et al., 1993	Cohort	Mortality of workers (through 1987) exposed and unexposed to dioxin between March 8, 1949 and November 22, 1949 as indicated by presence of chloracne, compared to local population mortality rates	122 with chloracne 632 without chloracne	—

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Dow				
Ott et al., 1980	Cohort	Mortality experience among workers exposed to 2,4,5-T in manufacturing (1950-1971) compared to mortality experience of U.S. white men	204	—
Cook et al., 1980	Cohort	Mortality experience (through 1978) of male workers involved in a chloracne incident (1964) from TCDD exposure, compared to mortality experience of U.S. white men	61	—
Bond et al., 1987	Cohort	Extension of Cook et al. (1980) study, mortality through 1982	322	(1) U.S. white male population (2) 2,026 employees without chloracne
Bond et al., 1983	Cross-sectional	Study of differences in workers potentially exposed and unexposed to TCDD during chemical production for (1) morbidity and (2) medical examination frequency between 1976 and 1978	(1) 183 (2) 114	(1) 732 (2) 456
Cook et al., 1986	Cohort	Mortality experience (1940-1979) of men manufacturing chlorinated phenols compared to U.S. white men	2,189	—
Ott et al., 1987; Cook et al., 1987	Cohort	Expanded Cook et al. (1986) study an additional three years, through 1982	2,187	—

Bond et al., 1989b	Cohort	Extension of Ott et al. (1987) study through 1984	2,187	—
Bond et al., 1989a	Cohort	Study of incidence of chloracne among a cohort of workers potentially exposed to TCDD, and association with other risk factors	2,072	Internal comparison
Bond et al., 1988	Cohort	Study of mortality (through 1982) among workers potentially exposed to 2,4-D (1945-1983) compared to U.S. white males and all other male employees not exposed	878	(1) U.S. white male population (2) 36,804 employees not exposed
Sobel et al., 1987	Case-control	Study of STS among Dow chemical employees (1940-1979) compared to employees without STS for possible association with several chemical exposures	14	126
Townsend et al., 1982	Cohort	Study of adverse reproductive outcomes among wives of Dow chemical employees potentially exposed to TCDD (1939-1975) compared to reproductive outcomes among wives whose husbands were not exposed	370	345
Other chemical plants				
Thiess et al., 1982	Cohort	Study of mortality experience among BASF employees potentially exposed to TCDD during Nov. 17, 1953 accident compared to population and other workers not exposed	74	External controls: 180,000 town 1.8 million district 60.5 million Federal Republic of Germany Two groups of 74 each from other cohort studies

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Zober et al., 1990	Cohort	Mortality experience of workers exposed to TCDD (1954-1987) at BASF plant compared to population of Federal Republic of Germany	247	—
Manz et al., 1991	Cohort	Mortality experience of workers (1952-1984) at Hamburg plant of Boehringer exposed to TCDD compared to national mortality and workers from another company	1,184 men 399 women	(a) population (b) 3,120 gas workers —
Saracci et al., 1991	Cohort	Study of mortality experience of 20 international cohorts of herbicide sprayers and production workers compared to mortality experience expected for the nation	16,863 men 1,527 women	—
Coggon et al., 1986	Cohort	Study of mortality experience (through 1983) among workers manufacturing and spraying MCPA (1947-1975) compared to expected numbers of deaths among men of England and Wales and for rural areas	5,754	—
Coggon et al., 1991	Cohort	Mortality experience among four cohorts of workers potentially exposed (1963-1985) to phenoxy herbicides and chlorophenols compared to national (England and Wales) expected numbers and to the local population where factory is located	1,104 Factory A 271 Factory B 345 Factory C 519 Factory D	—

Jennings et al., 1988	Cohort	Assessment of immunological abnormalities among workers exposed to TCDD during accident manufacturing 2,4,5-T compared to matched controls	18	15
May, 1982, 1983	Cohort	Health outcomes among workers exposed and probably exposed to TCDD following a 1968 accident, compared to unexposed workers	41 exposed 54 possibly exposed	31
Bueno de Mesquita et al., 1993	Cohort	Mortality experience of production workers exposed to phenoxy herbicides and chlorophenols in the Netherlands compared to national rates	2,310	—
Bashirov, 1969	Cross-sectional	Descriptive results of examination of workers involved in production of herbicides and study of workers at examination of cardiovascular and digestive systems compared to unexposed controls	292 (descriptive) 50 (examined)	20 (examined)
Lynge, 1985	Cohort	Study of cancer incidence among Danish workers exposed to phenoxyherbicides compared to expected results from the general population	3,390 men 1,069 women	—
Pazderova-Vejlupkova et al., 1981	Descriptive	Study of development of TCDD intoxication among men in Prague (1965-1968)	55	No comparison group

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Poland et al., 1971	Cross-sectional	Assessment of PCT, chloracne, hepatotoxicity, and neuropsychiatric symptoms among 2,4-D and 2,4,5-T workers compared to other plant workers	73 total 20 administrators 11 production supervisors 28 production workers 14 maintenance workers	Internal comparison
Thomas, 1987	Cohort	Assessment of mortality experience as of Jan. 1, 1981, for white men employed in fragrance and flavors plant with possible exposure to TCDD, compared to U.S. white men and for cancers compared to local men	1,412	—
Agricultural/Forestry Workers				
Cohort studies				
Agricultural workers				
Burmeister, 1981	Cohort	Study of mortality of farmers compared to nonfarmers in Iowa (1971-1978)	6,402	13,809
Wigle et al., 1990	Cohort	Mortality experience of male farmers 35 years or older (1971-1985) in Saskatchewan, Canada, compared to age- and period-specific mortality rates expected for Saskatchewan males	69,513	—

Morrison et al., 1993	Cohort	Mortality experience of male Canadian farmers 45 years or older in Manitoba, Saskatchewan, and Alberta, Canada (1971-1987), compared to Canadian prairie province mortality rates	145,383	—
Morrison et al., 1992	Cohort	Mortality experience of male farmers 35 years or older (1971-1987) compared to Canadian prairie province rates	155,547	—
Ronco et al., 1992	Cohort	Study of cancer incidence (1970-1980) among male and female Danish farm workers 15 to 74 years old, compared to expected numbers of cancers among persons economically active, and study of cancer mortality (November 1981-April 1982) among male and female Italian farmers 18 to 74 years old compared to persons in other occupational groups	No *N*s given	No *N*s given
Corrao et al., 1989	Cohort	Study of cancer incidence among male farmers licensed (1970-1974) to use pesticides, compared to number of cancers expected among licensed nonusers	642	18,839
Lerda and Rizzi, 1991	Cohort	Study of farmers exposed to 2,4-D as measured in urine, compared to men unexposed for differences in sperm volume, death, count, motility, and abnormalities between March and June 1989	32	25

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Hansen et al., 1992	Cohort	Study of cancer incidence among male and female Danish gardeners compared to incidence expected among the general population	4,015; 859 women, 3,156 men	—
Wiklund, 1983	Cohort	Study of cancer incidence (diagnosed 1961-1973) among agricultural workers in Sweden compared to rates expected from the 1960 population census	19,490	—
Wiklund and Holm, 1986	Cohort	STS incidence among agricultural and forestry workers in Sweden compared to the general population of men, 1960 census	354,620	1,725,845
Wiklund et al., 1988a	Cohort	Malignant lymphoma incidence among agricultural and forestry workers in Sweden compared to the general population of men, 1960 census	354,620	1,725,845
Eriksson et al., 1992	Cohort	Study of incidence of NHL, HD, and multiple myeloma (1971-1984) among selected occupational groups in Swedish men and women, compared to expected rates of disease in general population	Number in occupational group unknown	—

Forestry workers				
Green, 1987	Cohort	Suicide experience in a cohort of Canadian forestry workers by number of years in forestry trade as a surrogate for exposure to phenoxy herbicides compared to population	1,222	—
Green, 1991	Cohort	Mortality experience of male forestry workers (1950-1982) in Ontario, compared to the expected mortality of the male Ontario population	1,222	—
van Houdt et al., 1983	Cross-sectional	Study of acne and liver dysfunction in a select group of Dutch forestry workers exposed to 2,4,5-T and unexposed	54	54
Herbicide/Pesticide Sprayers				
Axelson and Sundell, 1974	Cohort	Study of mortality and cancer incidence among cohorts of Swedish railroad workers spraying herbicides (>45 days) compared to the expected number of deaths (1957-1972) from Swedish age- and sex-specific rates	348 total herbicide exposure 207 phenoxy acids and combinations 152 amitrole and combinations 28 other herbicides and combinations	—
Axelson et al., 1980	Cohort	Additional years of follow-up to cohort established in Axelson and Sundell (1974)	348	—

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Blair et al., 1983	Cohort	Mortality experience of white male Florida pesticide applicators compared to U.S. and Florida men	3,827	—
Riihimaki et al., 1982	Cohort	Study of mortality among herbicide applicators exposed to 2,4-D and 2,4,5-T in Finland compared to mortality expected in the population	1,926	—
Riihimaki et al., 1983	Cohort	Cancer morbidity and mortality in cohort (Riihimaki et al., 1982)	1,926	—
Smith et al., 1981	Cohort	Study of chemical applicators (1973-1979) in New Zealand compared to agricultural contractors for differences in adverse reproductive outcomes	459	422
Smith et al., 1982	Cohort	Study of adverse reproductive outcomes among chemical applicators and agricultural contractors by category of exposure: none; chemicals not 2,4,5-T; 2,4,5-T	113 pregnancies (chemicals not 2,4,5-T) 486 pregnancies (2,4,5-T)	401 pregnancies (not exposed)
Wiklund et al., 1987	Cohort	Risk of HD and NHL among Swedish pesticide applicators from date of license through 1982, compared to expected number of cases in the total population	20,245	—

Wiklund et al., 1988b	Cohort	Risk of STS in Wiklund et al. (1987) cohort through 1984	20,245	—
Wiklund et al., 1989a	Cohort	Risk of cancer in Wiklund et al. (1987) cohort through 1982	20,245	—
Wiklund et al., 1989b	Cohort	Risk of STS, HD, and NHL in Wiklund et al., 1987 cohort through 1984	20,245	—
Swaen et al., 1992	Cohort	Cancer mortality experience (through 1987) among Dutch male herbicide applicators licensed before 1980, compared to the total male Dutch population	1,341	—
Bender et al., 1989	Cohort	Cancer mortality of Minnesota highway maintenance workers compared to expected numbers based on white Minnesota men	4,849	—
Barthel, 1981	Cohort	Study of male agricultural production workers (1948-1972) for incidence of cancer, compared to incidence rates expected in the population	1,658	—
Case-control studies				
Hardell and Sandstrom, 1979	Case-control	Study of male cases of STS (26-80 years) diagnosed between 1970 and 1977 in northern Sweden, compared to population-based sample without cancer for association with occupational exposure to phenoxyacetic acids and chlorophenols	52	206

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Eriksson et al., 1979, 1981	Case-control	Study of cases of STS diagnosed between 1974 and 1978 in southern Sweden compared to population-based sample without cancer for association with occupational exposure to phenoxyacetic acids and chlorophenols	110	219
Hardell and Eriksson, 1988	Case-control	Study of male cases of STS (25-80 years) diagnosed between 1978-1983 in northern Sweden compared to two referent groups: (1) population based, (2) with other cancers, for association with occupational exposure to phenoxyacetic acids and chlorophenols	55	330 population based 190 other cancers
Wingren et al., 1990	Case-control	Study of male cases of STS (25-80 years) diagnosed 1975-1982 in southeast Sweden, compared to two referent groups: (1) population-based sample, (2) with other cancers, for association with phenoxyacetic acids and chlorophenols	71	315 population based 164 other cancers
Eriksson et al., 1990	Case-control	Study of male cases of STS (25-80 years) diagnosed 1978-1986 in central Sweden compared to population-based sample without cancer for association with occupational exposure to phenoxyacetic acids and chlorophenols	218	212

Hardell et al., 1980 Hardell et al., 1981	Case-control	Study of malignant lymphomas (HD, NHL, unknown) diagnosed in men age 25-85, between 1974 and 1978 in northern Sweden, compared to population-based sample without cancer for association with occupational exposure to phenoxyacetic acids and chlorophenols	60 HD 109 NHL	338
Hardell and Bengtsson, 1983	Case-control	Study of HD diagnosed in men 25-85, between 1974 and 1978 in northern Sweden, compared to population-based sample without cancer for association with occupational exposure to phenoxyacetic acid and chlorophenols	60	335
Hardell, 1981	Case-control	Study (1) of cases of STS (Hardell and Sandstrom, 1979) and malignant lymphomas (Hardell et al., 1981) compared to colon cancer cases, and (2) study of colon cancer compared to population-based controls for association with occupational exposure to phenoxyacetic acids and chlorophenols	(1) 221 (2) 154	154 541
Hardell et al., 1982	Case-control	Study of nasal and nasopharyngeal cancers diagnosed 1970-1979 in men 25-85 years residing in northern Sweden, compared to controls selected from previous studies (Hardell and Sandstrom, 1979; Hardell et al., 1981) for association with occupational exposure to phenoxyacetic acids and chlorophenols	44 nasal 27 nasopharyngeal	541

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Hardell et al., 1984	Case-control	Study of primary liver cancer diagnosed 1974-1981 in men 25-80 years, residing in northern Sweden compared to population-based controls for association with occupational exposure to phenoxyacetic acids and chlorophenols	98	200
Persson et al., 1989	Case-control	Study of HD and NHL among living men and women in Sweden, compared with those without these cancers for association with occupational exposures, including phenoxy herbicides	54 HD 106 NHL	275
Olsson and Brandt, 1988	Case-control	Study of NHL (1978-1981) in Swedish men, compared to two groups of men without NHL for association with occupational exposures including phenoxy acids	167	50 same area 80 other parts of Sweden
Smith et al., 1983	Case-control	Preliminary report of men with STS reported 1976-1980 in New Zealand, compared to controls with other cancers for association with phenoxyacetic acid exposure	80	92
Smith et al., 1984	Case-control	Study of STS among New Zealand residents (1976-1980), compared to those without these cancers for association with occupational exposures, including phenoxy herbicides	82	92

Smith and Pearce, 1986	Case-control	Update of Smith et al. (1983) with diagnoses through 1982	51 in updated study 133 when combined with Smith et al., 1983	315 407
Pearce et al., 1985	Case-control	Study of malignant lymphoma and multiple myeloma in men diagnosed 1977-1981 in New Zealand, compared to men with other cancers for association with agricultural occupations	734	2,936
Pearce et al., 1986b	Case-control	Study of NHL cases (ICD 202) in men diagnosed between 1977 and 1981 in New Zealand, compared to sample with other cancers and population sample, for association with occupational exposure to phenoxy herbicides and chlorophenols	83	168 other cancers 228 general population
Pearce et al., 1986a	Case-control	Study of male multiple myeloma cases diagnosed 1971-1981 in New Zealand, compared to controls for other cancers for potential association with phenoxy herbicides and chlorophenols	76	315
Pearce et al., 1987	Case-control	Expanded (Pearce et al., 1986b) study of NHL to include ICD 200 diagnosed cases, and additional controls for association with farming exposures	183	338
Blair and Thomas, 1979	Case-control	Study of leukemia cases in Nebraska (1957-1974) compared to deaths from other causes for association with agricultural practices	1,084	2,168

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Blair and White, 1985	Case-control	Study of leukemia cases by cell type in Nebraska (1957-1974) compared to nonleukemia deaths for association with agricultural practices	1,084	2,168
Brown et al., 1990	Case-control	Population-based case-control study of leukemia in Iowa and Minnesota men for association with farming exposures	578	1,245
Cantor et al., 1992	Case-control	Population-based case-control study of NHL in Iowa and Minnesota men for assocation with farming exposures	622	1,245
Zahm et al., 1990	Case-control	Study of white men 21 years or older diagnosed with NHL (1983-1986) in Nebraska, compared to residents of the same area without NHL, HD, multiple myeloma, chronic lymphocytic leukemia for association with herbicides (2,4-D) on farms	201	725
Boffetta et al., 1989	Nested case-control	National study of multiple myeloma compared to other cancer controls for association with exposures including pesticides and herbicides	282	1,128
Burmeister et al., 1982	Case-control	Study of leukemia deaths (1964-1978) in white men 30 years or older in Iowa, compared to nonleukemia deaths for association with farming	1,675	3,350

Burmeister et al., 1983	Case-control	Study of multiple myeloma, NHL, prostate and stomach cancer mortality (1964-1978) in white men 30 years or older compared to mortality from other causes for association with farming practices including herbicide use in Iowa	550 multiple myeloma 1,101 NHL 4,827 prostate 1,812 stomach	1,100 2,202 9,654 3,624
Hoar et al., 1986	Case-control	Study of STS, NHL, HD in Kansas (1976-1982), compared to controls without cancer for association with 2,4-D, 2,4,5-T, and other herbicides in white men 21 years or older	133 STS 121 HD 170 NHL	948
Cantor, 1982	Case-control	Study of NHL in Wisconsin among males (1968-1976) compared to men dying from other causes for association with farming exposures	774	1,651
Dubrow et al., 1988	Case-control	Death certificate study (1958-1983) of NHL and HD among white male residents of Hancock County, Ohio, compared to a random sample of those dying from other causes for association with farming	61 NHL 15 HD	304
Morris et al., 1986	Case-control	Study of multiple myeloma (1977-1981) in four SEER areas compared to population controls for risk factors associated with the disease, including farm use of herbicides	698	1,683

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Carmelli et al., 1981	Case-control	Cases of spontaneous abortions occurring to women (1978-1980), compared to live births for association with father's exposure to 2,4-D	134	311
Woods et al., 1987	Case-control	Study of STS or NHL in men 20-79 years old (1983-1985) in western Washington State compared to a population sample without these cancers for association with occupational exposure to phenoxy herbicides and chlorinated phenols	128 STS 576 NHL	694
Woods and Polissar, 1989	Case-control	Study of NHL from the Woods et al. (1987) study for association with phenoxy herbicides in farm workers	576	694
Alavanja et al., 1988	PMR analysis with nested case-control	Mortality experience of USDA extension agents (1970-1979) evaluated for specific cancer excess; case-control study of specific cancers identified from PMR analysis	1,495	—
Alavanja et al., 1989	PMR analysis with nested case-control	Mortality experience of USDA forest/soil conservationists (1970-1979) evaluated for specific cancer excess; case-control study of specific cancers identified from PMR analysis	1,411	—

Hardell et al., 1987	Case-control	Study of Kaposi's sarcoma in AIDS patients (23-53 years of age) compared to controls for association with TCDD and pesticide exposure in Sweden	50	50
Donna et al., 1984	Case-control	Study of ovarian cancer in women (1974-1980) for association with herbicide use, compared to women without ovarian cancer	60	127
Musicco et al., 1988	Case-control	Study of brain gliomas diagnosed 1983-1984 in men and women in Italy, compared to (1) patients with nonglioma nervous system tumors and (2) patients with other neurologic diseases, for association with chemical exposures in farming	240	(1) 465 (2) 277
Vineis et al., 1986	Case-control	Study of cases of STS in men and women diagnosed 1981-1983 in northern Italy, compared to population sample of controls for association with phenoxy herbicide exposure	37 men 31 women	85 men 73 women
Balarajan and Acheson, 1984	Case-control	Study of STS (1968-1976) diagnosed in men in England and Wales compared to men with other cancers for association with farming, agriculture, and forestry occupations	1,961	1,961
Smith and Christophers, 1992	Case-control	Study of STS and malignant lymphomas in men diagnosed 1982-1988 in Australia, compared to other cancers for association with exposure to phenoxy herbicides and chlorophenols	82	82 other cancers 82 population

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
LaVecchia et al., 1989	Case-control	Study of Italian men and women with HD, NHL, and MM (1983-1988), compared to population of Italy for association with occupations and herbicide use	69 HD 153 NHL 110 MM	396
Paper/Pulp Workers				
Robinson et al., 1986	Cohort	Mortality experience through March 1977 of white male workers employed in five paper/pulp mills compared to expected number of deaths among U.S. population	3,572	—
Henneberger et al., 1989	Cohort	Mortality experience through August 1985 of white men employed in Berlin, N.H. paper and pulp industry, compared to expected mortality in U.S. white men	883	—
Solet et al., 1989	Cohort	Mortality (1970-1984) among white male United Paperworkers International Union members, compared to expected number of deaths in U.S. men	201	—
Jappinen and Pukkala, 1991	Cohort	Cancer incidence (through 1987) among male Finnish pulp and paper workers (1945-1961), compared to rates in the local central hospital district	152	Approximately 135,000

Other Occupational Studies				
Fitzgerald et al., 1989	Cohort	Health outcomes in group exposed to electrical transformer fire in 1981 compared to standardized rates among upstate New York residents	377	—

[a]The dash (—) indicates the comparison group is based on a population (e.g., U.S. white males, country rates), with details given in the text for specifics of the actual population.

least one year, with no work in either the paper or the pulp mill. Vital status was determined through August 1, 1985, using information from the 1961 study follow-ups, contacts with relatives and friends, telephone books, city directories, SSA, and the NDI. Comparisons were made to death rates from U.S. vital statistics.

The third study was a PMR analysis of 201 white males who died between 1970 and 1984 and who had been employed in pulp and paper production plants (Solet et al., 1989). The workers were identified from the United Paperworkers International Union and were vested (employed at least 10 years) in the union's Paper Industry Union-Management Pension Fund. Data were obtained from the pension fund files for those who were deceased; employment history consisted of the plant and years worked there. Expected mortality, adjusted for sex, race, age at death, and calendar year of death, among U.S. males was used as a comparison. No data on potential exposure to processes involving TCDD were available.

A small cohort study of Finnish paper mill workers investigated the association of potential exposure to PCDD compounds, including TCDD, with lung cancer as well as all sites combined (Jappinen and Pukkala, 1991). One hundred fifty-two male pulp and paper mill workers in Finland who had been exposed to TCDD and dibenzofurans in bleach (organic chlorine compounds) for one year or more from 1945 to 1961 were followed from January 1, 1953, until December 31, 1987, for cancer incidence. Data from the Finnish Cancer Registry were used to identify incident cancers; the National Population Register was used to identify mortality. Expected numbers were obtained from rates of the local central hospital district. Exposure assessment was not performed, but association was studied specifically among subgroups chosen to have different levels of exposure to chlorinated compounds. No smoking data were available.

Other Occupational Studies

Following an electrical transformer fire in 1981, in a building in Binghamton, New York, TCDD was identified in the soot samples analyzed; 482 persons potentially exposed to polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins, and dibenzofurans were followed for adverse health effects (Fitzgerald et al., 1989). Of those potentially exposed, 155 were not actually in the building, but in the vicinity. A questionnaire was sent in April 1984 to all participants who had been identified originally. Questions concerning health status and reproductive history were asked; repeated attempts were made to contact those not known to be deceased. Hospital, physician, and pediatric reports were sought to verify medical and reproductive outcomes reported; computer linkages of the cohort with New York State vital records and the Cancer Registry were used to identify deaths (including spontaneous abortions

and fetal or infant deaths) and cancers between 1981 and 1984. Expected numbers for comparison were obtained from corresponding population rates of upstate New York (state of New York exclusive of New York City). The final study sample included 377 respondents.

ENVIRONMENTAL STUDIES

The occurrence of accidents and industrial disasters has offered opportunities to evaluate the long-term health effects of exposure to dioxin and other potentially hazardous chemicals. Among these disasters is the accidental explosion, on July 10, 1976, of a factory in Seveso, Italy, that produced trichlorophenol, which resulted in the release of TCDD-containing compounds into the surrounding environment, exposing those living in the area. Details of the accident and exposure are included in Chapter 2. The epidemiologic studies on these populations are summarized in Table 7-2.

Seveso

The incidence of neurological disorders following exposure to TCDD was the focus of one study initiated shortly after the Seveso accident on July 10, 1976 (Boeri et al., 1978). The accident resulted in what are subsequently referred to as exposure zones based on contamination of the soil: zone A included residents who were evacuated from the area around the plant (N = 734); zone B included residents who were exposed, but to a lesser extent (N = 4,699); and zone R included residents who had the lowest exposure (N = 31,800). Residents from zone A were invited for neurological examination, and 470 of 723 residents volunteered; invitation was by letter or personal invitation during home screening visits (Filippini et al., 1981). Residents of zone R requested examination, although not originally designated for inclusion in the study, however, since examinations of controls were not completed, volunteers from this zone (N = 152) were examined for comparison with the zone A participants. Neurologic testing occurred in March 1977. Although actual individual exposures were unknown, residence in a high- versus low-exposure potential area was considered as the exposure. As a follow-up to the screening above, residents were invited to return in April 1978 for a second neurological screening, to be compared with results from neurological tests of those in unpolluted areas around Seveso (Filippini et al., 1981). Of the 709 Seveso residents invited, 308 who attended the second screening were eligible for inclusion; subjects were examined clinically, completed a medical history questionnaire, and underwent an electrophysiologic investigation. A nonexposed population of 305 provided referent levels of neurological functioning. Analyses were done by comparing those with symptoms of neuropathy or previously studied

indicators of TCDD exposure (chloracne, gamma-glutamyl transpeptidase, glutamic-oxalacetic transaminase, or glutamic-pyruvic transaminase) to those with neither symptoms nor indicators.

With chloracne being used as a marker of exposure to TCDD, 152 Seveso residents with chloracne or a history of the lesions agreed to be examined for the presence of peripheral nervous system involvement as a long-term effect of exposure (Barbieri et al., 1988). The control group consisted of 123 age- and sex-matched subjects living in nearby towns with similar environmental pollution. The study was conducted from October 1982 to May 1983; medical history, occupational exposures to neurotoxic agents, dermatologic exam, laboratory testing, and neurological examination were completed on all study participants.

Descriptive data results of selected health outcomes that occurred among residents of the Seveso area after July 10, 1976, were reported (Bisanti et al., 1980). The number of residents included in this report differs slightly from the numbers presented above; here, for zone A, $N = 730$; zone B, $N = 4{,}737$; and zone R, $N = 31{,}800$. Health outcomes reported included chloracne, birth defects, and spontaneous abortions, as well as crude birth and death rates.

The distribution of chloracne among the population exposed in zones A, B, and R, especially among children, and associations with other diseases among those with and without chloracne are presented (Caramaschi et al., 1981). After the accident, reports of skin lesions were made, and cases of chloracne were diagnosed; data regarding dermatological conditions were collected by the Department of Dermosyphilopatic Diseases of the University of Milan; additionally, dermatological screening of children under 15 years was conducted between February and April 1977. Two groups of children, one with chloracne ($N = 146$) and one without symptoms ($N = 182$), were clinically followed with medical examinations and biochemical tests. Cases of chloracne were included from each of the three zones, as well as from non-ABR zones.

Children in the zones around Seveso were examined periodically for chloracne and were tested for laboratory levels of several chemicals in the blood and urine, to compare them to normal levels expected among children not exposed to TCDD (Mocarelli et al., 1986). Children residing in zone A ($N = 69$), zone B ($N = 528$), a subset of more highly exposed children in zone B ($N = 83$), zone R ($N = 874$), and a subset from zone R as a control group ($N = 241$) were followed over six years for levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, cholesterol and triglycerides, and amino levulinic acid in urine; these values were compared to a sample of 1,000 subjects used by the laboratory to establish "normal" values.

In a follow-up to these studies (Caramaschi et al., 1981; Mocarelli et

al., 1986) in children, dermatologic findings and laboratory tests were conducted among a group of the children with chloracne compared to controls (Assennato et al., 1989a).

Adults and children in the area of Seveso and surrounding unexposed communities were the subjects of a study of urinary *d*-glucaric acid as an indicator of induced hepatic enzymes associated with the TCDD exposures in the area (Ideo et al., 1985); an earlier study evaluated these enzymes in children with and without chloracne (Ideo et al., 1982). As part of the monitoring plan of the Seveso area, residents of zone B had blood drawn for testing, and for one week in February 1978, urine was collected from 117 adults as well; 127 residents of an unexposed community contributed urine specimens as a control group. Nonparametric statistical methods were used to compare levels of liver enzyme activity between groups because of the relatively small size of the sample.

A 10 year follow-up of individuals exposed to TCDD following the accidental explosion in Seveso has been reported (Bertazzi et al., 1989a,b). All persons who resided in any of the 11 towns included in the two health districts that were in the contaminated zones of Seveso (A, B, and R) were eligible for study follow-up; information collected included demographics, residence at time of the accident and subsequently, and date of first residence for those moving into the area. Classification of exposed residents of the Seveso area was according to zones A (N = 556), B (N = 3,920), and R (N = 26,227), or outside the contaminated boundaries, based on residence at the time of the accident or at first entry to the area. Study subjects were followed through national records throughout the country as of December 31, 1986; cause of death for those deceased was as certified by the attending physician and reported to the National Statistics Institute of Italy. The reference population was the cohort residing outside the contaminated A, B, and R zones (N = 167,391).

Cancer incidence over the same period for this cohort has also been evaluated, using rates for the Lombardy region from hospital discharge registration as a comparison (Pesatori et al., 1992).

Reported separately are the results of a 10 year follow-up mortality study (1976-1986) of children age 1-19 at the time of the accidental explosion in Seveso of the TCDD factory (Bertazzi et al., 1992), with methods similar to those used for adults described previously. The 19,637 subjects that were exposed (zones A, B, and R) and a reference group of 95,339 people living in the surrounding districts formed the basis of this study. The follow-up was nearly 99 percent for vital status as of December 31, 1986. Exposure data are reasonably good for the amount of TCDD on the ground in different zones beyond the factory. However, there is no individual quantification of exposures.

A two year prospective controlled study was conducted of workers potentially

exposed to TCDD during the cleanup of the most highly contaminated areas following the Seveso accident (Assennato et al., 1989b). Preemployment examinations were performed from March to June 1980 to select the study groups. Workers who met certain criteria (age and certain health characteristics) were assigned to either the cleanup group or the comparison group. Periodic examinations were conducted every six months. The cleanup group was provided with protective clothing and was subject to safety measures designed to minimize the potential for exposure. At the conclusion of the study, hypothesized TCDD-related clinical disease (i.e., chloracne, liver disease, peripheral neuropathy, porphyria cutanea tarda) and differences in biochemical outcomes were compared between the two groups.

All live and stillbirths from January 1, 1977, to December 31, 1982, to women who were residents in zones A, B, R, and non-ABR were reported to an ad hoc birth defects registry, the Seveso Congenital Malformation Registry (Mastroiacovo et al., 1988). Classification of infant exposure was based on residence of the mother; ascertainment of the malformation data was obtained from maternity hospitals, pediatric departments, and primary care pediatric services. A special team examined all reports to the registry. Registered infant malformations were considered as major or mild. A total of 15,291 births were included in the registry for this time period.

A cytogenetic analysis of maternal and fetal tissues from a control sample of women not exposed to environmental mutagens, who underwent an induced abortion between 8 and 11 weeks gestation, was compared to similar tissues from women exposed to TCDD around Seveso (Tenchini et al., 1983). The frequencies of aberrant cells, the relative proportions of individual types of chromosomal aberration, the average number of lesions per damaged cell, and the frequencies of polyploids were compared in maternal blood and placenta in the two samples.

Times Beach and Quail Run

During early 1971, by-products of a hexachlorophene and 2,4,5-T production facility in Verona, Missouri, were mixed with waste oils and sprayed on various sites around the state for dust control. TCDD was a contaminant of the mixtures sprayed, and the contamination was reported by the Environmental Protection Agency (EPA). A pilot study to evaluate health effects from potential exposure was conducted in the state in 1983, and results were reported (Stehr et al., 1986; Webb et al., 1987). At the time the pilot was designed, the environmental data available were limited, direct measures of TCDD body burden were not available, and health effects to be investigated were uncertain; the pilot was designed to potentially direct future research. Assessment of potential health effects was determined through

three means, including a health effects survey questionnaire that included information on exposure-risk (residence, recreation, occupation), medical history, and potential confounding variables. Individuals were given the questionnaire if they lived near, worked at, or participated in activities near a contaminated site; persons were so identified from response to a media announcement. The second assessment of health effects was through a dermatology screening clinic for anyone in the population suspecting exposure, and the third assessment of health effects was through a pilot medical study from among completed questionnaire respondents. A high-risk group ($N = 68$) consisted of those who had lived or worked at least six months in highly TCDD-contaminated areas; low-risk comparison subjects ($N = 36$) were selected from among the lowest exposed of the 800, and matched on type of exposure site, age, sex, race, and socioeconomic status (SES). The clinical protocol included physical, neurologic, and dermatologic exams; lab analysis; immune response tests; and storage of serum. All comparisons made were between low- and high-risk exposure groups.

Participants from the original study who did not respond to any of the delayed-type hypersensitivity skin test antigens or who responded to only one antigen (50 exposed and 27 unexposed) were invited back for retesting, and results are reported for 28 exposed and 15 unexposed individuals (Evans et al., 1988).

One of the sites with extremely high levels [2,200 parts per billion (ppb)] of TCDD from the study described above, was the area of Quail Run Mobile Home Park; residents were followed for long-term health effects between 1971 and 1983 (Hoffman et al., 1986; Stehr-Green et al., 1987). Residents were eligible for inclusion in the study if they had lived at Quail Run for at least six months between April 1971 and May 1983; of 207 potentially eligible households, 95 were located and a total of 154 persons enrolled in the study. A comparison group consisted of residents for at least six months of another mobile home park of comparable size, with similar homes and upkeep; three mobile home parks with no TCDD contaminants in the soil on testing constituted 515 households. Of those eligible to participate, 155 individuals agreed. Medical examinations of all participants were conducted from November 1984 to January 1985 by one of two physicians, blinded to subject's exposure status. Additionally, urine and blood samples were collected, delayed-type hypersensitivity testing was done, sensory peripheral neuropathy was tested, neurobehavioral tests were administered, and a standard interview of SES and household occupation was conducted. Comparisons were made between the exposed and unexposed mobile home residents; for physician-diagnosed medical conditions, 137 of the 154 exposed subjects were matched to unexposed subjects on sex, race, and age (within five years for those ≥ 15 years of age, within two years for those <15 years old). Diagnoses of medical conditions for both pair members

were included only if the diagnosis occurred after the participant's first residence in Quail Run until November 1984.

Further epidemiologic investigation examined adverse human reproductive outcomes associated with exposure to soil contaminated during 1971 with TCDD in residential areas of Missouri (Stockbauer et al., 1988). A pregnancy was considered as potentially "exposed" to TCDD if the mother's address on the birth or fetal death certificate was in an area with documented TCDD contamination equal to or greater than 1 ppb in proximity to the house. Missouri vital statistics data for live births and fetal deaths between January 1, 1972, and December 31, 1982, were linked to map and environmental sampling data for nine residential sites determined to have been contaminated by TCDD. The linkage resulted in 410 potentially exposed pregnancies. The majority ($N = 319$) of the births were from residents of the Times Beach area. For comparison, two births from unaffected areas of Missouri were selected for each exposed birth matched on maternal age, race, hospital of birth, and year of birth. Further exclusions, such as one member from each twin set or unavailable medical records, resulted in 402 exposed and 804 unexposed births that were reviewed for the presence of birth defects. Newborn medical records were reviewed (blind to exposure status) for the presence of selected structural, chromosomal, and biochemical defects. In addition, a statewide search of every hospital and major clinic in Missouri was undertaken to identify cases of birth defects. Variables obtained from the birth certificates included birthweight, sex of the infant, maternal education, parity, marital status, prepregnancy weight, smoking during pregnancy, and history of previous spontaneous or induced abortions. An attempt was made to classify exposed births further on the basis of the extent of potential TCDD exposure. First, the birth data set was compared with a Missouri central listing of TCDD-exposed persons. This list was derived from self-reported information from persons who thought they had been exposed to TCDD. Individuals on this list were divided into a high-risk group (lived at least six months in TCDD > 100 ppb contaminated areas or two years in 20-100 ppb contaminated areas) and a low-risk group (similar contamination levels, but lived there for less than stated time or lived where contamination was 1-19 ppb), based on time lived in the area and soil concentrations of TCDD. The investigators also compared births from 1972 to 1974 with births from 1975 to 1982, assuming a higher likelihood of exposure during the period of spraying of dirt roads with TCDD-contaminated oil prior to 1974.

Vietnam

Studies of the population of Vietnam who were exposed to the spraying that occurred during the Vietnam conflict have been conducted by Vietnamese

investigators, and the unpublished results of these studies have been summarized in a review paper by Constable and Hatch (1985). These studies focus primarily on reproductive outcomes, since the early laboratory studies indicated that adverse pregnancy outcomes could result from exposure to TCDD. Studies were conducted comparing couples who lived in sprayed areas in the South with couples living in unsprayed areas; some studies compared couples living in the North with couples living in the South of Vietnam; other studies compared couples living in the North in which husbands did and did not serve in the South. There were nine reports included in the review article, which are summarized as follows: (1) Khoa (1983): rates of obstetric events 1965-1982 among Montagnards living in a heavily sprayed area of southern Vietnam; (2) Nguyen (1983): rates of obstetric events 1979-1981 in a provincial hospital in a heavily sprayed area of southern Vietnam; (3) Trung and Chien (1983): comparison of rates of miscarriage and birth defects before and after spraying in an exposed and an unexposed village in southern Vietnam; (4) Huong and Phuong (1983): time trends analysis of reproductive events 1952-1981 with a case-control study of herbicide exposure and hydatidiform mole in Obstetric Hospital, Ho Chi Minh City; (5) Phuong and Huong (1983): comparison of reproductive problems in women exposed to herbicides in southern Vietnam and unexposed in southern and northern Vietnam; (6) Lang et al. (1983a): comparison of birth defects in offspring of soldiers from agricultural villages in northern Vietnam who did and did not serve in the South; (7) Lang et al. (1983b): comparison of miscarriage rates according to degree of northern Vietnam veterans' herbicide exposure; (8) Can et al. (1983b): comparison of reproductive events among wives of exposed and unexposed northern Vietnam veterans from three areas; (9) Can et al. (1983a): case-control study of birth defects in relation to northern Vietnam veterans' service in the South. Three studies in Vietnam were later published, and detailed methods follow.

The mortality experience from 1966 to 1986, in two villages that were sprayed with Agent Orange in Vietnam was compared with mortality in a village that remained unsprayed (Dai et al., 1990). Estimates of amount of exposure received during spraying for the three villages were obtained from published spray records; the village was considered as having been hit if the mission passed within 10 km of the village center. Since most babies in Vietnam are breast-fed, the possible effects of exposure of the mother on the offspring were of concern. Study subjects from the villages were interviewed on residential history, life-style, demographics, economic status, occupational history, chemical exposures (fertilizer and insecticides at least once a year), health status, medical history, and births and deaths of children; interviewers were not blinded to exposure status. Classification of exposure was into four groups, with two criteria to be met for each level of

exposure; if only one criterion per level was met, the exposure level assigned was the next lower. The categories were "heavy" (lived more than three years in sprayed area, and directly sprayed three or more times); "moderate" (lived in sprayed area one to three years, and directly sprayed one or two times); "light" (lived less than one year in sprayed areas, and not directly sprayed); "none" (lived in nonsprayed area during the Vietnam conflict).

To evaluate the potential association between mother's exposure to Agent Orange and TCDD during the Vietnam conflict, and the occurrence of birth defects or hydatidiform mole, a case-control study was conducted in Ho Chi Minh City, Vietnam (Phuong et al., 1989a). Cases were babies born with gross congenital anomalies during the period May 1-June 14 and July 18-August 25, 1982, in the Ob-Gyn Hospital of Ho Chi Minh City; also included were patients producing placentas with hydatidiform mole, with or without choriocarcinoma. Controls for birth defect cases were live births in the same time period; controls for the hydatidiform mole were patients admitted for vaginitis or exocervicitis. Matching variables for cases and controls included date of admission within seven days, SES, and residing originally in the southern part of Vietnam. Exposure was determined by mother's residence in villages known to be heavily sprayed primarily between 1965 and 1970.

Another study in southern Vietnam (May 2, 1982-June 15, 1982) evaluated reproductive abnormalities occurring among births to families resident in the heavily sprayed village of Thanh Phong, compared to births from families in Ho Chi Minh City (Phuong et al., 1989b). Mothers were interviewed for demographic information including residence from 1952 to 1982 and possible herbicide exposure. Data on behavioral habits including diet, smoking, and alcohol were also collected. Obstetrical history and determination of incidence of congenital anomalies were obtained in a separate interview. A total of 7,327 births occurred to Thanh Phong families and 6,690 births in commune 10, Ho Chi Minh City. Exposure in Thanh Phong was historically estimated to be heavy, moderate, or light. Heavy exposure included multiple spraying episodes and always resident in Thanh Phong. Moderate exposure included two sprayings and resident at least two years in Thanh Phong. Light or no exposure included less than two sprayings or indirect spraying, or residence in Thanh Phong after 1971.

Other Environmental Studies

In response to the concern of women in Alsea, Oregon, who believed their miscarriages were associated with local herbicide spraying, a study was undertaken by the Human Effects Monitoring Branch in the EPA Office of Pesticide Programs (U.S. EPA, 1979). An initial health questionnaire

survey (Alsea I) of nine women who had experienced 13 confirmed miscarriages from May 1973 to March 1978 prompted a study of the six year spontaneous abortion rates in three areas of Oregon (Alsea II). The definition of the study area, which was primarily rural, was around the "Alsea basin" in Oregon, as defined by U.S. Postal Service zip codes. Four hospitals in the largest towns were the source of birth information for the Alsea II study. The control study area was a primarily rural area, with little or no reported use of 2,4,5-T, where hospital facilities and physician practices were anticipated to be similar to those in the "exposed" area; the two hospitals in the control area were located in the two largest towns. Additionally, an urban area located in the agricultural nonforested Willamette Valley of Oregon was identified. The main hospital in this area was used by women delivering babies; it was used by women in other study areas as well. Spraying in the area of interest was determined through information supplied by major organizations using the chemicals, and consisted of date of application, rate of application, formulation, number of acres sprayed, and location of the sprayed areas. The areas sprayed were plotted on a map; seasonal usage patterns were also noted. All hospitals noted above were sources of spontaneous abortion data; for each spontaneous abortion, ICD code, age of patient, date of spontaneous abortion, gestation period, and patient's zip code were obtained from all records between 1972 and 1977. Physicians in the area were also contacted to assess their estimates of the number of spontaneous abortions that had been treated in the time period of interest; these interviews were not conducted in the control area. Computer tapes of births per month were obtained from the Vital Statistics Section of the Oregon State Health Department; birth certificate data were also obtained from this source. A spontaneous abortion index was developed for each area to account for number of births occurring in the area.

In an area of Northland, New Zealand, rates of occurrence of all diagnosed malformations, excluding miscarriages less than 28 weeks, were correlated with densities of 2,4,5-T sprayed during the same period (Hanify et al., 1981). Seven areas, as defined by location of the hospitals, were used in determining births in the hospitals and spraying as indicated by company records for those doing the spraying. Little spraying was done between 1959 and 1965; therefore, January 1, 1960-August 31, 1966, was used as the "unexposed" period, and September 1, 1972-August 31, 1977, as the "exposed" period.

An ecological study design was used to examine the relationship between the prevalence of oral cleft palate and the use of 2,4,5-T in Arkansas (Nelson et al., 1979). In order to estimate 2,4,5-T exposure, herbicide application reports were obtained from the Arkansas State Plant Board for 1970-1974. It was found that 58 percent of the acreage treated with 2,4,5-T was planted with rice; since rice is the major crop treated with 2,4,5-T, the

investigators assumed that the ratio of rice acreage to total acreage could be used to divide the 75 Arkansas counties into high (5 percent or more of total acreage planted with rice in 1974), medium (1.3-4.5 percent), and low exposure (0-0.9 percent). Ascertainment of oral clefts was conducted by using birth certificates (1943-1974) and records from the Crippled Children's Services of Arkansas.

A case-control study in Iowa and Michigan was undertaken to evaluate the potential association between agricultural use of chemicals and cleft lip or palate (Gordon and Shy, 1981). Based on a number of criteria (number of births, adequate birth defect reporting, agricultural status), Iowa and Michigan were chosen as the study area; National Center for Health Statistics data for 1974-1975 were used to identify cases and controls. Cases were infants born with cleft lip and/or palate in either Iowa or Michigan in 1974 or 1975. Controls were a random sample (2 percent) of live births in each state, which yielded approximately a 5:1 ratio of controls to cases. The study sample was restricted to white, liveborn singletons from rural areas; children with multiple malformations were excluded, for a final sample of 187 case and 985 control births. Two surrogate measures of exposure were used. The first was a ratio of the number of acres of a specific crop to the number of acres of farmland by county. The second measure was a ratio of the number of acres of cropland in which chemicals (herbicides and pesticides) were applied to the number of acres of farmland by county. For each state, exposures were categorized as crops only, all pesticide chemicals and fertilizers, and "suspect" chemicals (pesticides only). Within each of these categories, each county was assigned either a high or a low score on each crop and chemical. Finally, the county score, the sum of the high and low scores, was calculated within the three exposure categories and assigned to each study subject in that county. Further rescoring of the exposure variables was done, resulting in a dichotomous variable with 0 equal to a high score on no more than two chemicals and 1 for a high score on three or more chemicals.

Stillbirths and birth defects were compared to control births for association with exposure to chemicals used in agriculture in areas where the births occurred in New Brunswick, Canada, between 1973 and 1979 (White et al., 1988). Statistics Canada was the source of stillbirth information, and the Canadian Congenital Anomalies Surveillance System was the source of birth defect data. Chemical use data were compiled from the New Brunswick Department of Environment through maps and records from the spray application companies. Since there were no records for most of the period in question for chemical use in agriculture, an index of chemical exposure opportunity was developed based on where agricultural production might occur. Included in the pesticides under consideration as exposures were fentrothion formulations, aminocarb formulation, other forest insecticides,

herbicides with some phenoxy component, herbicides with only phenoxy, chlorinated herbicides, and nonchlorinated herbicides. Birth defects identified included neural tube defects, facial clefts, and bilateral renal agenesis. Correlational analyses, comparisons of mean rates of birth defects by periods of spraying, and a case-control analysis were conducted. For the case-control study, controls were identified from the Provincial Registry of Live-births and Statistics Canada; approximately two controls per case were selected and matched in one set by date of birth and sex, and in another set by county and date of birth, but from a different year. The residence in which the mother lived when giving birth determined the "exposure-risk window," calculated for the first trimester as the 2 weeks before and the 13 weeks after conception, and in the second trimester, from 14 to 27 weeks gestation. To calculate the exposure, (1) the occurrence of the spraying and the mother's residence were plotted on a map; (2) a radius was centered on the residence; and (3) potential exposure to forestry insecticides was quantified by the number of dots in the circle that occurred in the sprayed area.

A case study and a registry-based study were conducted in the county of Skaraborg, Sweden, in 18 boroughs with incinerators (Jansson and Voog, 1989). The case study was a patient investigation for six children born with cleft lip and/or palate between April and August 1987, and an exposure assessment including meteorological and dispersal calculations. The registry study compared numbers of cleft lip and palate occurring in the county of Skaraborg and the constituent boroughs between 1975 and 1987, compared to expected numbers. Rates of cleft lip before and after incinerators were introduced were compared during 1973-1986 for boroughs with cities excluded. The exposure of interest was municipal incinerators that were suspected of discharging high doses of TCDD in the area.

Since the mortality rates from STS and connective tissue cancers were apparently elevated in Midland County, Michigan, between 1970 and 1979, compared to the state of Michigan, an investigation of this potential excess was evaluated in conjunction with residence of the cases (Michigan Department of Public Health, 1983). Between 1960 and 1981, 20 deaths were coded as STS or connective tissue cancer in the Michigan Death Statistics. Rates of mortality were compared for each Michigan county, based on data for the period 1970-1981 from Michigan death certificates; comparisons were made with national and state rates. Residence for cases reported was investigated beyond what was reported on the death certificate as last known residence. Residence history, particularly for the period of interest (1960-1981), was obtained through interviews with next of kin; occupational history information was also obtained. Of particular interest was whether the decedent had been employed at Dow Chemical or Dow Corning, which would have potentially involved exposure to TCDD. Mortality rates were therefore examined for the 29 U.S. counties in which

TABLE 7-2 Epidemiologic Studies—Environmental Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Seveso				
Boeri et al., 1978	Cohort	Evaluation of neurological disorders among Seveso residents exposed to TCDD on July 10, 1976, compared to residents in unexposed areas	470 zone A	152 zone R
Filippini et al., 1981	Cohort	Comparison of prevalence of peripheral neuropathy on two screening examinations among Seveso residents, compared to residents in unexposed areas	308	305
Barbieri et al., 1988	Cohort	Comparison of prevalence of peripheral nervous system involvement among Seveso residents with chloracne, compared to residents in unexposed areas	152	123
Bisanti et al., 1980	Descriptive	Descriptive report of selected health outcomes among residents of Seveso located in zones A, B, R	730 zone A 4,737 zone B 31,800 zone R	No comparison group
Caramaschi et al., 1981	Cohort	Evaluation of chloracne among children in Seveso, compared to children with no chloracne, and association with other health outcomes between chloracne and no chloracne groups	146	182

Mocarelli et al., 1986	Cross-sectional	Study of laboratory measures of serum and urine in Seveso zone A and B children measured over 6 years (1977-1982), compared to zone R children	69 zone A 528 zone B 874 zone R	241, subset of zone R
Assennato et al., 1989a	Cohort	Comparison of dermatologic and laboratory findings in children during periodic exams following accident in Seveso	193 with chloracne	123
Ideo et al., 1982	Cross-sectional	Evaluation of hepatic enzymes in children exposed to Seveso compared to normal values	16 zone A 51 zone B	60 Bristo Assizio 26 Cannero
Ideo et al., 1985	Cross-sectional	Evaluation of levels of enzyme activity among residents of Seveso zone B and an uncontaminated community	117 adults	127 adults
Bertazzi et al., 1989a,b	Cohort	Comparison of mortality experience (1976-1986) of residents of contaminated zones (A, B, R) around Seveso to the mortality experience of unexposed residents in neighboring towns	556 zone A 3,920 zone B 26,227 zone R	167,391
Pesatori et al., 1992	Cohort	Cancer incidence (1976-1986) among those in zones A, B, R around Seveso compared to residents of uncontaminated surrounding areas	Data given in person-years	Data given in person-years

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Bertazzi et al., 1992	Cohort	Comparison of mortality of children (1976-1986) exposed during Seveso accident compared to children in uncontaminated areas	306 zone A 2,727 zone B 16,604 zone R	95,339
Assennato et al., 1989b	Cohort	Study of health outcomes in workers assigned to cleanup or referent group following Seveso accident	36	36
Tenchini et al., 1983	Cross-sectional	Cytogenetic analysis of maternal and fetal tissue among Seveso exposed compared to control sample	19	16
Mastroiacovo et al., 1988	Cohort	Comparison of birth defects occurring among zone A, B, R mothers with live and stillbirths to mothers with births from non-A, B, or R residents	26 zone A 435 zone B 2,439 zone R	12,391 (non-A, -B, -R)
Times Beach/Quail Run				
Stehr et al., 1986; Webb et al., 1987	Cross-sectional	Pilot study of Missouri residents exposed to TCDD in the environment (1971) for health effects, comparing potentially high-exposed to low-exposed residents	68 high-exposed	36 low-exposed
Evans et al., 1988	Cross-sectional	Comparison of retesting for skin delayed-type hypersensitivity among nonresponders in earlier test (Stehr et al., 1986)	28	15

Hoffman et al., 1986; Stehr-Green et al., 1987	Cohort	Study of the health effects (1971-1984) of residents of Quail Run Mobile Home Park compared to residents in uncontaminated mobile parks	154	155
Stockbauer et al., 1988	Cohort	Study of adverse reproductive outcomes (1972-1982) among mothers potentially exposed to TCDD-contaminated areas of Missouri (1971) compared to births among unexposed mothers	402 births	804 births
Vietnam				
Dai et al., 1990	Cohort	Study of infant mortality (1966-1986) in two South Vietnam villages exposed to Agent Orange spraying compared to infant mortality in unsprayed area	5,609	3,306
Phuong et al., 1989b	Cohort	Comparison of reproductive anomalies among births to women (May 1982-June 1982) living in areas heavily sprayed with herbicides in southern Vietnam, to women from Ho Chi Minh city	7,327 births	6,690 births
Phuong et al., 1989a	Case-control	Study of deformed babies and hydatidiform mole compared to normal births (1982) in Ho Chi Minh City for association with mother's exposure to Agent Orange and TCDD in Vietnam conflict	15 birth defects 50 hydatidiform moles	104 134

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Constable and Hatch, 1985	Review	Summaries of reproductive outcomes among Vietnamese populations, includes nine unpublished studies		
Other environmental studies				
Cartwright et al., 1988	Case-control	Study of living cases of NHL (1979-1984) in Yorkshire, England, compared to other hospitalized patients for association with a range of exposures including fertilizers/ herbicides	437	724
Gordon and Shy, 1981	Case-control	Study of agricultural chemical exposures and potential association with cleft palate/ lip in Iowa and Michigan, compared to other live births	187	985
Hanify et al., 1981	Ecological design	Study of adverse birth outcomes occurring 1960-1966, compared to 1972-1977 for association with 2,4,5-T spraying in the later time period	9,614 births	15,000 births
Jansson and Voog, 1989	Cohort/ Case study	Case study of facial cleft (April-August 1987) and study of facial clefts (1975-1987) compared to the rates expected in Swedish county with incinerators	20,595 births after incineration 6 case study	71,665 births before incineration

Lampi et al., 1992	Nested case-control/Cohort	Study of cancer incidence among a community in Finland exposed to water and food contaminated with chlorophenols (1987), compared to other communities; study of several cancers compared to population controls for association with potential risk factors including food and water consumption	56 colon cancer 40 bladder cancer 8 STS 7 HD 23 NHL 43 leukemia	688
Nelson et al., 1979	Ecological design	Study of prevalence of oval cleft palates in high, medium, and low 2,4,5-T-sprayed areas in Arkansas (1948-1974)	—	—
Vineis et al., 1991	Ecological	Presentation of rates (1985-1988) of NHL, HD, and STS in men and women 15-74 years living in provinces in Italy where phenoxy herbicides are used in riceweeding and defined in two categories	63 HD 253 NHL 49 STS	No control/unexposed
White et al., 1988	Case-control and ecological	Study of chemical exposures in agricultural activity for potential association with birth defects and stillbirths in New Brunswick, Canada, 1973-1979	(a) 392 defects (b) 298 stillbirths	(a) 384 matched date of birth/sex; 386 matched county/date of birth (b) 299 matched date of birth/sex; 302 matched county/date of birth

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Michigan, 1983	Descriptive	Comparison of Michigan county rates of mortality for STS and connective tissue cancer (1960-1981), compared to state and national rates for potential excess in areas where dioxin may be in the environment	County rates	State and national rates
U.S. EPA, 1979	Ecological design	Study of spontaneous abortions occurring during 1972-1977 in herbicide-sprayed areas around Alsea, Oregon, compared to spontaneous abortions occurring in unsprayed areas	2,344 births	(a) 1,666 control births—unsprayed area (b) 4,120 births—urban area

[a]The dash (—) indicates the comparison group is based on a population (e.g., U.S. white males, country rates), with details given in the text for specifics of the actual population.

chemical manufacturing may have produced dioxin contaminants; also examined were U.S. counties where STS death rates were in the 95th percentile, where rates in 1970-1979 were increased over those between 1950 and 1959, and where in 1970-1978 an excess of deaths occurred compared to national rates. Although these national comparisons were made, no information was available concerning possible dioxin contamination in the environment around these areas. There were no comparison groups for the cases examined in this study.

A case-control study was conducted in the Yorkshire Health Region where cases of NHL were identified from pathology departments in hospitals in Yorkshire between October 1979 and December 1984 (Cartwright et al., 1988). All pathology was reviewed and confirmed. The Yorkshire Regional Cancer Registry and the Regional Histopathology Lymphoma Panel were also checked for potential additional cases. The controls were selected from in-patients with a wide variety of nonmalignant conditions, and matched 2:1 to each case by residential health district, sex, and age ±3 years. Identical interviews were conducted by trained interviewers for cases and controls; information included a variety of occupational, personal, life-style, hobby, medical history, and drug history questions. The exposure of relevance was an item reported concerning use of fertilizers/herbicides for at least three months at work or at home. The study included 437 living cases and 724 living controls.

A study of a community in Finland exposed to chlorophenol through drinking water and fish contamination in 1987, and through occupational exposure in a lumber mill, was conducted, with both cohort (the entire town) and nested case-control components (Lampi et al., 1992). The cancer incidence for the health care district of interest, as obtained from files in the Finnish Cancer Registry between 1953 and 1986, was compared to the cancer incidence for neighboring municipalities and for the larger Cancer Control Region (CCR) (approximately 1.1 million population); neighboring municipalities were also compared to the expected incidence of the CCR. Additionally, a case-control study of cancers of the colon, bladder, ureter and urethra, soft tissues, lymphomas, and leukemia occurring in the health care district between 1967 and 1986 was conducted. The national population registry was the source of random selection of four controls per case, living in the same health care district at the time the cancer was diagnosed, of the same sex, and within two years of age. The study subjects or next of kin responded to a mailed questionnaire with items focused on work history, particularly in sawmills or on farms; source and duration of drinking and other household water supplies; source and quantity of fish eaten; frequency of eating soup; and smoking habits. Exposures were combined into groups, related to work in the sawmill, fungicide exposure, exposure to contaminated water source, and eating fish from contaminated lakes. A person was

exposed to the grouped variable if any item constituting the group had a positive response. Additional information on probable drinking water exposure was added based on history of residence, by a study member blinded to case or control status.

An ecological study of lymphomas and STS correlated with environmental exposures to phenoxy herbicides in two northern Italian provinces was conducted where phenoxy herbicides have been used since 1950 in rice weeding (Vineis et al., 1991). Cases of HD, NHL, and STS diagnosed between 1985 and 1988 in men and women between the ages of 15 and 74, living in the two provinces of interest, were identified. Hospitals in the provinces and a referral hospital outside the province were contacted for reports of newly diagnosed cases; a total of 63 cases of HD, 253 cases of NHL, and 49 cases of STS were identified. Soil and water contaminated by 2,4-D and 2,4,5-T, as indicated in analyses performed in 1974-1975, were used to indicate exposure areas. Categories of exposure were defined as category A, where 2,4-D or 2,4,5-T were detected in water or soil in at least one measurement, or category B, where the highest levels of water contamination were found. Areas did overlap partially. Rates were compared between the areas for men and women separately. No unexposed areas were identified as control areas for comparison.

VIETNAM VETERAN STUDIES

Studies of Vietnam veterans who were potentially exposed to Agent Orange have been conducted in the United States at the national and state levels, as well as in Australia (see Table 7-3). Exposure measures in these studies have been done on a variety of levels, and evaluations of health outcomes have been made utilizing a variety of different comparison or control groups. This section is organized primarily by the sponsors of the research, as this format was more conducive to the methodologic presentations of the articles. Within these studies, the exposure measures fall along a crude scale of measurement, from the individual level for the Ranch Hands, as reflected in the serum measurements of the amount of dioxin present, to some of the individual state studies, which examined groups of veterans serving in Vietnam as a surrogate for TCDD exposure.

It should also be noted that comparison groups for the veteran cohort studies vary to include unexposed Vietnam veterans who were stationed in areas essentially not exposed to active herbicide missions, and were unlikely to have been in areas sprayed with herbicides; Vietnam-era veterans, who were in the service at the time of the Vietnam conflict, but did not serve in Vietnam; non-Vietnam veterans, who served in other wars or conflicts, such as Korean or World War II; and various U.S. male populations (either state or national).

United States

Ranch Hands

The men responsible for the majority of the aerial spraying of herbicides were volunteers from the Air Force who participated in Operation Ranch Hand. Participants in this operation are referred to as "Ranch Hands." To determine whether there are adverse health effects associated with exposure to herbicides, including Agent Orange, the Air Force made a commitment to the Congress and the White House in 1979 to conduct an epidemiologic study of the Ranch Hand participants (AFHS, 1982).

A retrospective matched cohort study design was implemented to examine morbidity and mortality, with follow-up scheduled to continue until 2002. The National Personnel Records Center and the U.S. Air Force Human Resources Laboratory records were searched and cross-referenced to completely ascertain all Ranch Hand personnel (AFHS, 1982; Michalek et al., 1990). A total of 1,269 participants were originally identified through this process (AFHS, 1983). A control population of 24,971 C-130 crew members and support personnel assigned to duty in Southeast Asia but not occupationally exposed to herbicides (AFHS, 1983) was selected from the same data sources as used to identify the Ranch Hand population. Controls were matched on age, type of job using Air Force specialty code, and race (white/not white). The rationale for matching on these variables was to control for the clinical aging process, education and socioeconomic status, and potential differences by race in development of chronic disease. Since Ranch Hands and controls performed similar combat or combat-related jobs, many potential confounders related to the physical/psychophysiologic effects of combat stress and the Southeast Asia environment were potentially controlled (AFHS, 1982).

Ten matches for each exposed subject formed a control set. For the mortality study, each exposed subject and a 50 percent random sample of the subject's control set is being followed for 20 years, in a 1:5 matched design. The morbidity component of follow-up consists of a 1:1 matched design, using the first control randomized to the mortality ascertainment component of the study. If a control is noncompliant, another control from the matched "pool" will be selected; controls that die will not be replaced.

The baseline exam occurred in 1982, and future exams are scheduled until 2002. Morbidity is ascertained through questionnaire and physical examination, which emphasizes dermatologic, neuropsychiatric, hepatic, immunologic, reproductive, and neoplastic conditions. There were 1,208 Ranch Hands and 1,668 comparison subjects eligible for baseline examination. Initial questionnaire response rates were 97 percent for the exposed

cohort and 93 percent for the unexposed; baseline physical exam responses were 87 and 76 percent, respectively (Wolfe et al., 1990). For the 1987 examination and questionnaire (Wolfe et al., 1990), 84 percent of the Ranch Hands (N = 955) and 75 percent of the comparison subjects (N = 1,299) were fully compliant. Mortality outcome was obtained and reviewed by using U.S. Air Force Military Personnel Center records, Veterans Administration (VA) Death Beneficiary Identification and Record Location System (BIRLS), and the IRS database of active Social Security numbers. Death certificates were obtained from the appropriate health departments (Michalek et al., 1990).

The Ranch Hands were potentially exposed to large quantities of herbicides and categories were developed to account for their potential exposures:

1. low potential, including pilots, copilots, and navigators—exposure was primarily through preflight checks and during actual dissemination of the spray;
2. moderate potential, including crew chiefs, aircraft mechanics, and support personnel—exposure was possible by contact during dedrumming and aircraft loading operations, on-site repair of aircraft, and spray equipment;
3. high potential, including spray console operators and flight engineers.

Exposure occurred in loading the aircraft, from ground test equipment, from tank leakage, and during dissemination. An exposure index was calculated by evaluating known factors that would influence exposure, such as date of tour with Ranch Hands in Vietnam; number and length of tours with Ranch Hands; number of herbicide dissemination missions (as reflected by flying hours and air medals); herbicides employed; crew position; and routes of personal exposure. Data from spray mission tapes were employed to estimate the amount of herbicide used by the Ranch Hands, based on time and month active. Details on the exposure index are given in Chapter 6.

Results have been published for the baseline morbidity (AFHS, 1984a) and baseline mortality studies (AFHS, 1983); first (1984) and second (1987) follow-up examinations (AFHS, 1987, 1990); and reproductive outcomes study (AFHS, 1992). Mortality updates have been published for 1984-1986, 1989, and 1991 (AFHS, 1984b, 1985, 1986, 1989, 1991a). Serum dioxin levels were measured in 1982 (36 Ranch Hands) (Pirkle et al., 1989), 1987 (866 Ranch Hands) (AFHS, 1991b), and 1992 (results have not yet been published). The serum dioxin analysis of the 1987 follow-up examinations was published in 1991 (AFHS, 1991b). Details on serum dioxin studies are discussed further in Chapter 6. Continued follow-up and results will be forthcoming.

Centers for Disease Control

The Centers for Disease Control has undertaken a series of studies to examine various health outcomes of Vietnam veterans, as directed by Congress (Veterans Health Programs Extension and Improvement Act of 1979, Public Law 96-151, and Veterans' Health Care, Training, and Small Business Loan Act of 1981, Public Law 97-72). One of the first studies of Vietnam veterans was of birth defects among offspring of fathers serving in Vietnam (Erickson et al., 1984a,b). In response to the congressional mandate, the Vietnam Experience Study (VES; CDC, 1989b) was initiated. Since the cohort was young and the incidence of cancers reported was low, it was not possible to evaluate the association with Vietnam experience in a cohort study design; therefore, the Selected Cancers Study (SCS; CDC, 1990a) was initiated to evaluate the association among several rare cancers, Agent Orange exposure, and military service in Vietnam. To examine the concerns about Agent Orange more directly, the CDC originally proposed a study of the health of Vietnam veterans exposed to Agent Orange, compared to unexposed veterans, by using records of military unit locations and herbicide spray locations to assess exposure; this assessment was eventually determined by CDC not to be feasible. To make this determination, the Agent Orange Validation Study was conducted to evaluate TCDD levels in U.S. Army veterans, compared to exposure estimates based on military records and TCDD levels of veterans who did not serve in Vietnam. The Agent Orange Validation Study (CDC, 1989a) is discussed in Chapter 6 of this report.

Birth Defects Study The birth defects study by CDC was a case-control interview study (Erickson et al., 1984a,b) using cases and controls ascertained in the Atlanta area. Cases were selected from the Metropolitan Atlanta Congenital Defects Program (MACDP) registry, which has attempted to ascertain all liveborn and stillborn babies with structural and biochemical congenital defects. Mothers of these babies had to be residents of a five-county metropolitan Atlanta area at the time of the baby's birth. Cases were identified by a regular search of all records at hospitals that have obstetric or pediatric services, including examination of pathology department records, obstetric logs, and hospital disease indices. Initial identification of cases was also based on state of Georgia vital records and cytogenetic laboratories. Hospital charts were examined and abstracted for a confirmation of the initial diagnosis. A congenital anomaly was included if it was diagnosed prior to 1 year of age and occurred in a child who weighed > 500 grams with > 20 weeks' gestation.

Only cases with serious or major birth defects were included in this study. These defects are generally considered to include defects that affect survival or result in serious physical or psychological handicaps. Birth

defect diagnoses (coded according to the ICD-8 system) were grouped into three categories, namely: category 1, which included any baby with one or more "serious" or "major" defects; category 2, which mostly included defects coded as "other specified …," or "anomalies of …," and defects such as syndactyly and hip dislocation; and category 3, relatively minor defects and "unspecified" defects of organs. Babies with only category 3 minor defects were excluded. The initial ascertainment included a total of 7,529 cases; after exclusions, a final sample of 7,133 cases was eligible for the interview study.

The control group consisted of babies born without birth defects, chosen from among the 323,421 live births that occurred in hospitals in the MACDP surveillance area from 1968 to 1980. The control group was chosen by a stratified random sampling procedure to frequency match the controls on race, year and quarter of birth, and hospital of birth. This sampling process identified 4,246 eligible control babies.

After efforts to locate respondents, telephone interviews of parents of case and control babies were conducted in 1982 and 1983. Trained interviewers conducted a two-part interview with each parent. Two different interviewers conducted parts of the computer-assisted telephone interview. The first part of the interview with each parent asked about pregnancy history, and the second part covered items such as medical history, medication use, alcohol and tobacco use, and occupational history. The interviewer that conducted the second part of the interview was blinded to case or control status. For many exposures, mothers were asked about fathers, and vice versa.

The father's interview also involved a detailed history of military service including occupational specialties, periods, and locations of service. The potential for an individual Vietnam veteran exposure to Agent Orange was estimated in this study by using interview information and a review of military records. A task force of military specialists (Agent Orange Task Force) reviewed the interview data and military records, and estimated the Agent Orange "exposure opportunity" for each veteran without knowledge of case or control status. The exposure opportunity index (EOI) that was created represented five ordered categories from no or minimal exposure to numerous Agent Orange exposure opportunities. One EOI was developed based on interview data, another from military records. The records-based EOI used location data derived from the Operations Reports-Lessons Learned, which were prepared quarterly, indicating general battalion-sized unit locations. Information on day-to-day location of units was not available for this study. The general unit locations were compared to various Agent Orange and herbicide applications by using the Ranch Hand HERBS tapes and other data such as base perimeter spraying records. The EOI based on interviews used self-reported data on service locations, service periods, and

occupational specialties. The EOI ranged from a value of 1 (e.g., service in Vietnam prior to Agent Orange, stationed offshore) to a value of 5 (e.g., Ranch Hand personnel).

Vietnam Experience Study The VES was a historical cohort study of the health experience of Vietnam veterans. The study was divided into three parts: physical health; reproductive outcomes and child health; and psychosocial characteristics (CDC, 1987, 1988a-c, 1989b). The VES involved a health assessment of Vietnam veterans based on a random sample of enlisted men who served in the U.S. Army from 1965 through 1971. A random sample of U.S. Army veterans was ascertained, of whom 9,078 Vietnam veterans and 8,789 Vietnam era veterans who served only one term of enlistment and were discharged as enlisted men were eligible for telephone interview (CDC, 1988a, 1989b). Of these, 7,924 Vietnam (87 percent) and 7,364 Vietnam era (84 percent) veterans completed telephone health interviews. A random subsample of the veterans underwent extensive physical and psychological health examinations, involving 2,490 Vietnam (75 percent) and 1,972 Vietnam era (63 percent) veterans (CDC, 1989b). All examinations were conducted at a single medical facility, according to standardized procedures. To the extent possible, examiners did not know the status of the examinee. At the time of the study, the two groups of veterans were similar in terms of level of education, employment, income, marital status, and satisfaction with personal relationships (CDC, 1988a, 1989b). As Agent Orange exposure was not a focus of the study, military service in Vietnam was regarded as a surrogate for herbicide exposure. Results of the physical examination study have been reported (CDC, 1988a,b, 1989b).

The VES assessment of reproductive outcomes and child health included three components: (1) a telephone interview; (2) hospital record review of birth defects for a subsample of veterans who underwent a medical examination; and (3) a medical record review of selected birth defects for all study subjects (CDC, 1988c). A total of 28,724 eligible pregnancies (15,009 Vietnam veterans, 13,715 Vietnam era veterans), 24,698 eligible births (12,788 Vietnam veterans, 11,910 Vietnam era veterans), and 24,436 eligible live births (12,659 Vietnam veterans, 11,777 Vietnam era veterans) were reported. The telephone interview with veterans included pregnancy history (miscarriage, induced abortion, tubal pregnancy, live birth, stillbirth, birth defects), cancer, infant and childhood death, and other major health conditions in the first five years of life. In addition, information on a number of covariates (potential confounders and/or effect modifiers) was obtained from either military records or interview. These covariates included paternal age at birth of a child, veteran's race, the Army general technical placement exam score, enlistment status, primary military occupational

specialty, year of entry into the Army, years between entry and child's birth, paternal smoking history, alcohol use, educational attainment, marital status at interview, and illicit drug use in the service.

The interim results of the VES interview study prompted the CDC to conduct two substudies as an attempt to validate or verify the veterans' self-reports of birth defects among their offspring. The first substudy (the General Birth Defects Study) involved the comparison of the occurrence of birth defects recorded on hospital records of the children of Vietnam and Vietnam era veterans. The veterans chosen for this study were already part of the medical exam component of the VES, the veterans (1,237 Vietnam, 1,045 Vietnam era veterans) who attended the Lovelace Medical Foundation from January 1 through September 30, 1986.

There were a total of 4,462 veterans who participated in both the telephone interview and the medical examination. Of these, 2,282 veterans were examined after January 1, 1986, and were asked to provide information on the birth and hospital for each offspring. After excluding births prior to entry into the Army, 3,683 births (1,945 Vietnam veterans, 1,738 Vietnam era veterans) were ascertained. An attempt was made to obtain birth and hospital records for each veteran's offspring, and a variety of information was abstracted from eligible records. A total of 3,366 birth records were obtained. For 1,791 Vietnam veterans (92 percent) and 1,575 Vietnam era veterans (91 percent), birth records were obtained.

The second substudy (the Cerebrospinal Malformation Study, CSM) involved the acquisition of medical records for all active and suspected cases of cerebrospinal malformations (spina bifida, anencephalus, hydrocephalus) and stillbirths reported by veterans in the interview study. All veterans who reported a cerebrospinal malformation in the telephone interview study were included in the CSM substudy. Birth records were sought for all children with a reported or suspected CSM and all reported still-births. There were a total of 294 eligible births in this substudy (154 Vietnam veterans, 140 Vietnam era veterans), and 221 birth records were received overall, with 127 (83 percent) records obtained for Vietnam veterans and only 94 (67 percent) for Vietnam era veterans.

In both substudies, information on potential birth defects (major and minor) was abstracted, along with data on perinatal mortality, low birthweight, and maternal age and gravidity (number of pregnancies). A birth defect was defined as a structural abnormality present at birth or diagnosed prior to hospital discharge or transfer during the first 28 days after birth among liveborn or stillborn infants.

The CDC also examined the postservice mortality (through 1983) of a cohort of 9,324 U.S. Army veterans who served in Vietnam, compared to 8,989 Vietnam era Army veterans who served in Korea, Germany, or the United States (Boyle et al., 1987; CDC, 1987). The veterans came from all

branches of the service. Mortality follow-up was conducted from the time of discharge until January 1, 1984, by using a variety of methods, including personal contact with next of kin and computer linkages to the SSA, VA, IRS, and the NDI (Boyle et al., 1987). A veteran had to have served in Vietnam at any time during his term of enlistment, with no minimum. Results were also calculated by using the total U.S. male population as the reference group. Analyses were conducted utilizing both the cause of death recorded on the death certificate and the cause of death assigned by a medical review panel from supplemental sources including hospital records and autopsy reports.

An additional study (O'Brien et al., 1991) combined the mortality and interview data to identify all veterans with NHL. Medical reports and records were obtained for all self-reported cases. Four veterans who had stated in the interview that they had cancer had been diagnosed with NHL. All four were Vietnam veterans. In the mortality study, three Vietnam veterans and one Vietnam era veteran were found to have NHL. In total, therefore, seven Vietnam veterans and one Vietnam era veteran had some type of NHL. No information was available regarding specific exposure to Agent Orange, because none of the NHL cases had military job titles that suggested they had been exposed to herbicides while in Vietnam.

To evaluate whether self-reported assessment of exposure to herbicides influences the reporting of adverse health outcomes, a study was designed using VES subjects (Decoufle et al., 1992). Respondents from the VES cohort were asked to report patterns of health outcomes as well as exposure to combat and herbicides in Vietnam. An index of combat exposure and herbicide exposure was developed. Six questions were asked to determine ways in which veterans believed they were exposed to herbicides in Vietnam, including whether they had actually sprayed herbicides, handled spray equipment or containers, been present when others were spraying, gotten herbicides on skin or clothing, passed through areas that appeared defoliated, or been exposed in any "other" way. Crude comparisons between Vietnam and Vietnam era veterans for health effects were reported, as well as comparisons of differences in reported health outcomes by herbicide and combat exposure indices.

Selected Cancers Study The Selected Cancers Study (CDC, 1990a-d) was undertaken by the CDC to investigate the effects of military service in Vietnam and exposure to herbicides on the health of American veterans. This was a population-based case-control study. Cases were restricted to men born between 1929 and 1953. All cases of NHL; soft tissue and other sarcomas; HD; nasal, nasopharyngeal, and primary liver cancers diagnosed from December 1984 to November 1988 in the geographic regions covered by eight tumor registries were considered eligible. The tumor registries

covered three states (Connecticut, Kansas, Iowa) and five large metropolitan areas (Miami, Detroit, San Francisco, Seattle, Atlanta), comprising approximately 10 percent of the U.S. population (CDC, 1990a). The control group was selected by random-digit dialing, and was frequency matched to the cases for geographic area covered by the tumor registry and five year date-of-birth intervals. A second control group, pair matched to deceased cases, consisted of deceased individuals from the same registry area, date of birth, race, and time interval between death and proxy interview. Pathology experts confirmed the diagnosis for each of the cases by independent review of the microscopic slides and tissue blocks. Trained personnel interviewed study participants by telephone with the use of a standardized questionnaire, collecting information on medical history, occupation, contact with pesticides, personal characteristics and habits, and military service. The participation rate for the controls identified through random-digit dialing was 83 percent and averaged 87 percent for all types of cancer cases combined. Participation of cases was indicated by consent to interview, either by subject or next of kin. Subjects who reported having served on active duty in the U.S. military were asked if they had been stationed in Vietnam or off the coast of Vietnam. Information was also obtained regarding rank, dates, and branch of service. Military records were reviewed for the men who reported that they had been stationed in Vietnam. Any exposure to phenoxy acid herbicides and chlorophenols other than in Vietnam was ascertained from answers to questions. The interviewed cases included 310 men with HD, 48 with nasal cancer, 80 with nasopharyngeal cancer, 130 with primary liver cancer, 342 with STS, 1,157 with NHL, and 1,776 controls. The same control group was used for comparison to each series of cancer site cases. Potential AIDS cases were excluded from the analysis.

Exposure was indirectly assessed as Vietnam service. Study participants were classified by branch of service, duration of service, calendar year of service, job duties, self-perceived exposure to herbicides, and location in Vietnam according to military region or in the Navy, whether blue water, brown water, or on shore. The units were categorized as support, combat support, or combat units. This classification was done by the U.S. Army and Joint Services Environmental Support Group (ESG) (CDC, 1990a-d).

Analyses were conducted using three statistical models: (1) controlling only for simple design variables such as age; (2) controlling for socioeconomic factors (race and educational achievement); and (3) including characteristics that could be found in association with herbicides (e.g., occupational contact other than in Vietnam). For most analyses, the referent group was composed of men who did not serve in Vietnam. The authors stress that probably few of the Vietnam veterans were actually exposed to Agent Orange, so this is not a study of Agent Orange per se. Results are reported

for an association between military service in Vietnam and NHL (CDC, 1990b), STS and other sarcomas (CDC, 1990c), and HD, nasal, nasopharyngeal, and primary liver cancers (CDC, 1990d).

Department of Veterans Affairs

A proportionate mortality study was conducted by the U.S. Veterans Administration, (Breslin et al., 1988; Burt et al., 1987); a nested case-control study of the cases of NHL was conducted by Burt and colleagues, with controls selected from among the cardiovascular disease mortality deaths. The study subjects were ground troops who served in the U.S. Army or Marine Corps at any time from July 4, 1965, through March 1, 1973. Air Force, Navy, and Coast Guard personnel were excluded. The VA's BIRLS was used to select potential study subjects. This file is estimated to be 94 percent complete. A list of 186,000 Vietnam era veterans who served in the Army or Marine Corps, whose service dates included 1964-1975, and who were reported deceased as of July 1, 1982, was assembled from BIRLS. A random sample of 75,617 names was selected from this group, the aim being to get a sample size of approximately 50,000 eligible veterans. The military personnel records of these 75,617 potential study subjects were obtained and reviewed, and there were 52,253 men who died between July 4, 1965, and July 1, 1982, and who had served in the U.S. Army or Marine Corps during the period in question. Death certificates were obtained for 97 percent of the cases (881 were not obtained), and the cause of death was ascertained for 51,421 veterans. Sources of death certificates included the VA, Federal Archives Records Centers, and state vital statistics offices. Of these 51,421 men, 26,685 had not served in Southeast Asia, whereas 24,235 had served in Vietnam. Of those excluded from the analysis, 501 had served elsewhere in Southeast Asia, or their place of service was unknown. No data were available regarding exposure to herbicides. The analyses in this study compared 24,235 Vietnam veterans and 26,685 Vietnam era veterans. More than 50 percent of the veterans died between the ages of 25 and 34.

An additional study was conducted by the Department of Veterans Affairs, using the Vietnam veteran mortality experience reported above compared with three different referent groups and with additional follow-up through 1984 (Watanabe et al., 1991). Of these 62,068 men, 32,422 had not served in Southeast Asia, while 29,646 had served in Vietnam, and 711 had served elsewhere in Southeast Asia (Watanabe et al., 1991) or their place of service was unknown; the final group was excluded. The final study group of 62,068 veterans included 50,743 from an earlier mortality study (Breslin et al., 1988). Adjustments were made for age, race, and calendar year of death. Separate analyses were performed for Army and Marine Vietnam

veterans, because of potential differences in environmental exposures among those serving in the different military branches. Three separate comparison groups were identified: (1) branch-specific (Army, Marine) Vietnam era veterans, (2) all Vietnam era veterans combined, and (3) the U.S. male population.

A study examined whether Army I Corps Vietnam veterans had cancer mortality experiences similar to other Army Vietnam era veterans (Bullman et al., 1990). Army I Corps experience is the exposure surrogate measure. Information regarding the location of military service was obtained from military personnel records regarding the unit to which the veterans had been assigned in Vietnam, matched with a computerized file containing grid coordinates for the geographic location of each unit in Vietnam over time. In addition, station listings of units in Vietnam and the *Vietnam Order of Battle* (Stanton, 1981) were used to assign locations. There were 6,668 Army veterans who served in I Corps at least once during their tour of duty in Vietnam; 27,917 Army Vietnam era veterans were used as a comparison group. Mortality was standardized by age, race, and calendar year; data were analyzed by calendar year (five year intervals).

The DVA also examined the morbidity and mortality experience of a subgroup of Vietnam veterans potentially exposed to high levels of herbicides (Thomas and Kang, 1990). There were 22 U.S. Army Chemical Corps units assigned to South Vietnam between 1966 and 1971. These units were responsible for the storage, handling, mixing, and application of riot control agents (tear gas, burning agents) and herbicides. The Chemical Corps applied herbicides around the perimeters of base camps and was responsible for aerial spraying from helicopters. Morning reports for all Army Chemical Corps units known to have been assigned to Vietnam between 1966 and 1971 were examined, and a roster of information on all men who served in at least one of these units was created. The result was information on 954 men. Military service records were all obtained from the National Personnel Records Center and from the U.S. Army. The final study size was 894 men who were eligible and had available records. Their vital status on December 31, 1987, was determined through computer searches of the VA, SSA, IRS, and NDI data bases. Death certificates for those men determined to be deceased were obtained. Standardized mortality ratios were calculated by comparing the Chemical Corps cohort to mortality rates among U.S. men. Adjustment was made for race, age, and calendar period. Information on morbidity was obtained from VA hospital records and the Agent Orange Registry medical examinations.

The veterans' studies described up to this point include only men in the analysis. The DVA also conducted a study of mortality among women Vietnam veterans (Thomas et al., 1991). Women who served in Vietnam were identified from the service branches by various means: (1) Army

women were identified from morning report records of 91 Army hospital and administrative support units that were likely to have had female personnel; (2) Air Force women were identified from a computerized personnel file maintained by the Air Force Human Resources Laboratory; (3) Navy women were identified through a review of all personnel on the muster rolls of the four Navy facilities in Vietnam; (4) the Marine Corps provided listings of all women assigned to Vietnam. Women Vietnam veterans were eligible for inclusion in the study if they were on active duty in the U.S. Armed Forces at any time between 1964 and 1972, and had a tour of duty that included service in Vietnam during that same period.

Women who had never served in Vietnam were selected in a manner similar to that for the Vietnam cohort to serve as a comparison group. For the Army, ESG identified 90 units with female personnel stationed in the United States between 1964 and 1972; the other service branches selected women at random who had never served in Vietnam from their automated personnel files. These women were frequency matched to the Vietnam veterans by rank and military occupation. All personnel records for the potential study subjects were obtained from the National Personnel Records Center and the Army Reserve Personnel Center; 89 percent of the records were available for abstracting. Initially, 4,644 of the Vietnam cohort and 6,575 of the comparison cohort met the eligibility criteria. Vital status on December 31, 1987, was determined for study subjects using the DVA Beneficiary Records and the SSA, IRS, National Death Index, and military personnel records. An official certificate of death was obtained for deceased subjects. Deaths on active military duty before March 28, 1973, were excluded from the analyses.

The final study cohorts consisted of 4,582 women Vietnam veterans and 5,324 women who had served in the U.S. military but not in Vietnam or the Pacific theater. Person-years for risk of dying were calculated for each study subject starting with either the date she left military service or March 28, 1973. Analyses were adjusted for rank, military occupation (nurse or non-nurse), duration of military service, age at entry, and race. The mortality experiences in the cohort of women serving in Vietnam and the non-Vietnam cohort were each compared to the mortality experience of U.S. women, adjusted for race, age and calendar year. No information on any individual exposures, particularly to herbicides, was available.

Specific diseases and health outcomes have also been evaluated in studies conducted by the DVA, including case-control studies of STS (Kang et al., 1986, 1987) and NHL (Dalager et al., 1991), posttraumatic stress disorder (True et al., 1988; Bullman et al., 1991), and suicide (Farberow et al., 1990) as well as a co-twin study of self-reported physical health in a series of Vietnam era monozygotic twins (Eisen et al., 1991).

The DVA conducted two case-control studies of STS (Kang et al., 1986,

1987). In the first study, the Veterans Administrations patient treatment file (PTF) was used to identify all Vietnam era veterans diagnosed with STS between 1969 and 1983 (Kang et al., 1986) for a case-control study of these tumors. The PTF is a computerized hospital discharge abstract system covering all in-patient discharges from 172 VA medical centers. Vietnam era veterans were defined as veterans who served in the military sometime between August 5, 1964, and May 7, 1975. In all, 418 cases were identified; a pathology report was obtained for each case and reviewed by the study pathologist. After review, there were 234 remaining STS cases available for analysis. The comparison group consisted of 14,931 patients systematically sampled from the same Vietnam era veteran patient population. Military service information, particularly regarding Vietnam service, was obtained for STS cases and controls by review of the military personnel records. Records were located and abstracted for all of the 234 STS cases and 13,496 (90 percent) of the controls. Branch of service was used as a surrogate for exposure to Agent Orange.

In the second study, cases were drawn from the files of the Armed Forces Institute of Pathology (AFIP) (Kang et al., 1987). STS cases were restricted to men who were diagnosed between January 1, 1975, and December 31, 1980, and who were born between 1940 and 1955 (i.e., were between 18 and 25 years of age during the Vietnam conflict). In addition, their cancer specimens would have been referred for diagnostic evaluation before the publicity regarding Agent Orange. Controls were selected from the patient logs of referring pathologists or referring pathology departments, excluding patients with STS, NHL, and HD. For each case, a pathologist in the referring pathology department was asked to select three male patients born between 1940 and 1955. A total of 440 STS cases were identified. A letter was then sent to the attending physicians of the STS cases and controls for permission to approach the study subjects or their next of kin for a telephone interview. The telephone interview elicited information on occupational exposure, medical exposure, and life-style and sociodemographic factors. Interviews with study subjects or next of kin were conducted for 217 of the cases (78 percent) and 599 of the controls (74 percent).

Information was also obtained on military history. Military and Vietnam service was documented by reviewing military personnel records. As a surrogate for Agent Orange exposure, service in the Army or Marine Corps was used; military occupation, broad geographical location of the individual's unit in Vietnam in reference to recorded herbicide spray missions, and a combination of the above were identified.

By use of the PTF, a review for the years 1969 through 1985 identified all malignant lymphomas among male Vietnam era veterans born between 1937 and 1954 (Dalager et al., 1991). Pathology reports were requested from VA medical centers that had 10 or more lymphoma cases. These

reports were reviewed by a pathologist who identified 450 cases that were consistent with the diagnosis of NHL. Two controls were selected from the PTF from the same Vietnam era veteran population discharged from the VA inpatient hospital as the cases, matched by hospital, discharge year of hospitalization for NHL, and birth date. Persons with a diagnosis of lymphoma were excluded from the control group. In this way, 900 matched controls were identified. For both groups, the National Personnel Records Center was searched for military personnel records, and information was obtained on dates of service, military occupational specialty codes, and military region of service. These were found for 91 percent of the cases and 88 percent of the controls. The study population was restricted to men serving in the military sometime between July 1965 and March 1973. Because of the possibility of selection bias for those who were wounded during the Vietnam conflict, those persons who were first seen at a VA medical center as an immediate consequence of Vietnam duty were excluded.

Surrogate measures of Agent Orange exposure were specific military branch of service, with Army and Marine ground troops considered most likely to have been exposed directly and indirectly to Agent Orange; certain military regions (I, II, III, and IV) in Vietnam; or combat role determined by military occupation specialty (Dalager et al., 1991). The analysis in this study was limited to those persons who had been discharged from military service at least 5 years before their first hospitalization at a VA medical center. The final study population consisted of 201 NHL cases and 358 controls.

Psychological and behavioral outcomes among Vietnam veterans have also been examined by the DVA (True et al., 1988; Faberow et al., 1990; Bullman et al., 1991). In many of these studies, exposure to Agent Orange is not discussed, but exposure to "combat" is evaluated as the risk factor of interest. Some of the readjustment problems of Vietnam veterans have been attributed to posttraumatic stress disorder (PTSD). A case-control study of PTSD compared demographic and military characteristics of 374 Vietnam veterans' cases of PTSD to 373 healthy Vietnam veterans (Bullman et al., 1991). A computerized data base of approximately 200,000 Vietnam veterans who volunteered for physical examinations is maintained as the Agent Orange Registry in the Department of Veterans Affairs. Veterans were selected from this registry; cases and controls were frequency matched by age, year of registry exam, and race. Additional details of the Agent Orange Registry are given in Chapter 2. Crude odds ratios were used to evaluate the risk of PTSD associated with certain characteristics of Vietnam service, since there was no apparent confounding by other military factors. Combat exposure is evaluated for association with PTSD; it does not appear that combat is used as a surrogate for herbicide exposure.

A sample of 1,787 Army, Navy, and Marine veterans who entered the

service after August 4, 1965, was identified from the Survey of Veterans II conducted in 1979 by the VA and the U.S. Bureau of the Census (True et al., 1988). A summary index of combat exposure was developed from nine questions on combat roles and experiences. This index along with service in the Southeast Asia war zone was used to evaluate stresses of war. Symptoms related to PTSD were also measured. As with the previous study of PTSD, combat exposure was not used as a surrogate for herbicide exposure. Potential confounding effects of military service and demographic factors were adjusted in the analysis.

The DVA computer file of Vietnam veteran mortality (Breslin et al., 1988) was used to identify all Vietnam era veterans who died from suicide and motor vehicle accidents in Los Angeles county between 1977 and 1982 (Farberow et al., 1990). By searching backward from 1982, 100 consecutive veteran suicides and 100 consecutive veteran motor vehicle accidents were selected from the lists. The 175 veteran deaths that were matched to the medical examiner's data were divided into 38 Vietnam veteran suicides, 46 Vietnam veteran motor vehicle accidents, 43 Vietnam era veteran suicides, and 48 Vietnam era veteran motor vehicle accidents. The actual numbers available for analysis were less, as complete data were not available for all study subjects. Information on demographic data, toxicology tests, and police and suicide notes obtained from the medical examiner's record was combined with data abstracted from military personnel records. Unblinded psychological autopsies were conducted for 82 of the 175 veterans. Comparisons were done for demographic, military, and psychological factors and for preservice variables between (1) Vietnam veteran suicides and Vietnam veteran motor vehicle accidents; (2) Vietnam era veteran suicides and Vietnam era veteran motor vehicle accidents; (3) Vietnam veteran suicides and Vietnam era veteran suicides; and (4) Vietnam veteran motor vehicle accidents and Vietnam era veteran motor vehicle accidents. No information on individual exposures to herbicides was available.

Finally, the DVA studied monozygotic twins, born between 1939 and 1957, both of whom served during the Vietnam era (1965-1975), which defined a unique cohort to minimize differences in genetic and early environmental experiences. The twins were identified from the Vietnam Era Twin Registry (Eisen et al., 1991). One twin of the pair served in Southeast Asia, defined as being stationed in Vietnam, Laos, or Cambodia; serving in the waters around these countries; or flying missions over these areas. Individuals who were too ill to be interviewed or who had died were excluded. A questionnaire survey in 1987 by mail, telephone, or in-person interview asked about selected aspects of military service, physical health (hearing; skin, cardiovascular, gastrointestinal, and respiratory conditions; joint or skeletal disorders; kidney, bladder, or urinary disorders; blood; and cancer), symptoms, and use of alcohol and cigarettes. Included were twin pairs in

which the siblings were monozygotic and both members of the pair responded to the questionnaire, totaling 2,260 pairs.

A combat exposure index was developed by asking each veteran about 18 specific types of combat activities, resulting in a five-level index of exposure for analysis, which approximated quartiles of the combat index score distribution. The index of combat exposure was defined as the sum of all positive responses of an individual to each item. A pilot study of 150 twin pairs was conducted to test the methodology used in the full survey. This also provided a validation for those responding in both the pilot and the full study.

American Legion

The American Legion conducted a cohort study concerning the health and well-being of Vietnam veterans that belonged to the American Legion, a voluntary veterans service organization. Approximately 700,000 Vietnam era veterans are members of the American Legion at that time (Stellman et al., 1988a) Participants were selected from the membership rosters as of October 15, 1983, in six departments of the American Legion, encompassing the states of Colorado, Indiana, Maryland, Minnesota, Ohio, and Pennsylvania (Stellman et al., 1988a). Each man was given a self-administered questionnaire that included questions regarding medical history as well as combat exposure and possible Agent Orange exposure. Questionnaire return rates varied from a high of 64 percent in Minnesota to a low of 53 percent in Pennsylvania (Stellman et al., 1988a). Extensive discussion of the exposure measurement is included in Chapter 6. The questionnaire-derived service locations in Vietnam were compared with combined Air Force and U.S. Army joint services environmental records or spraying locations (HERBS tapes). Based on this, a numerical probability score was assigned to each subject. Subjects were stratified by combat (three levels) or by herbicide score (three levels). Age adjustment was not done because of the narrow age range within the study group. Of the 6,810 Vietnam era veterans who returned completed questionnaires, 2,858 had served in Southeast Asia and 3,933 served elsewhere (Stellman et al., 1988b). A series of studies examined physical health and reproductive outcomes (Stellman et al., 1988b), social-behavioral consequences (Stellman et al., 1988c), and PTSD (Snow et al., 1988).

State Studies

Several states including Hawaii, Iowa, Maine, Massachusetts, New Jersey, New Mexico, New York, Pennsylvania, Texas, West Virginia, and Wisconsin

have conducted studies of Vietnam veterans. Most of these studies remain unpublished in the scientific literature.

Hawaii A random sample of Vietnam era veterans residing in Hawaii was generated (Rellahan, 1985). Of 511 Vietnam era veterans agreeing to participate in the study, 418 questionnaire responses were received. The Vietnam experience group consisted of 232 respondents who had been stationed in Vietnam between 1962 and 1972. The control group was 186 respondents whose Vietnam era service was not in Southeast Asia. Respondents received general health questionnaires. Exposure of interest was the Vietnam experience (Rellahan, 1985).

Iowa The report prepared for the Iowa State Department of Health in 1985 was based on a questionnaire survey of Iowa Vietnam veterans in the Iowa Agent Orange Registry (Wendt, 1985). An Agent Orange Exposure Questionnaire was mailed to 45,181 veterans who served in Southeast Asia, listed in the Iowa Agent Orange Registry. There were 10,846 respondents (24 percent). Percentages of self-reported direct exposure to Agent Orange, length of exposure, and illness within 48 hours of exposure are reported. There was no comparison group.

Maine Vietnam veterans in Maine were surveyed by the Maine Commission on Vietnam and Atomic Veterans to assess health status, risk factors, and reproductive experience compared to veterans who were exposed during atomic nuclear weapons tests, and to compare observations from previous population studies examining similar outcomes (Deprez et al., 1991). Of 1,700 surveys sent to veterans in Maine, 249 were received from Vietnam veterans and 113 from atomic test veterans. Exposure to herbicides in Vietnam was by self-report, through descriptions of passing through sprayed areas, ingesting water or food in local areas, or clearing vegetation.

Massachusetts In Massachusetts, computerized statewide mortality files for 1972 to 1983 were linked to the computerized list of veterans who applied for a military service bonus, which was available through the Massachusetts Office of Veterans Services; the results were compiled for overall mortality (Kogan and Clapp, 1985), as well as for cause-specific mortality, with a focus on STS (Kogan and Clapp, 1988). To be eligible for a bonus, veterans must have served for at least six months between July 1, 1958, and April 1, 1973; must have been Massachusetts residents for at least six months immediately prior to entering the service; must have applied for the bonus; and must have been honorably discharged. It was estimated that 95 percent of all eligible Massachusetts residents received the bonus. A mortality file provided information on age at death, sex, race, cause and year of death, and Vietnam service. Cause of death codes on the mortality file were found to be 99 percent accurate compared to death certificates. The report is

limited to white males, because nonwhites and females constituted less than 2 percent of the population. The cause-specific mortality (1972-1983) was compared among deceased Vietnam veterans (N = 840), deceased Vietnam era veterans (N = 2,515), and all other deceased Massachusetts resident white men (excluding Vietnam era veterans). The standardized PMRs were calculated. The standardized mortality odds ratios were also calculated in certain instances by using circulatory disease as the comparison. No information was available regarding actual exposure to herbicides in Vietnam; the Vietnam experience is assumed to be the exposure. Information on potential confounding factors, such as smoking and drinking habits, and complete occupational histories were not available for analysis.

As a follow-up to the mortality study of veterans in Massachusetts (Kogan and Clapp, 1985, 1988), cases of selected cancers were identified from the Massachusetts Cancer Registry between 1982 and 1988 (Clapp et al., 1991). The data were linked to status as Vietnam era veterans or Vietnam bonus recipients. Controls for each cancer site analysis were veterans with other cancers, excluding STS, NHL, and kidney cancer. The study focused on males between the ages of 30 and 59 at the time of cancer diagnosis. There were 727 male Vietnam era veterans and 214 Vietnam veterans identified for inclusion in the analysis. STS and NHL were evaluated in greater detail in the analysis. Exposure of interest was Vietnam service.

Vietnam veterans receiving bonuses as previously described in Massachusetts and living in the greater Boston area were contacted regarding the presence of chloracne skin lesions as a surrogate for exposure to TCDD (Levy, 1988). Six current cases of chloracne and 25 control subjects from the same bonus list were identified. Controls were matched to cases on age, education, and time of Vietnam service. Neuropsychological tests were administered to all study subjects to determine the presence of PTSD in association with exposure to TCDD, as indicated by the presence of chloracne.

New Jersey The Agent Orange Commission in the state of New Jersey developed and supported what is known as the Pointman Project (I and II), which investigated exposure among veterans handling herbicides (Part I) (Kahn et al., 1988) and among ground combat troops (Part II) (Fiedler and Gochfeld, 1992; Kahn et al., 1992a,c). The first Pointman project was not considered a valid epidemiologic study, because it was based on only 10 Vietnam herbicide-exposed veterans, 10 Vietnam nonexposed veterans, and 7 Vietnam era veterans; immune status testing results have been reported (Kahn et al., 1992b). The second Pointman study, which focused on ground troops not specifically handling herbicides, involved 15 Army and 20 Marine veterans from battalion units that operated in heavily defoliated areas, and 20 Navy veterans from units operating on canals and rivers in heavily

defoliated areas (Fielder and Gochfeld, 1992). Vietnam veterans with no known exposure were chosen as controls ($N = 15$). Study subjects had blood drawn and tested for serum levels of 14 dioxins and furans (Kahn et al., 1992a). Outcomes evaluated included neurobehavioral assessments (Fielder and Gochfeld, 1992), semen analysis (Kahn et al., 1992c), and dibenzodioxin and dibenzofuran congener levels (Kahn et al., 1992a).

New Mexico A cohort study of Vietnam veterans in the Agent Orange registry of the VA medical center in Albuquerque, New Mexico, was designed to ascertain the presence of abnormal pulmonary pathology on radiographs among this cohort compared to an unexposed group of veterans who did not serve in Vietnam (Pollei et al., 1986). The exposure of interest was service in Vietnam. Veterans ($N = 422$) were between 18 and 45 years at the time of service and between 26 and 62 years at radiographic examination. Control, unexposed veterans were Air Force staff who did not serve in Vietnam, between 19 and 45 years of age, who had received a flight physical examination. A subset of films was available and analyzed separately for 27 veterans who reported handling Agent Orange.

New York A mortality study of New York State veterans used New York State Vital Records, Defense Manpower Data Center (DMDC) military information, and the VA BIRLS data base. Service in Vietnam was considered indicative of dioxin-contaminated herbicide exposure. In the first analysis, all deaths that occurred from 1965 to 1967, and 1970 to 1980, among males who were 18 to 29 years of age during the Vietnam era (1965-1971) were identified (Lawrence et al., 1985). Military service information was obtained from death certificates. Adjusted mortality odds ratios for 26 different causes of death were calculated for Vietnam era veterans ($N = 4,558$) and nonveterans ($N = 17,936$). Verification of veteran status as indicated on the death certificate was 90 percent accurate compared to next of kin interview. However, Vietnam service information showed a high degree of misclassification and needed to be assessed elsewhere. Therefore the DMDC and the BIRLS were used to obtain information on Vietnam service. A separate comparison was made of mortality (1970-1980) of veterans who served in Vietnam ($N = 555$) compared to mortality of non-Vietnam veterans of the same era ($N = 941$).

The New York State Cancer Registry (Greenwald et al., 1984) was used to identify all men with STS diagnosed from 1962 to 1980 who in 1962-1971 were between the ages of 18 and 29 years. Of the cases identified, 281 patients or their next of kin agreed to be interviewed; 151 cases were alive and 130 had died. Living controls were matched to all cases by five year age groups and zip code of residence, and identified by New York State Department of Motor Vehicle files. Additionally, deceased controls were matched to deceased cases from death certificates for year of death,

five year age group, years of education, race, and health systems area. Cancer deaths were excluded. Information for deceased controls was collected in a similar manner as for deceased cases, through interviews with next of kin. Medical records and hospital pathology information were collected for all cases. STS specimens were reviewed by a pathologist. Of the cases, 9 percent were not STS. All cases and controls or next of kin were interviewed by telephone using a standardized questionnaire. Information was obtained about military service experience, occupational history involving herbicide exposure (chemical production, highway or railroad maintenance or construction, park or garden maintenance, farming, herbicide spraying, outdoor or rural work); exposure to a list of specific chemicals including Agent Orange, dioxin, or 2,4,5-T; past illnesses; family medical history; and smoking and drinking histories. Dose information was not available for chemical exposures.

Pennsylvania A case-control study on the association of STS, NHL, and other selected rare cancers in Pennsylvania males who had served in Vietnam was conducted through a review of Pennsylvania death records of men dying between 1969 and 1983 at the ages of 18 to 50 (Goun and Kuller, 1986). A total of 349 deceased men and 349 men dying of other cancers excluding the cancers of interest and HD, were identified from the Pennsylvania death records, with matching on year of death, age of death, race, and county of residence. A living control group was also selected from the Pennsylvania Department of Transportation drivers' license files, matched for sex, year of birth, and county of residence. Military service information for cases and deceased controls was determined by using death certificates, questionnaires mailed to next of kin, official military personnel files, and records of the Pennsylvania Vietnam Conflict Veterans Compensation Bureau. Military service data for the living controls were obtained from computerized record linkage to the Pennsylvania Vietnam Conflict Veterans Compensation Bureau and the National Personnel Records Center. Vietnam military service was accepted as a surrogate for herbicide exposure, because no data on Agent Orange exposure were available.

Texas Preliminary studies of cytogenetics, sperm, and immune response were conducted among Texas Vietnam veterans compared to a control group (Newell, 1984). The exposure of interest was Agent Orange; military, medical, and other supporting documents were reviewed to determine Vietnam Agent Orange exposure and how it occurred. Included in the exposure evaluation was a determination of amount of exposure to herbicides (other than from Operation Ranch Hand), reported symptoms of chloracne at and after exposure, current medical problems believed to be associated with Agent Orange exposure, current or past chemical exposure (in fact an exclusion criterion), miscarriages, stillbirths, birth defects, and dates and

types of service duty. Exposures were categorized into six levels; those in the "highly exposed" category were included in these studies reported. The study group was intentionally skewed by researchers to reflect high Agent Orange doses. The amount of herbicide sprayed in the area in which the veteran was assigned was noted. Controls were selected as having no possible Agent Orange exposure in Vietnam. All three laboratory studies were performed on Vietnam veterans and controls, although total samples varied by test. Study 1 examined chromosome changes including breaks, exchanges, fragment rings, etc.; study 2 examined semen specimens for sperm count and morphologic abnormalities; and study 3 examined measures of T cells and blood lymphocytes.

West Virginia The West Virginia Health Department conducted a proportionate mortality study among veterans of the Vietnam era for the purposes of generating hypotheses regarding the possible association of specific causes of death with service in Vietnam (Holmes et al., 1986). A record of all deaths by cause was compiled from the death records of the Health Statistics Center of the state of West Virginia (1968-1983). This record was matched with a list of all individuals who had qualified for a bonus under the state's Vietnam era bonus program. Four groups of deaths were used for comparison purposes: all deceased male veterans, deceased male Vietnam era veterans, deceased Vietnam veterans, and all nonveteran West Virginia males. The analyses conducted were proportionate mortality analyses stratified on age at death (by five year period) and on year of death (by two year intervals). Of the total of 1,225 male veteran deaths identified, 610 were Vietnam era veterans and 615 were Vietnam veterans. No exposure data were available.

Wisconsin The Wisconsin Division of Health examined the mortality of Vietnam veterans in a study conducted in three phases. Phase 1 compared all Wisconsin veteran deaths (1960-1979) to Wisconsin nonveterans for white men, black men, and white women. Death records from the Wisconsin Center for Health Statistics were used to identify mortality (Anderson et al., 1986a,b). Phase 2 compared Wisconsin Vietnam veterans and Wisconsin Vietnam era veterans to Wisconsin nonveterans, and other Wisconsin veterans, for mortality experience from 1964 to 1983. Cause of mortality information was obtained from the Wisconsin Department of Veterans Affairs Grave Registration files. A Vietnam veteran was defined as someone serving for a minimum of six months on active duty in Vietnam at some time between January 1964 and December 1975. Vietnam era veterans did not serve in the Vietnam theater but served during the Vietnam era (Anderson et al., 1986a).

Phase 3 of the Wisconsin study of a cohort of Vietnam era veterans was an SMR study (Anderson et al., 1986b). Veterans were followed in this

phase through December 1984. Follow-up started at the time of discharge and ended at the time of death or December 1984. Mortality was evaluated by using four reference populations: U.S. men, Wisconsin men, male Wisconsin nonveterans, and Wisconsin veterans. There was no information regarding exposure, specifically, to Agent Orange or other herbicides.

Other U.S. Vietnam Veteran Studies

Additional studies have been conducted in Vietnam veterans to examine a number of health outcomes including testicular cancer (Tarone et al., 1991), spontaneous abortion in spouses of veterans (Aschengrau and Monson, 1989), late adverse pregnancy outcomes (Aschengrau and Monson, 1990), and PTSD (Goldberg et al., 1990).

A case-control study of testicular cancer in men was conducted following a published study indicating a potential association with testicular cancer in dogs that served in Vietnam (Hayes et al., 1990). Incident cases, newly diagnosed with testicular cancer between the ages of 18 and 42 years, between January 1, 1976, and June 30, 1981, and referred to one of three Washington, D.C., area hospitals (the National Naval Medical Center, Walter Reed Army Medical Center, and the National Institutes of Health Clinical Center) were identified (Tarone et al., 1991). Controls were male patients at the same hospital as the case but newly diagnosed during the same period with cancers other than cancer of the genital tract, matched to the cases by age (±2 years). A questionnaire was administered to each study participant, and included information on military service and occupational history. Successful interviews were obtained for 271 testicular cancer patients (88 percent) and 259 controls (90 percent). All patients with either cryptorchidism or low birthweight were eliminated from the study. Analysis was restricted also to the 137 testicular cancer cases and 156 controls who were born before 1955. NHL cases were also excluded as controls because of a potential association with Vietnam service, resulting in 130 controls for the study. Service in Vietnam was considered to be the exposure of interest.

Women with spontaneous abortion loss at Boston Hospital for Women between July 1976 and July 1978 were eligible for inclusion in a study comparing husband's military occupational service to husbands of women having full-term babies during this same time (Aschengrau and Monson, 1989). Cases were women with spontaneous abortions through 27 weeks; controls were randomly selected from those patients with delivery within one week of the case's pregnancy loss. Following exclusion, 201 cases and 1,119 controls were selected for study. Paternal military service was identified from patient medical record information, Massachusetts bonus records, and national military records. Military service for veterans was classified as Vietnam veteran, Vietnam era veteran, and no known military service.

Aspects of interest of Vietnam service were branch, rank, occupational specialty, and calendar years; combat jobs were designated by the Director of the Massachusetts Agent Orange Program.

By using methods similar to those described above (Aschengrau and Monson, 1989), the relationship between paternal military service in Vietnam and the risk of three late adverse pregnancy outcomes, was evaluated in women who delivered infants from August 1977 until March 1980 at Boston Hospital for Women (Aschengrau and Monson, 1990). Paternal military service was compared among 857 cases of congenital anomaly, 61 stillbirth cases, and 48 neonatal death cases with paternal military service for 998 normal control births.

A cohort of 2,092 male monozygotic twin pairs, in which both twins served during the Vietnam era (1965-1975), was identified (Goldberg et al., 1990). Evaluation of the presence of PTSD among twin pairs was determined from responses to a health survey, and compared among pairs of twins discordant for Vietnam service (N = 715); that is, one twin served in Southeast Asia, and one did not. Twins were identified for study from the Vietnam Era Twin Registry, and detailed study methods are described earlier in the chapter (Eisen et al., 1991).

Australia

In 1980, the Commonwealth Institute of Health at the University of Sydney was commissioned by the Australian government to investigate Agent Orange claims (Evatt, 1985). In 1981, the scope of the investigation broadened to enable examination of all aspects of Vietnam service. The intention of the commission was to undertake three studies, involving birth defects, mortality, and morbidity. The morbidity protocol was denied by the coalition government, and only the birth defects and mortality studies were conducted. Several initial informal sessions were designed to ascertain, anecdotally, how service personnel were potentially exposed to Agent Orange. Formal hearings that were related to matters of toxicity, birth defects, morbidity, mortality, and neuropsychology followed (Evatt, 1985).

The study of birth anomalies was designed as a case-control study (Donovan et al., 1983, 1984; Evatt, 1985). Infants with anomalies diagnosed at birth, or in the first week of life, were individually matched to control infants born without an anomaly in the same hospital, to a mother of similar age, and at about the same date. The fathers of 8,517 cases and controls were identified (1966-1979) and compared with a list of men who served in the Australian Army during Australia's involvement in Vietnam (1962-1972). Fathers who served in the Army during the Vietnam era were classified according to the following: National Service or Australian Regular Army, Vietnam veteran status, year of first tour in Vietnam, length of

TABLE 7-3 Epidemiologic Studies—Vietnam Veterans

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Ranch Hands				
AFHS, 1983, 1984b, 1985, 1986, 1989, 1991a	Cohort	Mortality updates of Ranch Hands tasked with herbicide spraying operations during the Vietnam conflict, compared with Air Force C-130 air and ground crew veterans in Southeast Asia who did not participate in herbicide spraying missions	1,261 (original cohort)	19,101 (original cohort)
AFHS, 1984a, 1987, 1990, 1991b	Cohort	Baseline morbidity and follow-up exam results of the Air Force Health Study	1,208 (baseline)	1,668 (baseline)
AFHS, 1992	Cohort	Reproductive outcomes of participants in the Air Force Health Study	791	942
Michalek et al., 1990	Cohort	Mortality of Ranch Hands compared with Air Force C-130 air and ground crew veterans in Southeast Asia	1,261	19,101
Wolfe et al., 1990	Cohort	Health status of Ranch Hands at second follow-up, compared with Air Force C-130 air and ground crew veterans in Southeast Asia	995	1,299

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Centers for Disease Control				
Erickson et al., 1984a,b	Case-control	CDC birth defects study of children born in the Atlanta area between 1968-1980, comparing fathers' Vietnam experience and potential Agent Orange exposure between birth defects cases and normal controls	7,133	4,246
CDC, 1989b	Cohort	Vietnam Experience Study—random sample of U.S. Army enlisted men 1965-1971	2,490	1,972
CDC, 1988a	Cohort	Vietnam Experience Study—random sample of U.S. Army enlisted men 1965-1971: psychosocial outcomes	2,490	1,972
CDC, 1988b	Cohort	Vietnam Experience Study: physical health outcomes	2,490	1,972
CDC, 1988c	Cohort	Vietnam Experience Study: reproductive outcomes	12,788 children	11,910 children
CDC, 1987; Boyle et al., 1987	Cohort	Vietnam Experience Study: mortality	9,324	8,989

O'Brien et al., 1991	Cohort	Interview report and mortality for NHL based on Vietnam Experience Study	8,170	7,564
Decoufle et al., 1992	Cohort	Association between self-reported health outcomes and perception of exposure to herbicides based on Vietnam Experience Study	7,924	7,364
CDC, 1990a	Case-control	Selected Cancers study—population-based case-control study of all men born between 1921 and 1953; cases diagnosed area covered by eight cancer registries and controls selected by random-digit dialing	1,157 NHL 342 STS 310 HD 48 Nasal carcinoma 80 Nasopharyngeal carcinoma 130 Primary liver cancer	1,776
CDC, 1990b	Case-control	Selected Cancers study—population-based case-control study of all men born between 1921 and 1953; cases diagnosed area covered by eight cancer registries and controls selected by random-digit dialing: NHL	1,157	1,776
CDC, 1990c	Case-control	Selected Cancers study: soft tissue sarcomas	342	1,776
CDC, 1990d	Case-control	Selected Cancers Study: HD, nasal cancer, nasopharyngeal cancer, and primary liver cancer	310 HD 48 Nasal carcinoma 80 Nasopharyngeal carcinoma 130 Primary liver cancer	1,776

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Dept. of Veterans Affairs				
Burt et al., 1987; Breslin et al., 1988	Cohort	Mortality experience (1965-1982) of Army and Marine Corps Vietnam veterans, compared to Vietnam era veterans who did not serve in Southeast Asia standardized by age and race; nested case-control study of NHL	24,235	26,685
Bullman et al., 1990	Cohort	Mortality experience of Army I Corps Vietnam veterans compared to Army Vietnam era veterans	6,668	27,917
Watanabe et al., 1991	Cohort	Mortality experience (1965-1984) of Army and Marine Corps Vietnam veterans compared to: (1) branch-specific (Army and Marine) Vietnam-era veterans; (2) all Vietnam-era veterans combined; (3) the U.S. male population	24,145 Army 5,501 Marines	(1) 27,145 Army; 4,505 Marines (2) 32,422 Combined Vietnam era (3) U.S. male population
Thomas and Kang, 1990	Cohort	Morbidity and mortality experience (1968-1987) of Army Chemical Corps Vietnam veterans compared to U.S. men	894	—

Thomas et al., 1991	Cohort	Mortality experience (1973-1987) among women Vietnam veterans compared to women non-Vietnam veterans and for each cohort compared to U.S. women	4,582	5,324
Kang et al., 1986	Case-control	STS cases (1969-1983) in Vietnam era veterans for association with branch of Vietnam service as a surrogate for Agent Orange exposure	234	13,496
Kang et al., 1987	Case-control	STS cases (1975-1980) diagnosed at the Armed Forces Institute of Pathology, compared to controls identified from patient logs of referring pathologists or their departments for association with Vietnam service and likelihood of Agent Orange exposure	217	599
Dalager et al., 1991	Case-control	Cases of NHL diagnosed 1969-1985 among Vietnam era veterans compared to cases of other malignancies among Vietnam era veterans for association with Vietnam service	201	358
True et al. 1988	Cross-sectional	PTSD and Vietnam combat experience evaluated among Vietnam era veterans	775	1,012
Bullman et al., 1991	Case-control	PTSD cases in Vietnam veterans compared to Vietnam veterans without PTSD for association with traumatic combat experience	374	373

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Farberow et al., 1990	Case-control	Psychological profiles and military factors associated with suicide and motor vehicle accident (MVA) fatalities in Los Angeles County Vietnam era veterans (1977-1982)	22 Vietnam suicides 19 Vietnam era suicides	21 Vietnam MVA 20 Vietnam era MVA
Eisen et al., 1991	Cohort	Health effects of male monozygotic twins serving in the armed forces during Vietnam era (1965-1975)	2,260	2,260
American Legion				
Snow et al., 1988	Cohort	Assessment of PTSD in association with traumatic combat experience among American Legionnaires serving in Southeast Asia (1961-1975)	2,858	Study group subdivided for internal comparison
Stellman et al., 1988b	Cohort	Assessment of physical health and reproductive outcomes among American Legionnaires who served in Southeast Asia (1961-1975) for association with combat and herbicide exposure	2,858	3,933
Stellman et al., 1988c	Cohort	Assessment of social and behavioral outcomes among American Legionnaires who served in Southeast Asia (1961-1975) for association with combat and herbicide exposure	2,858	3,933

State studies				
Rellahan, 1985	Cohort	Study of health outcomes in Vietnam era (1962-1972) veterans residing in Hawaii associated with Vietnam experience	232	186
Wendt, 1985	Descriptive	Descriptive findings of health effects and potential exposure to Agent Orange among Iowa veterans who served in Southeast Asia	10,846	None
Kogan and Clapp, 1985, 1988	Cohort	Mortality experience (1972-1983) among white male Massachusetts Vietnam veterans, compared to non-Vietnam veterans, and to all other nonveteran white males in Massachusetts	840 deaths	2,515 deaths in Vietnam era veterans
Clapp et al., 1991	Case-control	Selected cancers identified (1982-1988) among Massachusetts Vietnam veterans, compared to Massachusetts Vietnam era veterans with cancers of other sites	214	727
Levy, 1988	Cross-sectional	Study of PTSD in chloracne as indicator of exposure to TCDD and control Vietnam veterans in Massachusetts	6	25
Fiedler and Gochfeld, 1992; Kahn et al., 1992a,b,c	Cohort	New Jersey study of outcomes in select group of herbicide-exposed Army, Marine, and Navy Vietnam veterans, compared to veterans self-reported as unexposed	10 Pointman I 55 Pointman II	17 Pointman I 15 Pointman II

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Pollei et al., 1986	Cohort	Study of chest radiographs of New Mexico Agent Orange Registry Vietnam veterans, compared to control Air Force servicemen radiographs for pulmonary and cardiovascular pathology	422	105
Lawrence et al., 1985	Cohort	Mortality experience of New York State (1) Vietnam era veterans compared to nonveterans and (2) Vietnam veterans compared to Vietnam era veterans	(1) 4,558 (2) 555	17,936 941
Greenwald et al., 1984	Case-control	Cases of STS in New York State compared to controls without cancer for Vietnam service and herbicide exposure including Agent Orange, dioxin, or 2,4,5-T	281	281 live controls 130 deceased controls
Goun and Kuller, 1986	Case-control	Cases of STS, NHL, and selected rare cancers compared to controls without cancer for Vietnam experience in Pennsylvania men (1968-1983)	349	349 deceased
Anderson et al., 1986a	Cohort	Mortality experience of Wisconsin veterans compared to nonveterans (Phase 1); mortality experience of Wisconsin Vietnam veterans and Vietnam era veterans compared to nonveterans and other veterans (Phase 2)	110,815 white male veteran deaths 2,494 white male Vietnam era veteran deaths 923 white male Vietnam veteran deaths	342,654 white male nonveteran deaths 109,225 white male other veteran deaths

Anderson et al., 1986b	Cohort	Mortality experience of Wisconsin Vietnam era veterans and Vietnam veterans compared to U.S. men, Wisconsin men, Wisconsin nonveterans, and Wisconsin other veterans	122,238 Vietnam era veterans 43,398 Vietnam veterans	—
Holmes et al., 1986	Cohort	Mortality experience (1968-1983) of West Virginia veterans, Vietnam veterans, Vietnam era veterans compared to nonveterans; Vietnam veterans compared to Vietnam era veterans	615 Vietnam veterans 610 Vietnam era veterans	—
Newell, 1984	Cross-sectional	Preliminary (1) cytogenetic, (2) sperm, and (3) immune response tests in Texas Vietnam veterans compared to controls	(1) 30 (2) 32 (3) 66	30 32 66
Deprez et al., 1991	Descriptive	Study of Maine Vietnam veterans compared to atomic test veterans and general population for health status and reproductive outcomes	249	113 atomic test veterans
Other U.S. veteran studies				
Aschengrau and Monson, 1989	Case-control	Association between husband's military service and women having spontaneous abortion at 27 weeks compared to women delivering at 37 weeks	201	1,119
Aschengrau and Monson, 1990	Case-control	Study of cases with late adverse pregnancy outcomes compared to normal control births for association with paternal Vietnam service (1977-1980)	857 congenital anomalies 61 stillbirths 48 neonatal deaths	998

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Goldberg et al., 1990	Cohort	Study of male twin pairs who served in Vietnam era (1965-1975) for association between Vietnam service and PTSD	2,092	2,092
Tarone et al., 1991	Case-control	Study of cases between January 1976 and June 1981 with testicular cancer (18-42 years old) compared to hospital controls for association with Vietnam service	137	130
Australian studies				
Donovan et al., 1983, 1984	Case-control	Australian study of cases of congenital anomalies in children born (1969-1979), compared to infants born without anomalies for association with paternal Vietnam service	8,517	8,517
Fett et al., 1987a	Cohort	Australian study of mortality experience of Vietnam veterans compared to Vietnam era veterans through 1981	19,205	25,677
Fett et al., 1987b	Cohort	Australian study of cause-specific mortality experience of Vietnam veterans compared to Vietnam era veterans through 1981	19,205	25,677

Forcier et al., 1987	Cohort	Australian study of mortality in Vietnam veterans by job classification, location, and time of service	19,205	Internal comparison
Field and Kerr, 1988	Cohort	Tasmanian study of Vietnam veterans compared to neighborhood controls for adverse reproductive and childhood health outcomes	357	281

[a]The dash (—) indicates the comparison group is based on a population (e.g., U.S. white males, country rates), with details given in the text for specifics of the actual population.

Vietnam service before conception, and time to conception following return from Vietnam. Of the men identified with Army service, there were a total of 127 fathers of infants who were born with anomalies and 123 fathers of control infants who were Vietnam veterans. Numbers of specific defects were given.

An independent study in Tasmania evaluated numerous reproductive and childhood health problems for association with paternal Vietnam service (Field and Kerr, 1988). Tasmanian servicemen serving in Vietnam between 1965 and 1972 were identified and asked to identify a neighborhood control family with children in which the father was of similar age to the veteran. Of 1,395 Tasmanian servicemen identified as serving in this period, 357 veterans agreed and had conceived one or more children since service. Interviews of veterans and control families focused on 22 aspects of reproductive and childhood health, veterans' Vietnam service including rank and type of service, father's exposure to chemicals during civilian life, and family history of disease.

Mortality (Commonwealth Institute of Health, 1984a-c; Evatt, 1985; Fett et al., 1987a,b; Forcier et al., 1987) was examined in a retrospective cohort study among men drafted into the Australian National Service during the Vietnam era (1965-1971). Excluded from the study were those who died in Vietnam or within two years of enlistment, those less than 18 years old at enlistment, and those serving fewer than 90 days. The final study population consisted of 19,205 Vietnam veterans and 25,677 Vietnam era veterans who had served at least 12 months. Type of service within the Army (infantry, engineers, armor, artillery, minor field presence, nonfield corps), dates, and lengths of service were obtained from military records. Follow-up was from discharge through January 1, 1982. Vital status was determined by matching study subjects against death certificates from the State Registrar-General; information on where death occurred and whether from external causes was obtained from transcripts of court proceedings, police investigations, and reports of postmortems from hospital and clinical records. Analyses were also examined in each corps group by calendar year of Vietnam service or duration of service, with mortality rates of Vietnam veterans compared to those of Vietnam era veterans. Deaths from all causes (Fett et al., 1987b) and cause-specific mortality (Fett et al., 1987a) have been reported. An additional publication using the study methods described cross-tabulates veteran corps groups by phase of Vietnam conflict service for differences in mortality patterns (Forcier et al., 1987).

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8

Cancer

Cancer is the second leading cause of death in the United States. There has been a gradual rise in the proportion of deaths from cancer in the United States during the twentieth century, due in large part to increased tobacco use. At present, more than 30 percent of Americans will develop a malignancy at some time in their lives, and approximately half of them will die from it (Seidman et al., 1985). As a result of the high incidence of cancer, the often disfiguring and uncomfortable approaches to treatment, and the general lack of success in treating many types of cancer, it has become a particularly dreaded disease.

Many types of cancers are thought to be related to herbicides and/or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), but the evidence for the associations is uneven. Following this introduction about cancer and its epidemiology, the committee summarizes and reaches conclusions about the strength of the evidence in epidemiologic studies regarding associations between exposure to herbicides and TCDD and each type of cancer. The cancer types are discussed in the order in which they are listed in the International Classification of Diseases (U.S. DHHS, 1991). A summary at the end of the chapter compares the cancer types for which the strength of the epidemiologic evidence is similar, and discusses the nature of the evidence that led the committee to its conclusion.

Cancer is a disease of the cell. Cancer cells are malignant: that is, they lack normal growth control, and have the ability to invade and metastasize into surrounding tissues and other organs. These properties are inherent in the cells themselves, so that, for example, a cancer cell could be transplanted

into a normal host and a new malignant tumor would arise. This implies that the underlying abnormality that led to the transformation of a normal cell into a malignant cell is present in the genetic material of the cell itself or in the way the genetic information is expressed.

It is now well accepted that a malignant tumor develops from a single cell that has passed through a series of steps or stages of transformation. The initial stage of transformation, or "initiation," is thought to involve a mutation—that is, damage to DNA. The initiated cell and its progeny, most of which are destroyed by the body, then must pass through one or more additional stages (progression) before a clone of fully transformed malignant cells will acquire the essential properties of a malignant tumor. These generally include loss of normal specialization, faster than normal rates of cell division, and loss of normal limits on cell division.

Many carcinogens that have been identified thus far are initiators and are believed to interact directly with DNA. It is also known, however, that certain substances may promote tumor formation by initiated cells, even though they are not capable of the initial cell mutation. Based on its effects in animal studies, TCDD is considered a tumor promoter, not a tumor initiator. The potential mechanisms by which TCDD can act as a tumor promoter are discussed in Chapter 4.

It follows from an initiation/promotion model that tumor initiators should act early in the carcinogenic process, often decades before a cancer is diagnosed, while tumor promoters may exert their effect at any time between initiation and clinical diagnosis.

The experimental evidence suggesting that TCDD acts as a tumor promoter comes from studies in laboratory animals. It is possible, though not proven, that TCDD could also promote the formation of cancer in humans after exposure to another potential carcinogen. Understanding the biological mechanism whereby TCDD interacts with the process of cancer production is critical to the committee's analysis of the plausibility of an association between human cancer and exposure to Agent Orange and other herbicides. Although direct evidence may not be available regarding the biologic plausibility of a specific tumor site, this does not preclude examination of epidemiologic data for potential association in a population.

In evaluating the epidemiologic studies, the committee noted that in many studies, insufficient time had passed since exposure for many types of tumors to develop; this is an issue of minimum latency needed for an adequate study. However, if TCDD is acting as a promoter, studies that evaluate health outcomes before the usual minimum latency period has passed may be appropriate since this function may require a shorter latency period for its hypothesized mechanism of action.

CANCER EPIDEMIOLOGY

Cancer is a popular subject for discussion in the lay press and media. Hardly a day goes by without an article trumpeting some chemical or environmental hazard as playing some role in the etiology of some malignancy. In truth, it is difficult to establish causal relationships for cancers. There are several characteristics of cancer that strongly affect and influence the ability to establish etiologic relationships.

The first major characteristic in this regard is the long latency period that is thought to exist between exposure and onset of disease in most instances. Also, most etiologic agents (except cigarette smoking) probably contribute to only a relatively small fraction of cases. Furthermore, although cancer overall is relatively common, the term "cancer" actually represents a rubric of more than 100 different subtypes, categorized by anatomic site and histology, as well as by stage and other factors. Any specific subtype of cancer is a relatively rare event, and this makes its study difficult. For example, to study the impact of hypertension on the incidence of a relatively common outcome, such as coronary artery disease, an appropriately selected cohort of 1,000 to 2,000 people, followed for five to ten years, might suffice. A similar attempt to relate a risk factor to even a relatively common malignancy, such as lung cancer, would require a cohort of 10,000 to 20,000 individuals followed for five to ten years. For a less common cancer, such as non-Hodgkin's lymphoma, it would be even more difficult, requiring substantially more people.

Another characteristic—cancer's relatively high case fatality rate—is an additional complication for analysis. As a consequence, many studies rely on cancer mortality rather than cancer incidence as an outcome. Depending on the specific malignancy under study and the time period being explored, this is often a reasonable approach. However, improvements in the early detection and treatment of cancer have led to improved survival rates for several types of cancer (testicular cancer, childhood leukemia, Hodgkin's disease, for example). To the degree that it is true for a given malignancy, fatality rates have declined, and conclusions derived from the use of mortality data must be viewed cautiously.

Another aspect of cancer epidemiology that it is important to understand is the wide availability of tumor registries since the 1970s. For various reasons, many cities, states, countries, and other political and geographical regions have kept population-based data regarding the reporting of cancer incidence—usually by subtype, and often with survival and follow-up data—within their boundaries. These data are usually available to epidemiologists and greatly facilitate the practice of their science.

Finally, an important aspect of cancer epidemiology is precision of diagnosis by pathologic criteria. Compared to diagnosis of many other

types of disease, which may involve only patient-described symptoms, a cancer diagnosis is usually definitive only when based on pathologic review. In addition, histologic and other subtyping can also be defined fairly accurately. In the review and critique of any study, great attention must be paid to the efforts utilized by the investigators in establishing the pathologic diagnosis. Particularly when population-based registry data are utilized, or when data are collected from less sophisticated regions or hospitals, difficulties may arise in the accuracy of the study. When death certificate diagnoses are used, accuracy may suffer as well (see Chapter 5).

There are a large number of different types of cancer as defined by site and histology. In situations in which many studies have been conducted on a certain exposure, and multiple analyses conducted within each study, there is a risk of finding occasional statistical associations purely on the basis of chance. Thus, one must beware of overinterpreting an isolated finding of excess risk for a given tumor type within a single study. Consistency across studies, with consideration of dose-response relationships and use of other statistical methods that evaluate plausibility, should be assessed before reaching any conclusions regarding associations between exposures and cancer. Additionally, the confidence intervals around the estimate of association will provide guidance as to the degree of precision and study size. Wide intervals indicate that the sample size, on which the estimate was based, was relatively small, and therefore the degree of precision attributed to that estimate is more variable. These considerations are outlined more fully in Chapter 5.

SPECIFIC ISSUES WITH REGARD TO HERBICIDE EXPOSURE IN VIETNAM

Aside from the issues discussed above, there are also several general problems with regard to relating herbicide exposure in Vietnam to the incidence of cancer overall or to specific malignancies. As a whole, the cohort in Vietnam consisted primarily of young males and a far smaller number of females who were potentially exposed to herbicides between 1965 and 1975. A veteran who was 20 years old in 1965 is 48 years old in 1993, still very young with respect to developing cancer. Because the incidence of most malignancies is strongly age dependent, the cohort under discussion, for the most part, has not yet reached the age range of highest risk, making the incidence of cancer, and certainly of cancer subtypes, rare within most studies of veterans. The two specific types of cancer most closely linked to herbicide exposure in the scientific literature, soft tissue sarcoma (STS) and non-Hodgkin's lymphomas, are noteworthy in that their incidence is relatively high in younger age groups even in the absence of any harmful exposures. Thus, it may well be that for the most common cancer types, such as

lung, colon, and prostate cancers, the length of follow-up since the Vietnam conflict remains too short to adequately evaluate possible associations with exposure.

The age-specific incidence rates for the cancer types of special significance for the Vietnam veteran population are shown in Figure 8-1 for men and in Figure 8-2 for women. For most cancers (illustrated as a "typical cancer"), the incidence rates increase with age (note that the vertical axis of the graph uses a logarithmic scale), but for some cancers, such as brain cancer, acute lymphocytic and all leukemias, and non-Hodgkin's lymphoma, the rate of increase with age is more gradual than the rest; thus the incidence rates under age 40 (compared to those over age 40) are relatively high. Hodgkin's disease, soft tissue sarcoma, testicular cancer for men, and

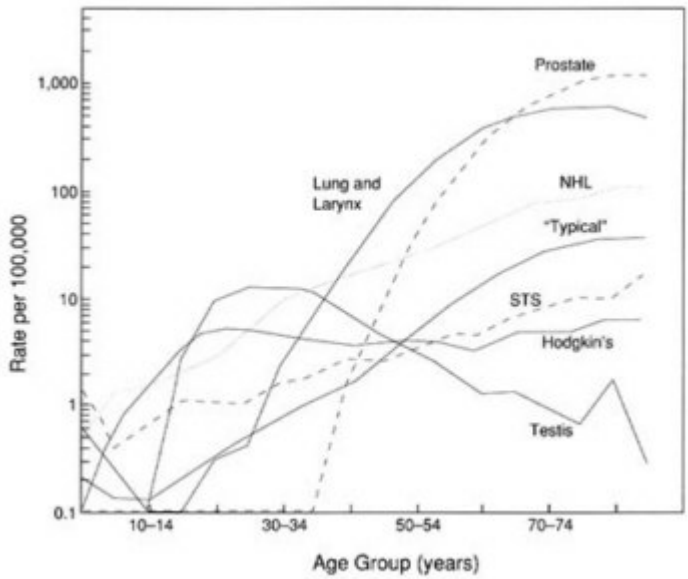

FIGURE 8-1 Average age-specific incidence rates for selected cancers in men for the period 1985-1989. The "typical" cancer illustrates a common pattern of age-specific rates seen in cancers not included in the figure; age-specific rates for all cancers combined have been rescaled (1:100) to construct the typical cancer. Rates are for 5 year age groups and are presented on a logarithmic scale. SOURCE: Miller et al., 1992.

cancer of the cervix for women are unique among the cancers under study in that their age-specific incidence rates peak below age 40.

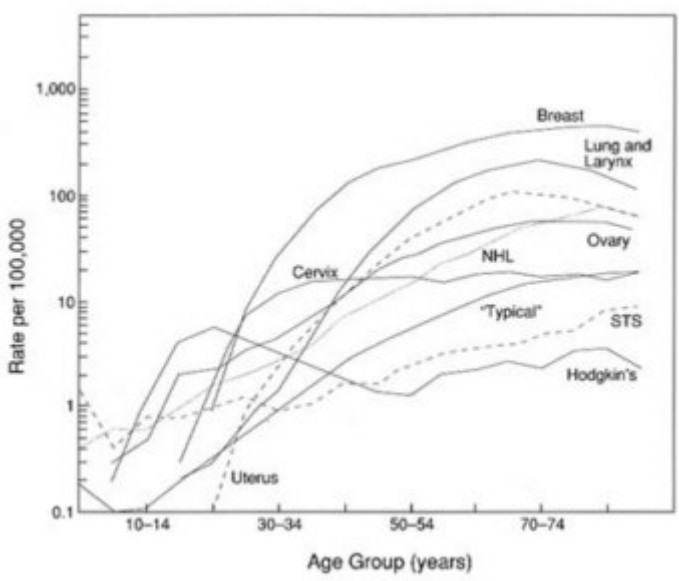

FIGURE 8-2 Average age-specific incidence rates for selected cancers in women for the period 1985-1989. The "typical" cancer illustrates a common pattern of age-specific rates seen in cancers not included in the figure; agespecific rates for all cancers combined have been rescaled (1:100) to construct the typical cancer. Rates are for 5 year age groups and are presented on a logarithmic scale. SOURCE: Miller et al., 1992.

EXPOSURE

It should be emphasized that for most of the studies reviewed for association between cancer outcomes and herbicide exposure, the actual exposure of each individual is in fact unknown. Some studies develop an index to approximate a scale of degrees of exposure; some studies use a surrogate measure of exposure, such as veterans service in Vietnam. The effect of this inadequate exposure measurement is a dilution of the statistical measure of the magnitude of the association. For example, if the odds ratio for a particular cancer and poor exposure measure to herbicides is 1.5, this risk

estimate is lower than what would be expected if good individual exposure data were available. The effects of misclassification of those who are exposed and unexposed are discussed in Chapter 6. Thus, studies with weak or no association should be considered in conjunction with the way in which exposure was measured.

PLAUSIBILITY DATA

Cancers of a variety of types have been identified in studies of animals exposed to TCDD, as described in detail in Chapter 4. These include liver, lung, and skin tumors in rats and mice. TCDD is not considered a genotoxic carcinogen, and in multistage models of carcinogenesis, TCDD acts as a tumor promoter and has little, if any, tumor-initiating activity. TCDD mediates carcinogenesis through a variety of biochemical effects that are dependent on the presence of a cellular receptor protein referred to as the Ah receptor. This receptor has been identified in both laboratory animals and humans, and appears to play a role in regulating cell proliferation and differentiation. The multiple site specificity of TCDD is likely to reflect its multiple mechanisms of action.

In contrast to TCDD, the experimental data supporting the carcinogenic activity of the herbicides used in Vietnam are considerably weaker. Only 2,4-D (2,4-dichlorophenoxyacetic acid) has produced positive results in an animal bioassay, and these are of controversial validity. The herbicides have not been adequately tested, however, so conclusions regarding their carcinogenicity in animals must be drawn with caution.

EXPECTED NUMBER OF CANCER CASES AMONG VIETNAM VETERANS

To provide some background for the consideration of cancer risks in Vietnam veterans, and to evaluate the possibilities for future epidemiologic studies of cancer in this group, the committee estimated the number of cancer cases that could be expected to occur in Vietnam veterans in the absence of any increase in risk due specifically to herbicide exposure, as follows. First, all Vietnam veterans were assumed to be born between 1946 and 1950, which corresponds to the largest five year age cohort. Second, the committee assumed that the most recent available national annual cancer incidence rates, those for 1985-1989 estimated by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program (Miller et al., 1992) would apply to Vietnam veterans. Further, if one assumes that 2,600,000 men and 7,000 women served in Vietnam (see Chapter 3), the expected number of specific cancers among Vietnam veterans in 1995 was estimated by applying the sex-specific SEER rates for ages 45-49

to male and female populations of the size given. SEER incidence rates for ages 50-54 were used to construct similar estimates for the year 2000. The incidence rates of most cancers increase with age, and some Vietnam veterans are older than the assumptions in this calculation, so the numbers given are perhaps underestimated. Also, 2.6 million is the minimum estimate of the number of Vietnam veterans. Furthermore, these calculations make no assumption of any higher risk among veterans because of exposure to herbicides in Vietnam or other aspects of the Vietnam experience. Because of these biases, the figures presented in the following section of this report should be interpreted as providing only order-of-magnitude estimates, not precise predictions for the Vietnam veteran cohort.

The results of the committee's calculations of the number of expected cancer cases in the Vietnam cohort are shown in Table 8-1. Based on rates in the general U.S. population, the expected numbers of new cancer cases per year in male veterans are relatively small. Overall, about 0.3 percent of male veterans and 0.4 percent of female veterans are expected to be diagnosed with cancer in 1995. In 2000, new cancers are expected in 0.5 percent of male and 0.6 percent of female veterans. The estimates range from as few as 21 cases for acute lymphocytic leukemia in 1995 to as many as 494 for non-Hodgkin's lymphoma. The exceptions to this pattern are cancers of the prostate, colon, and lung, for which the expected numbers of new cases among male veterans in 2000 are 855, 931, and 2,860, respectively. The number of cancers of each type expected among female veterans is very much smaller, owing to the smaller number of women (compared to men) who served in Vietnam; only for breast cancer is the expected number higher than ten new cases per year, and for many of the cancers under study, the expected number of new cases is less than one.

The estimates in Table 8-1, which are based on cancer incidence rates in the general population, show that each year, regardless of the effect that herbicides might have on cancer incidence, many veterans can be expected to be diagnosed with cancer. However, two factors make it difficult to conduct the epidemiologic studies needed to detect any *increased* risk for specific cancers that herbicide exposure might cause among veterans. First, only some of those who served in Vietnam were exposed to herbicides (see Chapter 6); therefore, any added risk for a specific cancer would best be studied in the smaller exposed population with a more limited number of cases. Second, some cancers are sufficiently rare that even among *all* Vietnam veterans there will be too few cases for reliable epidemiologic studies. This problem of inadequate numbers of cancer cases was also seen in the Centers for Disease Control's (CDC) Selected Cancers Study; even with a network of cancer registries that covered 10 percent of the U.S. male population, born between 1921 and 1953, the statistical power to detect an association between some of the cancers under study and Vietnam service was low.

TABLE 8-1 Number of Cancer Cases Expected Among Vietnam Veterans in 1995 and in 2000

	Males		Females	
Cancer Site	1995	2000	1995	2000
All Sites	6,689.8	12,126.4	28.6	39.6
Colon	460.2	930.8	1.1	2.4
Rectum	293.8	587.6	0.6	1.0
Pancreas	163.8	325.0	0.3	0.5
Stomach	192.4	340.6	0.3	0.4
Liver and intrahepatic bile duct	70.2	150.8	0.1	0.1
Lung, bronchus, and larynx	1,432.6	3,224.0	2.5	5.0
Bone	10.4	20.8	0.1	0.0
Soft tissue	65.0	85.8	0.1	0.2
Melanoma	486.2	631.8	1.1	1.3
Breast	23.4	26.0	13.2	15.5
Uterus	—	—	1.6	2.8
Cervix	—	—	1.2	1.2
Ovary	—	—	1.5	1.9
Kidney and renal pelvis	306.8	496.6	0.4	0.7
Bladder (invasive and in situ)	374.4	777.4	0.3	0.6
Prostate	179.4	855.4	—	—
Testis	117.0	85.8	—	—
Brain and other nervous system	226.2	267.8	0.4	0.4
Hodgkin's disease	93.6	109.2	0.1	0.1
Non-Hodgkin's lymphoma	379.6	494.0	0.5	0.7
Multiple myeloma	57.2	132.6	0.2	0.3
Leukemia	205.4	358.8	0.4	0.5
Acute lymphocytic	20.8	20.8	0.0	0.0
Chronic lymphocytic	44.2	122.2	0.1	0.1
Acute myeloid	41.6	83.2	0.1	0.2
Chronic myeloid	41.6	62.4	0.1	0.1

NOTES: Not applicable is designated as—. Estimates for breast cancer, cervical cancer, and melanoma do not include carcinoma in situ. Specific categories of cancer correspond to the following ICD-9 codes. All sites: 140-208; Liver and intrahepatic bile duct: 155.0-155.2; Lung, bronchus, and larynx: 161.0-161.9, 162.2-162.9; Soft tissue sarcoma: 171.0-171.9, 164.1; Breast: 174.0-174.9 (female), 175 (male); Uterus (corpus and not otherwise specified): 179, 182.0-182.1, 182.8; Cervix: 180.0-180.9; Ovary: 183.0; Hodgkin's disease: 201.0-201.9; Non-Hodgkin's lymphoma: 200.0-200.8, 202.0-202.2, 202.8-202.9; Multiple myeloma: 203.0, 203.2-203.8; Melanoma: 172.0-172.9; Bone and joint: 170.0-170.9; Colon: 153.0-153.9, 159.0; Rectum: 154.0-154.1; Pancreas: 157.0-157.9; Stomach: 151.0-151.9; Brain and other nervous system: 191.0-191.9, 192.0-192.3, 192.8-192.9; Kidney and renal pelvis: 189.0, 189.1; Bladder: 188.0-188.9; Prostate: 185; Testis: 186.0-186.9; Leukemia: 202.4, 203.1, 204.0-204.9, 205.0-205.9, 206.0-206.9, 207.0-207.2, 207.8, 208.0-208.9; Acute lymphocytic leukemia: 204.0; Chronic lymphocytic leukemia: 204.1; Acute myeloid leukemia: 205.0; Chronic myeloid leukemia: 205.1.

SOURCE: Calculated using data in Miller et al., 1992.

OVERALL CANCER

Background

The American Cancer Society estimated that 1,130,000 Americans were diagnosed with cancer in 1992, and approximately 520,000 people died from it (ACS, 1992). Overall, cancer mortality increased from 162 per 100,000 in 1973 to 173 per 100,000 in 1989 (Miller et al., 1992) (these rates are age-adjusted to the 1970 population). According to the committee's calculations, approximately 6,690 new cases of cancers of all types are expected among male Vietnam veterans in 1995 and approximately 29 among female veterans. In 2000, the expected numbers are approximately 12,126 new cases in male veterans and 40 in female veterans.

Doll and Peto (1981), in their now classic monograph, studied attributable risks for cancer (the proportion of all cancer cases that can be attributed to a particular cause). The most important overall risk factor was tobacco exposure, which was estimated to account for about 30 percent of all cancer incidence. This reflects the strong carcinogenic effect of tobacco smoke, the numerous anatomic sites that are affected, and the prevalence of smoking in the population. Diet, which represents a large number of different types of exposure, was found to have an impact of similar magnitude, although the confidence intervals on this risk estimate were extremely wide.

Occupational and environmental exposures to carcinogens, the closest analogues to Agent Orange exposure, were found by Doll and Peto to have a smaller attributable risk for cancer overall (i.e., only a small proportion of cancer of all sites combined could be attributed to these exposures). Nevertheless, these environmental agents may be highly carcinogenic. For instance, only a fraction of the overall population is exposed to asbestos, and only a fraction of the work force is exposed to a particular carcinogen such as benzene or polycyclic hydrocarbons. Nonetheless, individuals exposed to these chemicals may have a high risk of developing cancer. Therefore, identifying environmental and occupational carcinogenic exposures, and developing preventive measures for them, are of great public health importance.

Epidemiologists and cancer specialists generally do not study risk factors for all cancers combined. The causal associations and other characteristics for anatomically and histologically defined subtypes of cancer are so variable that general statements regarding "cancer" are of little use. The usefulness of studying overall cancer would be evident if a slight increase in risk occurred for many different cancers. The small relative increase for a given site might, however, not be detectable because of low statistical power resulting from the small number of cases. An apparent increase in risk of "all cancers" in a given study might actually stem from an increase

in a single type of cancer. Nonetheless, a variety of attempts at defining cancer risk among veterans exposed to Agent Orange, other herbicides, and TCDD have been reported and deserve description and analysis.

Epidemiologic Studies

Occupational Studies

Many studies have examined cancer mortality among a number of different occupational groups. One occupational group of interest for the association between cancer and exposures to herbicides is agricultural workers and farmers. A study of agricultural extension agents in the cooperative extension service of the U.S. Department of Agriculture (USDA) found no excess of overall cancer mortality (Alavanja et al., 1988). No excess overall cancer was observed in a study conducted among Danish gardeners (Hansen et al., 1992) or among herbicide applicators in Finland (Riihimaki et al., 1982, 1983). The overall proportionate mortality ratio (PMR) was decreased among Iowa farmers (Burmeister, 1981) and Swedish agricultural workers (Wiklund, 1983). A study among licensed herbicide applicators in the Netherlands (Swaen et al., 1992) found no significant increase in cancer mortality. A possible decrease in overall cancer mortality among farmers might be due to healthier life-styles overall, with decreased tobacco usage (Sterling and Weinkam, 1976), or to increased physical activity.

Other occupational groups potentially exposed to herbicides and dioxins are forestry and paper workers. No excess cancer mortality has been found in several studies of these workers conducted in Canada (Green, 1991), Finland (Jappinen and Pukkala, 1991), or the United States (Robinson et al., 1986; Henneberger et al., 1989). One study of 201 deceased white men who had been employed in pulp and paper production did find a statistically significant PMR of 1.3 [confidence interval (CI) 1.0-1.7] for all malignant neoplasms (Solet et al., 1989), which reflected an excess risk of lung cancer.

The National Institute for Occupational Safety and Health (NIOSH) study (Fingerhut et al., 1991) of workers in 12 plants in the United States that produced chemicals contaminated with TCDD found a standardized mortality ratio (SMR) of 1.2 for all cancers (CI 1.0-1.3). The SMR was higher in the subcohort with more than one year of exposure and more than 20 years latency (SMR = 1.5, CI 1.2-1.8). A study among German production workers exposed to TCDD (Manz et al., 1991) found an SMR for total cancer mortality of 1.2 (CI 1.0-1.5) compared to the total population, with increased cancer mortality among men having 20 or more years of employment. Saracci and coworkers (1991) found no overall increase in cancer mortality in their international study of workers exposed to phenoxy herbicides

and chlorophenols. Studies among workers involved in the production of 2-methyl-4-chlorophenoxyacetic acid (MCPA), phenoxy herbicides and chlorophenols in the United Kingdom found no excess overall cancer (Coggon et al., 1986, 1991). A study among MCPA workers in Denmark did not show an excess incidence of cancer (Lynge, 1985).

No excess cancer mortality has been found among workers exposed in a flavor and fragrance chemical plant (Thomas, 1987), or in highway maintenance (Bender et al., 1989). A study of Swedish railroad workers (Axelson and Sundell, 1974; Axelson et al., 1980) did find an excess tumor mortality, especially linked to amitrole and the category of all other herbicides.

Zober and colleagues (1990) looked at the 34 year mortality of 247 workers who were partially or heavily exposed to TCDD following an accident at a BASF plant in Germany. No consistent statistically significant overall excess in cancer mortality was observed. Following an accident in a trichlorophenol process plant at Monsanto that resulted in TCDD exposure, the SMR for malignant neoplasms was 1.0 (Zack and Suskind, 1980). After an electrical transformer fire in a Binghamton, New York, office building, no excess in cancer incidence was noted after four years among those potentially exposed to polychlorinated biphenyls (PCBs) and dioxins (Fitzgerald et al., 1989).

Environmental Studies

Other studies have looked at overall cancer mortality in people exposed to herbicides through environmental accidents or other environmental contamination of water and soil. The overall cancer mortality and incidence in the Seveso population has not been shown to be increased (Bertazzi et al., 1989a,b, 1992; Pesatori et al., 1992). In a Missouri community exposed to TCDD in sludge waste from a chemical production facility, the sample size was too small to evaluate overall cancer mortality (Hoffman et al., 1986). A study of a community in Southern Finland (Lampi et al., 1992) exposed to chlorophenols in contaminated drinking water also did not show an excess risk of overall cancer incidence.

Vietnam Veterans Studies

Many of the studies of Vietnam veterans have reported total cancer mortality. A follow-up study of 19,205 Australian Vietnam veterans found no excess of overall mortality or overall cancer mortality compared to 25,677 Vietnam era veterans who served in Australia (Fett et al., 1987a,b). There was no difference in mortality by period of service during the Vietnam conflict (Forcier et al., 1987). A similar study of U.S. Vietnam veterans, the CDC Vietnam Experience Study, also showed no excess overall cancer mortality

(Boyle et al., 1987; CDC, 1987a), nor have studies of the more highly exposed Ranch Hands (Michalek et al., 1990); however, the sample size for the Ranch Hand study was too small to evaluate even overall cancer outcomes.

Studies in Wisconsin (Anderson et al., 1986a,b) and Massachusetts (Kogan and Clapp, 1985) showed no excess of overall cancer mortality among white Vietnam veterans compared to Vietnam era veterans.

Studies conducted by the Department of Veterans Affairs (DVA; formerly the Veterans Administration), have also explored total cancer mortality. A study conducted among ground troop veterans (Breslin et al., 1988) found no excess of cancer in Vietnam veterans compared to 26,685 Vietnam era veterans who served in areas other than Southeast Asia; no excess overall cancer mortality was shown in Army I Corps veterans (Bullman et al., 1990). A study conducted by the DVA (Watanabe et al., 1991) found an elevated PMR for cancer overall for Army Vietnam veterans compared to U.S. men and for Marines compared to Vietnam era Marines. Among female veterans who served in Vietnam (Thomas et al., 1991), results showed no excess overall cancer mortality, although this study was fairly small to evaluate overall cancer.

Summary

Because every cancer has a unique set of multifactorial risk factors, epidemiologists focusing on etiology and prevention of cancer do not usually study mortality from all cancers as a single outcome. Such studies would be weak, and the results difficult to interpret. Two studies of TCDD-exposed workers (Fingerhut et al., 1991; Manz et al., 1991), however, did show significant excesses with consistent dose-response relationships, and these were the only worker studies that had relatively large, highly exposed cohorts to investigate. Studies conducted among Vietnam veterans themselves were, for the most part, negative with regard to overall cancer mortality. Exposure measures are diluted because individual measures of exposure are lacking, which results in misclassification of individuals; however, important increases among specific cancer types are certainly possible.

GASTROINTESTINAL TRACT TUMORS

Background

As a group, this category includes the major cancers in the United States as well as in the world. Within this group, the committee reviewed the data on colon cancer (ICD-9 153.0-153.9), rectal cancer (ICD-9 154.0-154.1), stomach cancer (ICD-9 151.0-151.9), and pancreatic cancer (ICD-9 157.0-157.9). According to the American Cancer Society, 208,700 new

cases of cancers of these types were diagnosed in the United States in 1992, and some 96,600 men and women died of these cancers (ACS, 1992). These cases are divided approximately equally between men and women. According to the committee's calculations, 1,110 cases of these cancers are expected among male Vietnam veterans and 2.3 among female veterans in 1995. In 2000, the expected numbers are 2,184 cases in male veterans and 4.3 in female veterans.

Although incidence and mortality have been declining for stomach cancer for many years, the incidence of colorectal cancer has increased and pancreatic cancer has shown only a slight decline. Because all of these cancers occur primarily at older ages, the Vietnam veteran cohort is, as a whole, too young to have yet moved into the high incidence period for these malignancies. Malignancies of the upper and lower gastrointestinal tracts have frequently been associated with dietary practices.

Colorectal cancer appears to develop from malignant transformation of benign adenomas that grow on the inner surface of the large bowel. Risk factors include family history of the disease, a history of inflammatory bowel disease, and diet (Page and Asire, 1985). High dietary intake of fats has been linked to increased risk of colon cancer, whereas high intake of dietary fiber is linked to decreased risk (Page and Asire, 1985; Prentice and Sheppard, 1990; Weisburger, 1991).

Most stomach cancers are ulcerated adenocarcinomas arising from the cells that produce gastric acid and digestive enzymes (Mayer and Garnick, 1986a). The causes of stomach cancer are not clearly understood, but large differences among countries in incidence of the disease suggest that environmental and life-style factors may be important. Studies have consistently shown that immigrants and their offspring tend to assume the stomach cancer risk of their host country (Nomura, 1982). Associations have also been reported with low socioeconomic status, radiation exposure, and intake of nitrites and related compounds (Nomura, 1982).

There are about 28,000 new pancreatic cancer cases per year with a very high mortality rate. It is a "silent" cancer with no signs or symptoms until it is in advanced stages. The majority of pancreatic cancers are adenocarcinomas. Many risk factors have been suggested but most have proven false on further study. No preventive measures are known and treatment is usually ineffective. Only 3 percent of patients survive for five years.

Epidemiologic Studies

Studies included in this section on the gastrointestinal (GI) tract specifically involve cancer outcomes in the colon, rectum, pancreas, and stomach; hepatobiliary cancers are considered later in this chapter. Esophageal cancer and small bowel cancers were not considered in this group because they have not been the target of most epidemiologic studies that the committee

reviewed. Where studies have focused on cancer in one of the organs included in the GI system, it is noted in the text discussion.

A case-control study was conducted among white male Iowans over age 30 who died of stomach cancer between 1964 and 1978 (Burmeister et al., 1983). A statistically elevated increased risk of stomach cancer was observed for farmers (OR = 1.3). The odds ratio remained significantly increased when the association between birth cohort and age at death was examined. Deaths before 1970 also showed an association (OR = 1.4) with stomach cancer.

A case-control study in Sweden (Hardell, 1981) looked at risk for colon cancer and found no excess risk for agricultural workers or others exposed to phenoxy herbicides. Likewise, a case-control study of colon cancer, following a PMR analysis (PMR = 1.5, CI 1.1-2.0) among forest and soil conservationists (Alavanja et al., 1989) found no elevated risk of colon cancer associated with being a forest (OR = 1.4, CI 0.7-2.8) or soil (OR = 1.2, CI 0.7-2.0) conservationist.

There were many studies that examined one or more gastrointestinal tract cancers where no consistent associations were found. These included studies of chemical production workers in the U.S. and other countries (Lynge, 1985; Coggon et al., 1986; Thomas, 1987; Bond et al., 1988; Zober et al., 1990; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991), agricultural workers (Burmeister, 1981; Wiklund, 1983; Hoar et al., 1986; Alavanja et al., 1988; Wigle et al., 1990; Hansen et al., 1992; Ronco et al., 1992), pesticide applicators (Axelson et al., 1980; Blair et al., 1983; Swaen et al., 1992), paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989; Solet et al., 1989), the Seveso population (Bertazzi et al., 1989a,b; Pesatori et al., 1992), other environmental exposure (Lampi et al., 1992), and Vietnam veterans (Kogan and Clapp, 1985; Lawrence et al., 1985; Anderson et al., 1986a,b; Boyle et al., 1987; Breslin et al., 1988).

Summary

Results for these cancers are summarized in Tables 8-2 through 8-5. The epidemiologic studies examining stomach cancer, pancreatic cancer, rectal cancer, and colon cancer were evenly distributed around the null. Estimated relative risks were usually near 1.0, and only the rare study in this group found a statistically significant elevated relative risk.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of no association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic

TABLE 8-2 Selected Epidemiologic Studies—Stomach Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Fingerhut et al., 1991	NIOSH cohort	10	1.0 (0.5-1.9)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0.0-3.7)
Manz et al., 1991	German production workers	12	1.2 (0.6-2.1)
Zober et al., 1990	BASF production workers—basic cohort	3	3.0 (0.8-11.8)
Coggon et al., 1986	British MCPA production workers	26	0.9 (0.6-1.3)
Lynge, 1985	Danish male production workers	12	1.3
Saracci et al., 1991	IARC cohort	40	0.9 (0.6-1.2)
Thomas, 1987	Flavor and fragrance chemical production workers		1.4
Burmeister, 1981	Farmers in Iowa	338	1.1 ($p < .01$)
Wiklund, 1983	Swedish agricultural workers	2,599	1.1 (1.0-1.2)[b]
Wigle et al., 1990	Canadian farmers	246	0.9 (0.8-1.0)
Ronco et al., 1992	Danish male self-employed farm workers	286	0.9
Alavanja et al., 1988	USDA agricultural extension agents	10	0.7 (0.4-1.4)
Alavanja et al., 1989	USDA forest/soil conservationists	9	0.7 (0.3-1.3)
Blair et al., 1983	Florida pesticide applicators	4	1.2
Swaen et al., 1992	Dutch herbicide applicators	1	0.5 (0-2.7)[c]
Axelson et al., 1980	Swedish railroad workers—total exposure	3	2.2
Henneberger et al., 1989	Paper and pulp workers	5	1.2 (0.4-2.8)
Robinson et al., 1986	Paper and pulp workers	17	1.2 (0.7-2.1)
Solet et al., 1989	Paper and pulp workers	1	0.5 (0.1-3.0)
Case-control studies			
Burmeister et al., 1983	Iowa residents—farming exposures		1.3 ($p < .05$)
Environmental			
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	40	0.8 (0.6-1.2)
	Female residents—zones A, B, R	22	1.0 (0.6-1.5)
Bertazzi et al., 1989b	Seveso male residents—zone B	7	1.2 (0.6-2.6)
Pesatori et al., 1992	Seveso male residents—zones A and B	7	0.9 (0.4-1.8)
	Female residents—zones A and B	3	0.8 (0.3-2.5)
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	88	1.1 (0.9-1.5)
	Marine Vietnam veterans	17	0.8 (0.4-1.6)
Anderson et al., 1986a	Wisconsin Vietnam veterans	3	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	1	—

[a]Given when available.
[b]99% CI.
[c]Risk estimate is for stomach and small intestine.

TABLE 8-3 Selected Epidemiologic Studies—Pancreatic Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Fingerhut et al., 1991	NIOSH cohort	10	0.8 (0.4-1.6)
Coggon et al., 1986	British MCPA production workers	9	0.7 (0.3-1.4)
Lynge, 1985	Danish male production workers	3	0.6
Ronco et al., 1992	Danish self-employed male farm workers	137	0.6 ($p < .05$)
Saracci et al., 1991	NIOSH cohort	26	1.1 (0.7-1.6)
Thomas, 1987	Flavor and fragrance chemical production workers		1.4
Burmeister, 1981	Farmers in Iowa	416	1.1
Wiklund, 1983	Swedish agricultural workers	777	0.8 (0.8-0.9)[b]
Alavanja et al., 1988	USDA agricultural extension agents	21	1.3 (0.8-1.9)
Alavanja et al., 1989	USDA forest conservationists		1.2 (0.4-3.4)
	USDA soil conservationists		1.1 (0.5-2.2)
Blair et al., 1983	Florida pesticide applicators	4	1.0
Swaen et al., 1992	Dutch herbicide applicators	3	2.2 (0.4-6.4)
Robinson et al., 1986	Paper and pulp workers	4	0.3 (0.1-1.1)
Henneberger et al., 1989	Paper and pulp workers	9	1.9 (0.9-3.6)
Solet et al., 1989	Paper and pulp workers	1	0.4 (0.0-2.1)
Environmental			
Bertazzi et al., 1989b	Seveso male residents—zone B	2	1.1 (0.3-4.5)
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	9	0.6 (0.3-1.2)
	Female residents—zones A, B, R	4	1.0 (0.3-2.7)
Pesatori et al., 1992	Seveso male residents—zones A and B	2	1.0 (0.3-4.2)
	Female residents—zones A and B	1	1.6 (0.2-12.0)
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	82	0.9 (0.6-1.2)
	Marine Vietnam veterans	18	1.6 (0.5-5.8)
Thomas et al., 1991	Women Vietnam veterans	5	2.7 (0.9-6.2)
Anderson et al., 1986a	Wisconsin Vietnam veterans	6	5.5 (2.8-10.9)
Anderson et al., 1986b	Wisconsin Vietnam veterans	4	—

[a]Given when available.
[b]99% CI.

TABLE 8-4 Selected Epidemiologic Studies—Colon Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Fingerhut et al., 1991	NIOSH cohort	25	1.2 (0.8-1.8)
Bond et al., 1988	Dow 2,4-D production workers	4	2.1 (0.6-5.4)
Manz et al., 1991	German production workers	8	0.9 (0.4-1.8)
Thiess et al., 1982	BASF production workers		0.4
Zober et al., 1990	BASF production workers—basic cohort	2	2.5 (0.4-14.1)[b]
Coggon et al., 1986	British MCPA production workers	19	1.0 (0.6-1.6)
Lynge, 1985	Danish production workers—men	10	1.0
Saracci et al., 1991	IARC cohort	41	1.1 (0.8-1.5)
Thomas, 1987	Flavor and fragrance chemical production workers		0.6
Burmeister, 1981	Farmers in Iowa	1,064	1.0 (NS)
Ronco et al., 1992	Danish male self-employed farm workers	277	0.7 ($p < .05$)
Wiklund, 1983	Swedish agricultural workers	1,332	0.8 (0.7-0.8)[c]
Alavanja et al., 1988	USDA agricultural extension agents		1.0 (0.7-1.5)
Alavanja et al., 1989	USDA forest conservationists		1.4 (0.7-2.8)
	USDA soil conservationists		1.2 (0.7-2.0)
Blair et al., 1983	Florida pesticide applicators	5	0.8
Swaen et al., 1992	Dutch herbicide applicators	4	2.6 (0.7-6.5)
Henneberger et al., 1989	Paper and pulp workers	9	1.0 (0.5-2.0)
Robinson et al., 1986	Paper and pulp workers	7	0.4 (0.2-0.9)
Solet et al., 1989	Paper and pulp workers	7	1.5 (0.6-3.0)
Case-control studies			
Hoar et al., 1986	Kansas residents		
	No herbicide use		1.6 (0.8-3.6)
	Herbicide use		1.5 (0.6-4.0)
Hardell, 1981	Residents of Sweden		
	Exposed to phenoxy acids	11	1.3 (0.6-2.8)
	Exposed to chlorophenols	6	1.8 (0.6-5.3)
Environmental			
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	20	1.0 (0.6-1.5)
	Female residents—zones A, B, R	12	0.7 (0.4-2.2)
Pesatori et al., 1992	Seveso male residents—zones A and B	3	0.6 (0.2-1.9)
	Female residents—zones A and B	3	0.7 (0.2-2.2)
Lampi et al., 1992	Finnish community exposed to chlorophenol contamination	9	1.1 (0.7-1.8)
Vietnam veterans			
Breslin et al. 1988	Army Vietnam veterans	209	1.0 (0.7-1.3)[d]
	Marine Vietnam veterans	33	1.3 (0.7-2.2)[d]
Anderson et al., 1986a	Wisconsin Vietnam veterans	4	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	6	1.0 (0.4-2.2)

[a]Given when available.
[b]Colon and rectal cancer results are combined in this study.
[c]99% CI.
[d]Intestinal and other GI cancer results are combined in this study.

acid; and picloram) and gastrointestinal cancers (stomach, pancreatic, rectal, and colon cancers).

TABLE 8-5 Selected Epidemiologic Studies—Rectal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Fingerhut et al., 1991	NIOSH cohort	5	0.9 (0.3-2.1)
Bond et al., 1988	Dow 2,4-D production workers	1	1.7 (0.0-9.3)
Coggon et al., 1986	British MCPA chemical workers	8	0.6 (0.3-1.2)
Lynge, 1985	Danish production workers—men	14	1.5
Saracci et al., 1991	IARC cohort	24	1.1 (0.7-1.6)
Thomas, 1987	Flavor and fragrance chemical production workers		2.5
Wiklund, 1983	Swedish agricultural workers	1,083	0.9 (0.9-1.0)[b]
Ronco et al., 1992	Danish male self-employed farmers	309	0.8 ($p < .05$)
Alavanja et al., 1988	USDA agricultural extension agents	5	0.6 (0.2-1.3)
Alavanja et al., 1989	USDA forest/soil conservationists	9	1.0 (0.5-1.9)
Blair et al., 1983	Florida pesticide applicators	2	1.0
Henneberger et al., 1989	Paper and pulp workers	1	0.4 (0-2.1)
Environmental			
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	10	1.0 (0.5-2.0)
	Female residents—zones A, B, R	7	1.2 (0.5-2.7)
Bertazzi et al., 1989b	Seveso male residents—zone B	2	1.7 (0.4-7.0)
Pesatori et al., 1992	Seveso male residents—zones A and B	3	1.2 (0.4-3.8)
	Female residents—zones A and B	2	1.2 (0.3-4.7)
Vietnam veterans			
Anderson et al., 1986a	Wisconsin Vietnam veterans	1	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	1	—

[a]Given when available.
[b]99% CI.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

HEPATOBILIARY CANCERS

Background

According to the American Cancer Society, 15,400 new cases of hepatobiliary cancer (ICD-9 155.0-155.2) were diagnosed in the United States in 1992, and some 12,300 men and women died of cancer of the liver and the biliary passages (ACS, 1992). Similar numbers of cases are seen in men and women. According to the committee's calculations, 70 cases of cancers of the liver and the biliary passages are expected among male Vietnam veterans and 0.1 among female veterans in 1995, and 151 in male veterans and 0.1 in female veterans in 2000.

In the United States, liver cancers account for only about 1.4 percent of new cancer cases and 2.4 percent of cancer deaths. Misclassification of metastatic cancers as primary liver cancer can, however, lead to over-reporting of deaths due to liver cancer (Percy et al., 1990a). In developing countries, especially sub-Saharan Africa and Southeast Asia, liver cancers are common and are among the leading causes of death.

About 90 percent of primary liver cancers are hepatocellular carcinomas; tumors of the intrahepatic bile ducts (cholangiocarcinomas) represent approximately 7 percent of malignant tumors of the liver (Mayer and Garnick, 1986b). Each is a separate histological appearance of differentiated cells derived from a common progenitor in the early embryo derived from the foregut epithelium. As such, both forms have many of the same characteristics and can be rationally grouped together for epidemiologic studies. Often an individual tumor will have areas that resemble both bile duct and hepatic cells. Other liver malignancies, such as angiosarcomas, are extremely rare.

The plausibility of an association of TCDD with liver malignancy follows from the finding of increased risk of liver cancer or liver and biliary cancer among individuals exposed to similar compounds that also act through the Ah locus. Kuratsune and colleagues (1986) found a substantial increase

of liver cancer deaths among Yusho patients exposed to dibenzofurans. Nine deaths from liver cancer were observed among males and two among females. The expected numbers of deaths, based on rates of the Fukuoka and Nagasaki prefectures, were 2.3 and 0.8, respectively. A review by Nicholson (1987) of all data on the mortality of capacitor manufacturing workers exposed to PCBs showed 7 deaths to have occurred from cancer of the liver, biliary passages, and gallbladder, compared with 2.54 expected. Thus there is evidence that very high exposures to other compounds that interact with the Ah receptor increase hepatobiliary cancer risk.

Epidemiologists have established hepatitis B virus (HBV) infection as a major risk factor for primary liver cancer (Beasley and Hwang, 1984). HBV is endemic in the regions where liver cancer is most common but is also a factor in Western countries. Recent evidence also links primary liver cancer to the hepatitis C virus (Yu et al., 1990). Alcohol consumption, with or without cirrhosis, appears to be a principal risk factor for liver cancer in Western countries (Yu et al., 1991). Other risk factors include disease-induced cirrhosis (Mayer and Garnick, 1986b), oral contraceptives (Palmer et al., 1989), and smoking. Primary liver cancer has also been linked to exposure to aflatoxin (a toxin contaminating poorly stored peanuts) (Yeh et al., 1989). Cancer of the intrahepatic bile duct has been attributed to liver flukes (*Clonorchis* and *Opisthorchis*), which are ingested by humans through uncooked fish and then reside primarily in the intrahepatic bile duct where they cause chronic damage (Belamaric, 1973). Hepatic angiosarcomas have been associated with exposure to arsenicals, thorotrast, and vinyl chloride (Greenwald and Greenwald, 1983). Animal experiments have shown aflatoxin to be a potent liver carcinogen, but its role in human hepatic carcinoma with or without concurrent HBV infection remains to be defined.

Epidemiologic Studies

Occupational Studies

Production Workers In combined data on production workers at 12 plants in the United States that produced chemicals contaminated with TCDD, reported by Fingerhut and colleagues (1991), six deaths were observed due to cancer of the liver and biliary tract, and the SMR was 1.2 (CI 0.4-2.5). When the exposure was limited to those who had more than 20 years of latency, only 1 death was observed due to liver cancer, somewhat lower than expected (1.7) giving an SMR of 0.6 (CI 0.01-3.3).

In the other combined cohort of workers exposed to herbicides, Saracci and colleagues (1991) identified four deaths due to liver cancer among those who were exposed, giving an SMR of 0.4 (CI 0.1-1.1). These reduced rates of liver cancer may reflect the contribution of the healthy worker

effect to the onset of this cancer; chance or differences in life-styles may also explain the results.

In the study of workers from Denmark involved in the manufacture of phenoxy herbicides, Lynge (1985) observed three cases of liver cancer among men, and none in women. Given that the expected number of cases was 3.1, the relative risk of liver cancer among men was 1.0, showing no elevation. Zack and Suskind (1980) studied 121 plant workers who developed chloracne following an accident; no cases of liver cancer were observed, 0.2 case was expected.

Agricultural/Forestry Workers A study of farmers in Denmark and Italy by Ronco and colleagues (1992) observed no evidence of increased liver cancer in the cohort from Denmark; the group included in this study from Italy was too small to be informative. Among the self-employed Danish men, 23 were diagnosed with liver cancer, giving a relative risk estimate of 0.4, compared to the Danish population. Among Danish men classified as farm employees, nine liver cancers were observed, giving a relative risk estimate of 0.8. Among self-employed women and employees, no cases of liver cancer were observed, but among those women classified as family workers (i.e., those who were actively involved in the work of the farm owned by their husbands), five cases of liver cancer were observed, giving an estimated relative risk of 0.5. In the study by Wiklund (1983), 103 cases of liver cancer were observed among agricultural workers. This was significantly lower than the expected number (306) giving an incidence ratio of 0.3 (99% CI 0.3-0.4).

In a case-control study in Sweden, Hardell and colleagues (1984) observed a positive relationship between exposure to phenoxy or dichlorophenoxy herbicides and risk of liver cancer. Based on 102 cases, these authors observed an odds ratio of 1.8 (CI 0.9-4.0).

Paper/Pulp Workers In a study of mortality among pulp and paper workers, Solet and colleagues (1989) observed two deaths due to liver cancer when one was expected. Based on this small number of cases the confidence interval was broad, ranging from 0.2 to 7.3 (PMR = 2.0).

Environmental Studies

Follow-up of the population involved in the Seveso incident (Bertazzi et al., 1989b) showed that during 10 years, only three deaths among males due to liver cancer occurred in the population of zone B, no higher than expected. Among those in zone R, only seven deaths due to liver cancer were recorded, giving a mortality ratio of 0.4 (CI 0.2-0.8). Additional data for this population-based on incident cases of liver cancer include these

same cases and show similar results (Pesatori et al., 1992). Data from U.S. populations living in contaminated areas do not add any useful information. The residents of the Quail Run trailer park were free from diagnosed liver cancer, whereas 1.5 cases were expected (Hoffman et al., 1986; Stehr-Green et al., 1987).

Vietnam Veterans Studies

Studies of liver cancer among veterans are also hampered by small study size. For example, in the study of Wisconsin Vietnam veterans (Anderson et al., 1986a,b), no men were observed to have died from liver cancer. In the mortality component of the Vietnam Experience Study (VES; Boyle et al., 1987) only one death from liver cancer among Vietnam veterans was observed. In the larger mortality study among U.S. Army and Marine Corps Vietnam veterans, Breslin and colleagues (1988) identified 34 liver cancer deaths among the Army veterans; the PMR was 1.0 (CI 0.8-1.4). With fewer deaths, the data from the Marines are consistent with this result. Based on six deaths from cancer of the liver or bile ducts, the PMR was 1.2 (CI 0.5-2.8).

The Selected Cancers Study (CDC, 1990c) included a pathologic review of studies to confirm the diagnosis of 130 men with primary liver cancer. Only 6 percent (N = 8) of the men with primary liver cancer served in Vietnam, compared to 7.5 percent of the control subjects. After adjusting for design and a range of established risk factors, the relative risk (RR) was 1.2 (CI 0.5-2.7). Of the eight Vietnam veterans with primary liver cancer, four were in the Navy and three were in the Army (for one, the proxy respondent did not know the branch of service). The risk for Vietnam veterans was slightly lower than for men who served elsewhere in the military.

Summary

There are relatively few occupational, environmental, or veterans studies of liver cancer (Table 8-6), and most of these are small in size and have not controlled for life-style-related risk factors. One of the largest studies (Hardell et al., 1984) indicates an increased risk for liver cancer and exposure to herbicides, but another study of Swedish agricultural workers (Wiklund, 1983) estimates a relative risk that is significantly less than 1.0. The estimated relative risks from other studies are both positive and negative. As a whole, given the methodological difficulties associated with most of the few existing studies, the evidence regarding liver cancer is not convincing with regard to either an association with herbicides/TCDD or the lack of an association.

TABLE 8-6 Selected Epidemiologic Studies—Hepatobiliary Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Zack and Suskind, 1980	Monsanto production workers	0	—
Bond et al., 1988	Dow 2,4-D production workers		1.2
Fingerhut et al., 1991	NIOSH cohort	6	1.2 (0.4-2.5)
	20 years latency	1	0.6 (0.01-3.3)
Lynge, 1985	Danish production workers	3	1.0
Saracci et al., 1991	IARC cohort	4	0.4 (0.1-1.1)
Wiklund, 1983	Swedish agricultural workers	103	0.3 (0.3-0.4)[b]
Ronco et al., 1992	Danish and Italian farm workers		
	Danish male self-employed farmers	23	0.4
	Employees of Danish farmers	9	0.8
	Female family workers	5	0.5
Solet et al., 1989	Paper and pulp workers	2	2.0 (0.2-7.3)
Case-control studies			
Hardell et al., 1984	Male residents of northern Sweden	102	1.8 (0.9-4.0)
Environmental			
Bertazzi et al., 1989b	Seveso male residents—zone B	3	1.2 (0.4-3.8)
	Male zone R residents	7	0.4 (0.2-0.8)
Pesatori et al., 1992	Seveso male residents—zones A and B	4	1.5 (0.5-4.0)
	Female residents—zones A and B	1	1.2 (0.2-9.1)
Stehr et al., 1986	Missouri residents	0	—
Hoffman et al., 1986	Residents of Quail Run Mobile Home Park	0	—
Vietnam veterans			
Cohort studies			
Breslin et al., 1988	Army Vietnam veterans	34	1.0 (0.8-1.4)
	Marine Vietnam veterans	6	1.2 (0.5-2.8)
Anderson et al., 1986a,b	Wisconsin Vietnam veterans	0	—
Case-control studies			
CDC, 1990	U.S. men born between 1921 and 1953	8	1.2 (0.5-2.7)

[a]Given when available.
[b]99% CI.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and hepatobiliary cancer.

Biologic Plausibility

When laboratory animals are administered TCDD, it interacts with an intracellular protein called the Ah receptor. Interaction between TCDD and the Ah receptor appears to play a role in susceptibility to carcinogenesis among laboratory animals. Humans also have intracellular proteins that have been identified as Ah receptors, so it is plausible that interactions between TCDD and Ah receptors could play a role in human health effects.

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites, especially the liver. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD. More than 120 chemicals have been identified as liver carcinogens in laboratory rodents.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

NASAL/NASOPHARYNGEAL CANCER

Background

Nasal and nasopharyngeal cancers (ICD-9 147.0-147.9, 160.0-160.9) can develop from any of the cell types present in any of these organs. The

epithelium of the nasal and nasopharyngeal cavities is partly squamous, partly columnar and ciliated pseudostratified columnar. Precise distribution is variable. Also, there are serous and mucous glands and lymphoid aggregates in close association with the epithelium. Several types of adenomas may develop in the nasal cavity ("nasal polyps" papillomas). Squamous cell carcinomas may develop in dysplastic epithelium at any surface site and are the most common type. They tend to spread locally, eroding into adjacent structures (orbit, cranial cavity, oral cavity) and may metastasize to cervical lymph nodes. Malignant mesenchymal tumors, especially rhabdomyosarcomas, are relatively frequent in this region and are derived from underlying connective tissues. Nasopharyngeal cancers occur in three histological variants: keratinizing squamous cell, nonkeratinizing squamous cell, and undifferentiated. Also, sarcomas and lymphomas (both Hodgkin's and non-Hodgkin's) are frequently seen in this region.

Surgery, radiation, and chemotherapy are used individually or in combination for treatment of these neoplasms. Because of the proximity of vital anatomic structures, success of treatment is limited unless the tumor is diagnosed early in the evolution of the tumor cells.

Associations have been found between nasal cancers and occupational exposure to nickel (Doll et al., 1977) and to chromates (Higginson and Muir, 1973). Exposure to wood dust is also a risk factor for nasal cancer (Anderson et al., 1977); smoking (Elwood, 1981) or exposure to formaldehyde (Luce et al., 1993) may increase the risk associated with wood dust. There is also evidence that leather workers have an increased risk for nasal cancers (Luce et al., 1993). A study in Shanghai, China, demonstrated an association between chronic nasal diseases and consumption of salt-preserved foods (Zheng et al., 1992b).

Although nasopharyngeal cancers are relatively uncommon, higher incidence is seen in southern China and Southeast Asia. Even among Chinese living in the United States, rates are higher than for whites or blacks (Burt et al., 1992). Dietary factors, including consumption of salt-preserved foods containing nitrosamines, appear to contribute to increased risk (Ablashi, 1978). A study in Shanghai of occupational risk factors found excess risks for workers in a variety of settings including textile weaving, baking, and metal smelting, forging, and grinding (Zheng et al., 1992a). Nasopharyngeal cancer has also been associated with the Epstein-Barr virus, but the role of the virus is not yet clear (Henle and Henle, 1981). A genetic risk has been suggested as well (Gajwani et al., 1980).

Incidence of nasopharyngeal cancer in the United States is highest among the Chinese population and lowest among whites (Burt et al., 1992). Rates are generally twice as high in men as in women. Incidence remained stable between 1973 and 1986, but survival appears to have improved (Burt et al.,

1992). Age, sex, and histologic type of the tumor each independently influence survival.

Epidemiologic Studies

The study by Saracci and colleagues (1991) of production workers and sprayers showed a relative risk of 2.9 for these cancers based on three cases. In addition, the study of MCPA chemical workers (Coggon et al., 1986) also showed an elevated risk of 4.9, based on the same three cases. A case-control study by Hardell and colleagues (1982) found an OR = 2.1 (CI 0.9-4.7) for those exposed to phenoxy acids, based on eight exposed cases. In the CDC Selected Cancers Study of Vietnam veterans (CDC, 1990c), there were 48 cases of nasal cancer and 80 cases of nasopharyngeal cancer with 2 and 3, respectively, having service in Vietnam. No significant associations for Vietnam service and these cancers were found.

Other studies showing inconclusive results included studies of agricultural workers (Wiklund, 1983; Ronco et al., 1992) and paper and pulp workers (Robinson et al., 1986). Results are summarized in Table 8-7.

TABLE 8-7 Selected Epidemiologic Studies—Nasal/Nasopharyngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Coggon et al., 1986	British MCPA production workers	3	4.9 (1.0-14.4)
Saracci et al., 1991	IARC cohort	3	2.9 (0.6-8.5)
Wiklund, 1983	Swedish agricultural workers		0.8 (0.6-1.2)
Ronco et al., 1992	Danish and Italian farm workers		0.6 (NS)
Robinson et al., 1986	Paper and pulp workers	0	—
Case-control studies			
Hardell et al., 1982	Residents of northern Sweden		
	Phenoxy acid exposure	8	2.1 (0.9-4.7)
	Chlorophenol exposure	9	6.7 (2.8-16.2)
Vietnam veterans			
CDC, 1990	U.S. men born between 1921 and 1953 Vietnam veterans	2	0.7 (0.1-3.0)

NOTE: NS = not significant.

[a] Given when available.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and nasal/nasopharyngeal cancer.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. Pharmacokinetic studies indicate that TCDD accumulates in the nasopharyngeal area of animals. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

RESPIRATORY CANCERS

Background

Carcinomas of the lung and bronchus (ICD-9 162.2-162.9) are now the leading causes of cancer death in the United States. According to the American Cancer Society, 168,000 new cases were diagnosed in the United States in 1992, and some 146,000 men and women died from respiratory cancers (ACS, 1992). Substantially more men (102,000) than women (66,000) were diagnosed with these cancers. According to the committee's calculations, 1,266 cases of cancer of the lung and bronchus are expected to be diagnosed among male Vietnam veterans and 2.3 among female veterans in

1995. For the year 2000, the expected numbers are 2,860 cases in male veterans and 4.6 in female veterans. The committee's calculations indicate that 166 cases of cancer of the larynx (ICD-9 161.0-161.9) are expected to be diagnosed among male Vietnam veterans and 0.1 among female veterans in 1995. For the year 2000, the expected numbers are 364 cases of cancer of the larynx in male veterans and 0.3 in female veterans.

The incidence and mortality rates for lung cancers have increased markedly during the last half century, reflecting the earlier patterns of adoption and continuation of smoking in the population. Decreases in recent years in the prevalence of smoking among men are now leading to small reductions in the incidence of lung cancer and will result in reductions in mortality. Incidence and mortality rates for women began increasing more recently than those for men. In 1987, women's lung cancer deaths exceeded those for breast cancer for the first time (ACS, 1992). For men and women, the incidence of lung cancer increases rapidly beginning at about age 40.

The principal types of lung neoplasms are identified collectively as bronchogenic carcinoma or carcinoma of the lung. Of these, squamous cell carcinoma accounts for 50-70 percent of lung tumors, adenocarcinoma for 10-25 percent, small-cell (oat cell) carcinomas for about 5 percent, and large-cell carcinomas for about 5 percent (McGee et al., 1992). Often a neoplasm may be made up of mixtures of these cell types. These different types of lung tumors are often combined in epidemiologic studies for several reasons: (1) there are frequently mixed patterns of a variety of different cell types; (2) there is abundant evidence that these tumors arise from a common stem cell that differentiates along one or more of these pathways; and (3) they often arise in a similar location near the hilum of the lung in the first- or second-order bronchi.

Cigarette smoking is the major risk factor for lung cancer, estimated by the American Cancer Society (1992) to be responsible for about 87 percent of lung cancer deaths in the United States. The risk increases with length of time and number of cigarettes smoked (U.S. DHHS, 1987). Tobacco smoke may include both tumor initiators and promoters. Other important epidemiologically identified risk factors include exposure to radon, arsenic, asbestos, chromium, nickel, and aromatic hydrocarbons. Asbestos and radon interact with cigarette smoking, increasing the risk of lung cancer beyond that predicted from the sum of the individual risks (ACS, 1992).

Epidemiologic Studies

Occupational Studies

Production Workers In a study of Dow Chemical Company workers involved in the production of 2,4-D (Bond et al., 1988), the SMR for lung

cancer was 1.0 (8 observed versus 7.7 expected deaths, CI 0.5-2.0). When other Dow workers are used as a comparison group, the effect estimate is slightly higher, as one might expect from a more comparable, relatively healthy comparison group (i.e., less bias from the healthy worker effect). The SMR for all respiratory cancers (lung alone is not given) is 1.2 (9 observed versus 7.4 expected deaths, CI 0.6-2.3). The authors estimated lifetime cumulative exposure to 2,4-D, and when the cohort was divided into three groups with 15 year exposure lag with respect to this estimate, the SMRs were low exposure, 0.7; medium exposure, 1.0; high exposure, 1.7. These subgroup SMRs were based on one, two, and five deaths, respectively; a test of the null hypothesis that there is no trend evidenced in these data has a *p*-value of .1.

Lung cancer mortality in a cohort employed in the production and spraying of MCPA and other phenoxy herbicides (Coggon et al., 1986) was close to that expected, with the national comparison population yielding a slight deficit in risk and the rural comparison a slight excess in risk (with national comparison: SMR = 0.9, CI 0.8-1.1; with rural comparison: SMR = 1.2, CI 1.0-1.4).

When the cohort was subdivided by estimated level of exposure to phenoxy acids, weak evidence of an increase in risk with increase in exposure was observed [background exposure: SMR = 1.0 (CI 0.7-1.4); low exposure: SMR = 1.1 (CI 0.8-1.6); high exposure: SMR = 1.3 (CI 1.0-1.8)]. These figures, based on the rural comparison population, may suffer from the problem of noncomparability of indirectly standardized rates, but the authors do not provide the data with which to perform the more appropriate internal analysis. Nevertheless, because these three categories are distinguished on "grade" or intensity of exposure and not exposure duration, they most likely do not differ dramatically in underlying age distribution, so comparisons of the three SMRs are probably appropriate. When the cohort was subdivided by duration of potential exposure into three categories, less than one month, one to six months, and more than six months, the first of these groups contained only seven lung cancer deaths, and unstable risk estimates. When the 8 1 month and 1-6 month groups are combined into "short" duration, SMR = 1.2 (CI 0.8-1.6); for the "long" duration (> 6 months), SMR = 1.3, (CI 1.0-1.7). The study included workers employed over a 29 year period, but maximum length of employment of individual workers was not reported. Note however that employment for more than six months does not necessarily imply substantial exposure and that this comparison may be affected by noncomparability of underlying age distributions, as just discussed.

Using very similar methods, Coggon and colleagues (1991) have recently reported on the mortality experience of the employees of four different British factories where phenoxy herbicides and other chemicals were

manufactured. When compared to either national or rural population, there was a slight excess of lung cancer mortality (SMR = 1.3, CI 0.8-2.1 with national rates; rural rates yield nearly identical results). However, when the analysis was restricted to those with any exposure above "background" levels, the risk dropped slightly (SMR = 1.2, CI 0.7-2.1), which is the opposite of what one would expect were this a true association because it would be expected that the overall association would be diluted by those with low exposure in the background group.

Among Danish phenoxy herbicide manufacturing workers (Lynge, 1985) the lung cancer incidence of the entire work force was about that expected (SMR for males = 1.2 based on 38 observed cases, SMR for females = 2.2 based on only 6 observed cases). However, when the cohort was restricted to those actually engaged in the manufacturing or packaging of phenoxy herbicides the risk in men increased (SMR = 2.1, CI 1.0-3.7, based on 11 observed cases). There was only one female lung cancer case in these areas of the plant. These results were obtained without application of a latency period, but the authors report that the results were the same when a 10 year latency period was used.

Lynge reports that the excess lung cancer risk was present in both plants studied and that the workers were generally recruited from the countryside where tobacco consumption was lower than the national average in the 1950s. No direct information on the smoking habits of the cohort was available, however. A review of the other occupational information for the lung cancer cases did not identify any known risk factors likely to explain the observed excess.

In a retrospective cohort mortality study of a population of chemical workers potentially exposed to TCDD in the production of hexachlorophene at a flavor and fragrance plant (Thomas, 1987) the SMR for lung cancer in white males was 1.2 (29 observed versus 25.1 expected deaths, CI 0.8-1.7). Because of the complex exposures of this cohort and the likelihood that only a small unidentifiable fraction was exposed to TCDD, these results are of very limited usefulness in evaluating the associations under consideration.

After an industrial accident involving the release of TCDD (Zober et al., 1990), 78 deaths were observed in a 34 year period. With the small number of total deaths, the results concerning lung cancer (six deaths due to trachea, bronchus, or lung cancer) are inconclusive. In the most heavily exposed subgroup, there were 4 deaths from lung cancer, compared to 2.0 expected from national mortality rates (SMR = 2.0, CI 0.6-5.2). Among those with chloracne, lung cancer appeared somewhat elevated, although the sample size (3,589 person-years) precludes a precise estimate of this effect. Among those with chloracne, there were 6 deaths from lung cancer and 3.3 expected (SMR = 1.8 CI 0.7-4.0).

The workers in a Hamburg, Germany, herbicide production facility heavily contaminated with TCDD were studied by Manz and colleagues (1991) in the first few years of its operation. The risk estimate for lung cancer was elevated compared to gas company workers (SMR = 1.7 based on 26 observed and 15.6 expected deaths, CI 1.1-2.4). Two comparison groups were available for this study, the general population and a cohort of gas workers previously studied by the authors. The gas worker comparison group yielded a somewhat higher risk estimate (shown above) probably because of the healthy worker effect. Smoking data were not available for all subjects, but in a subsample of 361 workers, 73 percent were self-reported smokers, compared to 76 percent of 2,860 gas workers who reported smoking. It is thus unlikely that smoking differentials could explain the observed excess of lung cancer.

Saracci and colleagues (1991) have reported on the mortality experience of a large international cohort, including both production workers and herbicide sprayers. The degree of exposure to TCDD is more uncertain than that of the large U.S. study by Fingerhut and colleagues (1991) described below, in that some of the cohorts included in the Saracci study are of individuals either spraying or producing compounds, such as 2,4-D, MCPA, or 2-(4-chloro-2-methylphenoxy) propanoic acid (MCPP), which are unlikely to contain significant quantities of TCDD. Mortality from cancer of the respiratory tract was normal, based on 173 observed deaths (SMR = 1.0, CI 0.9-1.2).

The workers in two British herbicide production plants were not included in the above calculations because job history information was not available. Nevertheless the authors are confident that the majority of the subjects in these two plants were indeed exposed to phenoxy herbicides to some degree. An excess lung cancer mortality risk was observed (SMR = 2.2, CI 1.1-4.0, based on 11 observed cases). No smoking information is available (Saracci et al., 1991).

A cohort of production workers in the Netherlands (Bueno de Mesquita et al., 1993) showed no excess lung cancer deaths. Where results from two factories were combined, factory A, where 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was produced, was the only source of the lung cancers that were reported.

In the NIOSH cohort (Fingerhut et al., 1991) of TCDD-exposed workers, an elevated risk of lung cancer was observed in those with more than one year of exposure (SMR = 1.3, CI 1.0-1.7, based on 59 observed deaths). An analysis of mortality according to duration of exposure in processes involving TCDD contamination shows increasing standardized rate ratios (SRRs) with increasing duration of exposure for cancer of the trachea, bronchus, and lung (<1 year: SRR = 1.0; 1-5 years: SRR = 1.1; 5-15 years: SRR = 1.7; > 15 years: SRR = 1.4; test for trend, $p = .2$). The increased risk for

cancer of the lung is unlikely to be the result of excess cigarette smoking in the cohort. Workers from two of the plants were interviewed in 1987 and their smoking histories ascertained.

Summary of Production Worker Studies The studies of Coggon and Lynge are largely subsumed under the European registry of Saracci and colleagues, and so should not be viewed as independent measurements of effect. Note, however, that certain findings in these two studies that are suggestive of an association with lung cancer are lost when the combined cohort of Saracci is presented. In the Coggon study (1986), weak evidence of a trend of increasing risk with increasing category of exposure was observed (see above). In the study by Lynge (1985) of Danish herbicide production workers, an elevated risk of lung cancer is observed, and this elevation is consistent over the two plants studied. In addition, the rural population from which the work force was derived would be expected to have lower than average smoking rates, thus indirectly reducing the likelihood that the lung cancer excess could be explained by smoking.

One might derive a pooled estimate of the lung cancer risk among production workers from the following studies (Table 8-8): Bond et al. (1988), Zober et al. (1990), Manz et al. (1991), Saracci et al. (1991), and Fingerhut et al. (1991). Of the six cohorts in these studies, there are three in which the committee is fairly confident that there was a substantial level of exposure to TCDD: those of Zober et al., (1990), Manz et al. (1991), and the high exposure group of Fingerhut et al. (1991). When just those three studies are combined, the pooled SMR is somewhat elevated: 1.4 (CI 1.2-1.8).

Because many of the workers smoked and were exposed to other chemicals it is not possible to rule out alternative explanations for this small excess risk. It is unlikely, however, that smoking explains the entire effect, since

TABLE 8-8 Selected Epidemiologic Studies of Production Workers—Lung Cancer

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Bond et al., 1988	Dow 2,4-D production workers	8	1.0 (0.5-2.0)
Zober et al., 1990	BASF production workers	4	2.0 (0.6-5.2)
Manz et al., 1991	German production workers	26	1.7 (1.1-2.4)
Saracci et al., 1991	IARC cohort	173	1.0 (0.9-1.2)
	Probably exposed	11	2.2 (1.1-4.0)
Fingerhut et al., 1991	NIOSH cohort		
	Exposed > 1 year	59	1.3 (1.0-1.7)

the studies of Fingerhut and Manz both found that smoking rates were only slightly different in samples of their study populations than in the comparison populations. Chemical production workers are often exposed to asbestos, which until recently was widely used wherever an industrial process involved high-temperature. This well-known lung carcinogen might confound the observed association with herbicide and TCDD exposure in many of these studies. But it is also unlikely that asbestos could fully explain these findings because the lung cancer risk from asbestos among chemical workers in general (as distinct from those whose occupations brought them into frequent and direct contact with the substance) is not elevated (Wong and Raabe, 1989). Thus, although tobacco and asbestos cannot be ruled out, the more likely explanation for the observed elevations in risk is one or more agents associated with the production of phenoxy herbicides and related compounds.

Agricultural/Forestry Workers Studies that compare the lung cancer experience of farmers as a group to that of other occupations or the general population show a consistent *deficit* of lung cancer among farmers. For example, studies by Burmeister (1981) and by Wigle and colleagues (1990) in North America, and by Wiklund (1983) in Sweden, all provide strong evidence for a reduced risk of lung or respiratory cancer in farmers. A cohort study of Danish gardeners (Hansen et al., 1992) observed neither a deficit nor an excess of lung cancer.

Several authors have attributed the deficit in lung cancer among men to decreased smoking among farmers, and there is evidence to support this supposition, at least in the United States (Sterling and Weinkam, 1976) and Sweden (Rylander, 1990). Another causal hypothesis that has been proposed is that farmers are exposed to high levels of bacterial endotoxins in a wide variety of organic dusts (Rylander, 1990). These biologically active compounds have been shown to retard cancer growth in laboratory animals and have been proposed as anticancer drugs (Engelhardt et al., 1991).

Several studies of cohorts whose members were engaged in agriculture-related activities have examined lung cancer risk, but the connection to herbicides is tenuous and does not add to the evidence of an association (Alavanja et al., 1988, 1989).

Herbicide Pesticide Applicators Studies of herbicide and pesticide applicators are more relevant than those just discussed because it can be presumed that applicators had more sustained exposures to herbicides, and the types of pesticides and durations of exposure can often be quantified generally. There are several weaknesses in many of these studies, however, including the lack of individual estimates of exposure in most studies, the fact that many different kinds of pesticides were often used, and the limited sample size.

Axelson and Sundell (1974) conducted a cohort study of railroad right-of-way herbicide sprayers in Sweden, initially covering the period 1957-1972, and then extended until 1978 (Axelson et al., 1980). Based on follow-up through 1978, the results for lung cancer were ambiguous because of the very small numbers of both observed and expected cancers; for example, the SMR for lung cancer was 1.4 (CI 0.3-4.0), based on three observed cases for all types of exposure.

A cohort of Finnish workers who sprayed the herbicides 2,4-D and 2,4,5-T was followed by Riihimaki and colleagues (1982). Good employment records were available, and follow-up through 1980 was nearly complete. Additional strengths of this study include the apparent lack of confounding by other chemical exposures (although the authors do not explore what the cohort members did when not spraying) and the relatively high exposures that the subjects probably experienced during spraying seasons. Follow-up beyond 1980 has not yet been reported, and as of that date, the numbers of observed and expected cancers were still small. No information on smoking habits for the cohort is available. By applying a 10 year latency period (the shortest latency for which data were provided), 12 lung cancer deaths were observed compared to 11.1 expected (SMR = 1.1, CI 0.6-1.9). Lung cancer incidence for the period 1972-1978 with 10 year latency applied, resulted in the SMR = 1.4 (9 cases observed versus 6.6 expected, CI 0.6-2.6; Riihimaki et al., 1983).

In a study of licensed pesticide applicators in Florida (Blair et al., 1983), the overall lung cancer SMR was 1.4 (34 observed deaths versus 25.1 expected, CI 0.9-1.9). The risk estimate rose with the number of years licensed from 1.0 for less than 10 years licensed, to 1.6 for 10-19 years, to 2.9 for 20 years or more (in a test for trend, $p = .13$). Increased lung cancer SMRs were found for workers licensed to apply pesticides for termites and other wood-infesting organisms, general household pests, rodents, and lawn and ornamental pests, and to apply fumigants. In a small group of firms that were licensed only to treat lawns and ornamentals, the SMR was not elevated (SMR = 0.9), but the numbers were small (observed deaths 7 versus 7.6 expected, CI 0.4-1.9). However, workers were exposed to a multiplicity of chemicals, some known carcinogens; individual exposure to phenoxy herbicides or to any TCDD-contaminated compound cannot be determined. It is unlikely that the elevated lung cancer risk in the entire cohort can be entirely attributable to smoking. The SMRs for other smoking-related diseases were depressed, the risk was related to duration of pesticide use, and implausibly high smoking prevalences would be necessary among this cohort to explain a lung cancer risk of this magnitude.

Green's (1991) cohort study of right-of-way sprayers in Ontario was based on records of spraying activities, but the number of subjects was small. Lung cancer mortality was essentially normal (5 deaths versus 4.6

expected, RR = 1.1, CI 0.4-2.5), but the small numbers of observed deaths preclude strong conclusions. Minnesota highway workers (Bender et al., 1989) were at reduced risk of lung cancer (SMR = 0.7, CI 0.5-0.9), as were Swedish pesticide applicators (Wiklund et al., 1989a); the standardized incidence ratio (SIR) was 0.5 (CI 0.4-0.7), with 38 observed cases. No association with lung cancer was found when the cohort was subdivided by years since license or by year of birth.

Licensed herbicide applicators in the Netherlands were studied by Swaen and colleagues (1992). The study is about the same size as that of Riihimaki and colleagues (1982) in Finland and yielded similar results for lung cancer: 12 deaths observed compared to 11.2 expected (SMR = 1.1, CI 0.6-1.9).

Summary of Pesticide Applicator Studies If the cohorts of Axelson (at second follow-up), Riihimaki (with minimum 10 year latency—all others have no latency restriction), Blair (lawn and ornamental sprayers only), and Green are considered roughly comparable studies of workers with likely exposure to phenoxy herbicides through manual spraying, the observed and expected deaths could be combined to yield a more precise estimate of risk. This yields 27 observed and 25.5 expected deaths and an SMR = 1.1 (CI 0.8-1.5) (Table 8-9).

Paper/Pulp Workers Unlike the studies of farmers, the reports of paper workers are not consistent with respect to their estimates of lung cancer risk. Some studies do report an excess (usually without adequate control for potential confounding by smoking) (Solet et al., 1989; Jappinen and Pukkala, 1991), while others do not (Robinson et al., 1986; Henneberger et al., 1989).

TABLE 8-9 Selected Epidemiologic Studies of Herbicide/Pesticide Applicators—Lung Cancer

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Axelson et al., 1980	Swedish railroad workers	3	1.4 (0.3-4.0)
Riihimaki et al., 1982[a]	Finnish herbicides applicators 10 year latency	12	1.1 (0.6-1.9)
Blair et al., 1983	Florida pesticide applicators		
	Licensed to spray herbicides on lawn and ornamentals only	7	0.9 (0.4-1.9)
Green, 1991	Canadian forestry workers	5	1.1 (0.4-2.5)

[a] Minimum 10 year latency restriction.

Environmental Studies

Studies of the population exposed by the industrial accident in Seveso in northern Italy to TCDD include estimates of lung cancer risk (Bertazzi et al., 1989b; Pesatori et al., 1992). Ten years of follow-up for mortality and cancer incidence demonstrate an inconsistent pattern of lung cancer rates in the different exposed groups, as well as between males and females. Those most heavily exposed from the accident itself (those living in zone A at the time of the accident, but subsequently permanently evacuated) are too few in number (two observed lung cancer deaths in 10 years) to provide any information. When those living in zone A are combined with those in zone B, the lung cancer incidence in males was slightly elevated, based on 20 observed cases (RR = 1.1, CI 0.7-1.7), while there were no observed cases among women (expected number not given). The largest and least contaminated area, zone R, was not found to have elevated lung cancer incidence rates in males (99 observed cases, RR = 0.9, CI 0.7-1.1), whereas a slight excess was observed in females (16 observed cases, RR = 1.3, CI 0.8-2.3). Lung cancer incidence in men was not different from what was expected either in zones A and B (RR = 1.1, CI 0.7-1.7) or in zone R (RR = 0.9, CI 0.7-1.1) (Pesatori et al., 1992).

Smoking is not likely to explain differences in lung cancer rates between exposed zones around Seveso and the comparison population because the latter consists of the residents of nearby towns that are economically and culturally similar to the contaminated region.

The studies of the Seveso population follow them only until 10 years after the accident. If the TCDD released in 1976 did increase the risk for cancers in the lung, this is not sufficient time for all these tumors to come to clinical attention. At least another 10 years is needed before the impact of the accident on cancer incidence can be meaningfully assessed.

Vietnam Veterans Studies

Ranch Hands The follow-up study of Ranch Hand veterans is too small to evaluate excess cancer risks (Michalek et al., 1990). Lung cancer mortality was similar in Ranch Hands and the comparison group, although based on only five Ranch Hand lung cancer deaths (incidence density ratio = 0.9, CI 0.3-2.1).

CDC The Vietnam Experience Study (Boyle et al., 1987) was too small to consider lung cancer risk; only one lung cancer death occurred in the comparison group of Vietnam era veterans.

DVA Studies Breslin and colleagues (1988) and Watanabe and colleagues (1991) have studied Army and Marine Vietnam veterans and Vietnam

era veterans; there was a small increase in lung cancer risk in Army and Marine Corps veterans who served in Vietnam. For both groups combined the PMR is 1.1 (CI 1.0-1.2), and the risk is only slightly higher in Marine than in Army veterans. No smoking data are available on this cohort, but other studies have suggested that the smoking habits of Vietnam and Vietnam era veterans were not significantly different from each other.

Investigators at the Department of Veterans Affairs designed an additional PMR study based on deceased Army veterans who served in Military Region I (I Corps), where the majority of Marines were stationed (Bullman et al., 1990). Lung cancer risk was comparable in Army I Corps veterans and Army Vietnam era veterans (PMR = 0.9, CI 0.8-1.1, based on 187 observed deaths).

The mortality experience of women who served in Vietnam has been studied by DVA investigators (Thomas et al., 1991). Lung cancer mortality was comparable or perhaps somewhat reduced in Vietnam veterans, although based on only eight lung cancer deaths in the exposed group (after adjusting for potential confounding factors, the relative risk was 0.6, CI 0.3-1.5).

Twenty-two U.S. Army Chemical Corps units assigned to South Vietnam between 1966 and 1971 have been followed for vital status through 1987 (Thomas and Kang, 1990). In the final cohort of 894 men there were only 2 deaths from lung cancer, against 1.8 expected based on the entire U.S. male population (SMR = 1.1, CI 0.1-4.0).

State Studies Studies of Vietnam veterans in four different states have examined lung cancer mortality rates: Wisconsin (Anderson et al., 1986a,b), Massachusetts (Kogan and Clapp, 1985, 1988), New York (Lawrence et al., 1985), and West Virginia (Holmes et al., 1986). In each case, lung cancer mortality rates were comparable between Vietnam veterans and Vietnam era veterans.

Australian Vietnam Veterans Among Australian Vietnam veterans compared to Vietnam era veterans serving in Australia, the relative risk was 2.7 (CI 0.2-30.0) for lung cancer (Fett et al., 1987b). This association is based on only two cases among the Vietnam veterans.

The studies of lung cancer risk performed to date in veterans are of limited usefulness for the evaluation of herbicide exposure, either because they are too small or because it is not possible to identify those soldiers who were likely to have been exposed to herbicides.

Epidemiologic Studies of Laryngeal Cancer

In nearly all studies analyzing respiratory cancers, the authors either group all of the different types of cancer in this broad group together (ICD codes 161 to 165 include trachea, bronchus, lung, larynx), or present data

for the largest category within this—ICD code 162—trachea, bronchus, and lung. Cancers of these last three sites are often simply called "lung cancer." In only a few cases are the data broken out to allow assessment of any other respiratory sites. Of note are five studies of production workers in which data for laryngeal cancer (ICD 161) are presented separately (Table 8-10). Although the numbers are too small to draw strong conclusions, the consistency of a mild elevation in relative risk is suggestive of an association for laryngeal cancer. Pooling all but the Coggon data (Coggon et al., 1986, 1991) yields an odds ratio (OR) of 1.8 (CI 1.0-3.2). Potential confounders of an occupational risk for this cancer include tobacco and alcohol consumption. As noted previously, these studies did not directly control for potential confounding by smoking, although its magnitude in the Manz et al. (1991) and Fingerhut et al. (1991) studies is not likely to be large. There is no information on alcohol consumption in any of the studies.

TABLE 8-10 Selected Epidemiologic Studies of Production Workers—Laryngeal Cancer

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Fingerhut et al., 1991	NIOSH cohort D1 year exposure, D20 years latency	3	2.7 (0.6-7.8)
Bond et al., 1988	Dow 2,4-D production workers	1	3.0 (0.4-16.8)
Coggon et al., 1986[a]	British MCPA production workers	4	2.3 (0.5-4.5)
Manz et al., 1991	German production workers	2	2.0 (0.2-7.1)
Saracci et al., 1991	IARC cohort		
	Exposed subcohort	8	1.5 (0.6-2.9)

[a] These workers are included in the European cohort of Saracci et al. (1991).

Summary

Among the many epidemiologic studies of respiratory cancers (specifically cancers of the lung, larynx, and trachea), positive associations were found consistently only in those studies in which TCDD or herbicide exposures were probably high and prolonged, especially the largest, most heavily exposed cohorts of chemical production workers exposed to TCDD (Zober et al., 1990; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991) (see Table 8-8) and herbicide applicators (Axelson and Sundell, 1974; Riihimaki et al., 1982; Blair et al., 1983; and Green, 1991). Studies of farmers tended to show a decreased risk of respiratory cancers (perhaps due to lower smoking rates), and studies of Vietnam veterans are inconclusive. The committee felt that the evidence for this association was limited/suggestive rather

than sufficient because of the inconsistent pattern of positive findings across populations with various degrees of exposure and because the most important risk factor for respiratory cancers—cigarette smoking—was not fully controlled for or evaluated in all studies.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and respiratory cancers (lung, larynx, trachea).

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. Lung cancer has been shown to be associated with TCDD exposure in male and ovariectomized female rats, which suggests a hormonal interaction. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

BONE CANCER

Background

According to the American Cancer Society, 2,000 new cases of bone and joint cancer (ICD-9 170.0-170.9) were diagnosed in the United States

in 1992, and some 1,050 men and women died of this cancer (ACS, 1992). These cases are divided approximately equally between men and women. According to the committee's calculations, 10 cases of bone cancers are expected among male Vietnam veterans and 0.1 among female veterans in 1995. In 2000, the expected numbers are 21 in male veterans and less than 0.05 in female veterans.

Malignant sarcomas arise in various kinds of skeletal tissues. Osteosarcoma develops in the bone itself, Ewing's sarcoma in the bone marrow, and chondrosarcoma in the cartilage cells. Sometimes osteosarcoma and chondrosarcoma are considered among the sarcomas for classification. Primary bone cancers are among the least common malignancies. The bones are, however, a frequent site for secondary tumors of other cancers that have metastasized. Only the primary cancers are considered here. Although bone cancers are seen at all ages, they are concentrated among young people under the age of 20 and among the elderly. Osteosarcoma and Ewing's sarcoma occur primarily at young ages, whereas chondrosarcoma occurs at older ages. The principal known risk factors for osteosarcoma are exposure to radiation and, at older ages, Paget's disease.

Epidemiologic Studies

Bone and joint cancers are relatively uncommon. As a result, a small number of cases may give an apparent excess in risk because of the low number expected, although the sizes of the cohorts generally studied in the investigations reviewed by the committee have been too small to detect a statistically significant risk, even if that should be present. The studies included studies of chemical production workers in the United States and other countries (Coggon et al., 1986; Bond et al., 1988; Zober et al., 1990; Fingerhut et al., 1991), agricultural workers (Burmeister, 1981; Wiklund, 1983; Ronco et al., 1992), and Vietnam veterans (Lawrence et al., 1985; Anderson et al., 1986a,b; Breslin et al., 1988). There has generally not been a consistent finding of excess bone cancer observed in the various exposure groups that have been investigated, as indicated in Table 8-11.

On the whole, the studies regarding bone cancer are evenly distributed in both a positive and a negative direction. Nonetheless, because of its rarity, very few of the studies are of sufficient size to have much statistical power, and the confidence limits are typically large.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an

association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and bone cancer.

TABLE 8-11 Selected Epidemiologic Studies—Bone Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Fingerhut et al., 1991	NIOSH cohort	2	2.3 (0.3-8.2)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0-31.1)
Zober et al., 1990	BASF production workers	0	— (0-70.0)
Coggon et al., 1986	British MCPA production workers	1	0.9 (0.0-5.0)
Burmeister, 1981	Farmers in Iowa	56	1.1 (NS)
Wiklund, 1983	Swedish agricultural workers	44	1.0 (0.6-1.4)[b]
Ronco et al., 1992	Danish male self-employed farm workers	9	0.9
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	27	0.8 (0.4-1.7)
	Marine Vietnam veterans	11	1.4 (0.1-21.5)
Lawrence et al., 1985	New York Vietnam veterans	8	1.0 (0.3-3.0)
Anderson et al., 1986a	Wisconsin Vietnam veterans	1	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	1	—

NOTE: NS = not significant.

[a]Given when available.
[b]99% CI.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental,

and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

SOFT TISSUE SARCOMAS

Background

According to the American Cancer Society, 5,900 new cases of soft tissue sarcomas (ICD-9 171.0-171.9, 164.1) were diagnosed in the United States in 1992, and some 3,300 men and women died of these cancers (ACS, 1992). New cases were slightly more common in men than in women, but similar numbers of deaths occurred. According to the committee's calculations, 65 cases of STS are expected among male Vietnam veterans and 0.1 among female veterans in 1995. In 2000, the expected numbers are 86 cases in male veterans and 0.2 in female veterans.

STSs arise in the soft somatic tissues that occur within and between organs. These tissues are derived from the primitive mesenchyme of the mesodermal layer of the embryo; they account for about 50 percent of the adult body weight. STSs appear in as many as 28 histological types with 14 subtypes (Sobin, 1978). These tumors, which can arise anywhere in the body, include fibrosarcoma and malignant fibrous histiocytoma, leiomyosarcoma (smooth muscle), rhabdomyosarcoma (striated muscle), liposarcoma (fat cells), synovial cell sarcoma (synovial and tendon cells), and angiosarcoma (blood vessels). Most occur de novo rather than from transformation of the much more common benign tumors of the soft tissues.

Three of the most common types of STS—liposarcoma, fibrosarcoma, and rhabdomyosarcoma—occur in similar numbers in men and women. A fourth common form, leiomyosarcoma, is much more frequent in women (often arising in the uterus). Among men, the gastrointestinal tract is the predominant site for leiomyosarcomas. These sarcomas are also found more often in blacks than in whites. The age pattern for incidence of STS is strongly dependent on cell type. Often the distribution is bimodal, with a peak incidence in infancy and childhood and another peak in adult life. For example, rhabdomyosarcoma has a peak incidence between ages 1 and 7, and another peak at 18 years. Based on Connecticut Tumor Registry data, the incidence of STS has shown an upward trend for both sexes since 1935. The slope was gradual until 1950-1954 after which there was a sharp increase to double the 1935 level. Whether this represents an actual increase or an artifact of case histologic definition, case finding, or reporting is uncertain.

Because of the diverse characteristics of STS described above, accurate diagnosis can be difficult. Diagnosis is usually based on the discovery of a mass followed by biopsy. The size of the mass may affect whether the tumor is perceived as being intraorgan or primarily within the soft tissue. Classification of STS by routine histological stains is often difficult. Because of the diversity and rarity of STSs, the average pathologist may not have detailed knowledge of the differential criteria, and even specialists in STS pathology disagree on some types. Overall, there is no clear histogenetic basis for registry classification of soft tissue sarcomas. Convention is based on arbitrary pathologic appearances. DVA conventions currently do not consider chondrosarcomas, osteosarcomas, mesothelioma, and Kaposi's sarcoma among those related to herbicide exposure for compensation. Accuracy of death certificates is another classification problem. At least half of the STSs are deep in body cavities or within organs; unless an autopsy and/or tissue biopsy is done, many are apt to be overlooked. An evaluation of the difficulty of diagnosis and classification of STS from death certificates has been published (Suruda et al., 1993).

A major difficulty in the epidemiologic study of STS has been the failure of the ICD-Oncology (Percy et al., 1990b) to categorize these tumors in a systematic way. The site organ-oriented code is well suited for organ-specific neoplasms—usually carcinomas; however, the "connective tissue cancers" exclude mesenchymal tumors arising in parenchymatous organs (approximately half the mesenchymal tissue is located within organs). The classification also fails to recognize the heterogeneity of cell types.

Although practices vary, many studies exclude malignancies that might be classified as STS: tumors of hematopoietic tissues (leukemia, Hodgkin's disease, lymphomas); osteosarcomas; and STSs of major visceral organs (heart, lung, kidney, liver, intestine). Mesotheliomas are sometimes included but are more often considered with lung cancer. In the studies reviewed here, mesothelioma was generally excluded from the category of STS. No specific evidence was uncovered, however, bearing on a possible link between this rare tumor and herbicide or TCDD exposure. Different investigators have used different conventions in identifying STS cases for inclusion in epidemiologic studies. As noted below, this adds further complexity to the interpretation of the epidemiologic data.

In dealing with STS, the epidemiologist is faced with two difficult choices: (1) lump all STSs together and deal with the data as a group, or (2) subdivide STS into its individual histopathologic subgroups. Because of their diverse origins, locations, behaviors, etc., the interpretation of a study using the first approach may be difficult. The second approach would result in very few cases per type, probably insufficient for reliable statistical analysis. Studies evaluated here use the first approach; no results are given for individual subtypes.

TCDD caused fibrosarcomas in at least two animal bioassays in both rats and mice (NTP, 1982a,b). TCDD is also known to suppress immune function in animals (see Chapter 4), and at least one sarcoma, Kaposi's, and several other types of cancer as well, are more frequent in humans suffering from chronic immune suppression, especially AIDS (Williams, 1991).

For the vast majority of STSs, risk factors have not been described. Of the few that are known, the associations are diverse. Familial and genetic factors have been associated with Gardner's syndrome (including fibromatosis, fibrosarcomas, and multiple skeletal tumors), neurofibromatosis (including von Recklinghausen's disease), schwannomas, lipomas, lymphangiomas, hemangiomas, acoustic neuromas, and Kaposi's sarcoma. A small fraction of STSs of all types can be induced by exposure to external radiation, including therapeutic exposure. Radium clock dial painters, for example, had an increased incidence of fibrosarcomas (Polednak et al., 1978).

Some animal risk factors do not appear to produce the same results in humans. Viral particles have been isolated from several animal sarcomas, but only indirect evidence of viral factors has been identified in human sarcomas. Fibrosarcomas have been attributed to plastic implants in rats, but there is no evidence that foreign bodies—metals, plastics, bullets, or heterologous tissue transplants—result in the development of sarcomas in humans.

Polycyclic aromatic hydrocarbons produce sarcomas in rats, mice, and guinea pigs. Although there is no direct evidence of a similar response in humans, it is well established that human exposure to polyvinyl chloride is associated with the development of hepatic angiosarcoma.

Epidemiologic Studies

Occupational Studies

Production Workers A cohort of German production workers (Zober et al., 1990) involved 247 persons, among whom no STS cases have occurred in the 34 years of follow-up. Other cohorts of production workers with no STS deaths observed and less than one death expected are those of Coggon et al. (1991) in the United Kingdom; Bond et al. (1988) in the United States; Manz et al. (1991) in Germany; and Bueno de Mesquita et al. (1993) in the Netherlands. Another study by Coggon and colleagues (1986) observed one STS death against 0.9 expected.

One study of phenoxy herbicide production workers in two Danish factories (Lynge, 1985) identified 5 cases of STS (all male), while 1.8 were expected (SMR = 2.7, CI 0.9-6.3). If a 10 year latency is applied, the relative risk increases slightly to 3.7 (CI 1.0-9.4). When analysis is restricted to workers engaged specifically in manufacturing jobs, only 1 observed

case remains versus 0.3 expected. However the author believes that workers elsewhere in the factory were probably exposed to the herbicides. No exposure data are available for the cohort, but the fact that these were phenoxy herbicide production facilities means that a presumption of substantial exposure is reasonable for some fraction of the cohort.

In the International Agency for Research on Cancer (IARC) cohort (Saracci et al., 1991), which includes both production workers and herbicide sprayers, four deaths from STS occurred in the exposed and probably exposed group compared to two expected (SMR = 2.0, CI 0.6-5.2). When an analysis was performed by years since first exposure, all 4 deaths occurred between 10 and 19 years, while the number expected was 0.7 (SMR = 6.1, CI 1.7-15.5).

The finding of an increased risk of soft tissue sarcoma in the IARC analysis is in accord with that of the NIOSH cohort (Fingerhut et al., 1991) described below. The addition of five cases among cohort members, but not included in the mortality analysis, gives increased credence to the mortality finding.

The NIOSH cohort includes exposure assessment, both environmental and biological (Fingerhut et al., 1991). For STS, 4 deaths were observed, while 1.2 were expected (SMR = 3.4, CI 0.9-8.7). Among those with at least 20 years of latency and one year of exposure, there were 3 STS deaths and 0.3 expected (SMR = 9.2, CI 1.9-27.0).

This evidence for an association between TCDD and STS is tempered by the difficult diagnosis of this rare class of tumors (Suruda et al., 1993). Because this was a cohort mortality study using national mortality rates as the standard, the authors had no choice but to define cases as those with an underlying cause of death by STS (ICD 171) listed on the death certificate, despite the frequent errors in diagnosis that doubtless arise from this approach. To use additional data to refine the case definition would have made the case series not comparable to the comparison population (based solely on death certificates), and introduced a potentially serious bias. The authors did in fact review tissue specimens and hospital records, although this supplementary information was not used in the mortality analyses. Two of the four deaths attributed to ICD 171 were found not to be STS at all. Hospital record review identified two additional cases of STS that were coded on death certificates as dying from other causes, although one of these was not considered a true STS when subjected to independent histologic review. A seventh STS death occurred in a small group of exposed workers who were not included in the cohort because they did not meet certain entry criteria. Thus studies of STS based on death certificates suffer because of the particular problems of diagnosis and classification of these tumors (see earlier discussion of STS diagnosis) and because they are so rare that even relatively large cohort studies such as the NIOSH study (Fingerhut et al., 1991) yield very small numbers of deaths.

Collins and his colleagues (1993) from Monsanto have recently hypothesized that heavy exposure to 4-aminobiphenyl alone or in combination with TCDD may explain the observed STS excess. A substantial body of evidence, however, points toward an association of STS with exposure to phenoxy herbicides and related compounds, whereas the possibility of a link to 4-aminobiphenyl has not previously been reported.

Summary of Production Worker Studies The production workers studied in pesticide and related industries have exposures that are likely to have been fairly high and sustained for long periods. The cohort assembled by NIOSH is particularly important because of its size and documented exposures (Fingerhut et al., 1991). An elevated risk of STS was found in this study, although based on few cases. The SMR for workers with more than a year of exposure 20 years or more before death was 9.2 (CI 1.9-27.0), although based on only three cases.

The IARC cohort also experienced an excess STS risk, but does not have consistent exposure documentation (Saracci et al., 1991); the cohort of Danish herbicide production workers studied by Lynge makes up a large fraction of the Saracci cohort and is responsible for the elevation in risk for STS observed in the larger IARC study (Lynge, 1985).

Agricultural/Forestry Workers Data from England were used to investigate association between work in agriculture or forestry—and thus possible exposure to TCDD as a contaminant of herbicides—and STS (Balarajan and Acheson, 1984). Current occupation in one of six broad classes of agricultural and forestry workers yielded a relative risk for STS of 1.2 (CI 0.8-1.6). This risk was restricted to just one of these six classes, that of farmers, farm managers, and market gardeners, who experienced a relative risk of 1.7 (CI 1.0-2.9). A limitation of the study, which would tend to diminish estimates of risk, is that the only exposure information was occupation at the time of cancer diagnosis. Nine of the 42 cases of STS among farmers occurred in those over 75 years of age, which the authors felt made phenoxy herbicide exposure unlikely. Therefore the relative risk among those under age 75 was calculated to be 1.4 (CI 0.8-2.6).

A unique record linkage system in Sweden permitted the calculation of risks of STS for large numbers of workers in agriculture and forestry (Wiklund and Holm, 1986). Between 1961 and 1979, 331 cases of STS occurred in the agriculture and forestry group—an incidence essentially identical to that observed in the comparison group (RR = 0.9, CI 0.8-1.0). None of the subgroups showed elevated risks, nor was there any evidence of a trend over time in STS risk among these groups. When the STS cases were subdivided by histologic type, there was no evidence of an elevation in risk for any particular type.

The authors discuss the discrepancy between their findings and those

from the Hardell case-control studies discussed below. They note that if the true relative risk of STS for exposed workers is about 6 (as observed in the first two case-control studies), and if approximately 15 percent of agriculture and forestry workers are so exposed (based on Swedish data), then they would have expected to find a relative risk of about 1.5 in this study.

Two limitations of this registry-based study noted by the authors seem particularly relevant for the present discussion. First, Swedish agricultural workers utilize medical services less frequently than other sectors of society and so may be subject to a general problem of underdiagnosis. Second, STS is a an especially difficult cancer to diagnosis, and registry-based studies without confirmatory histopathologic review probably involve numerous inaccuracies in tumor classification. As many as 20 percent of the cases initially called STS may be eliminated from a case series upon review (Woods et al., 1987).

A study of forest or soil conservationists (Alavanja et al., 1989) showed no increase in mortality from STS, although only two deaths were observed (PMR = 1.0, CI 0.1-3.6). A weakness of the study is that no data exist on potential exposure to phenoxy herbicides, either individually or as a group. A similar study of agricultural extension agents found no deaths from STS (Alavanja et al., 1988).

Danish gardeners (859 females, 3, 156 males) exposed to a variety of pesticides were followed for cancer incidence by Hansen and colleagues (1992). Male gardeners had an excess risk of STS [standardized morbidity ratio (SMbR) = 5.3, CI 1.1-15.4)], based however on only three cases. No cases of STS were observed among female gardeners. It is difficult to link the elevated risk in men to phenoxy herbicides with any confidence because of the diversity of exposures in gardeners, but the finding is nevertheless suggestive of an association.

Summary of Agricultural Worker Studies The studies of agricultural workers are largely uninformative for STS because workers with substantial exposure to herbicides are probably interspersed among large numbers of subjects without herbicide exposure, which would dilute measures of association. Also, herbicide exposures are inseparable from exposures to many other potentially toxic agents. Nevertheless two findings from these studies are suggestive:

1. A broad class of British farm workers (farmers, farm managers, and market gardeners) was found to have a slightly elevated risk of STS in a study by Balarajan and Acheson (1984), compared to those with other cancers (RR = 1.7, CI 1.0-2.9). There was no measurement of individual exposure in this study.
2. Danish gardeners experienced an elevated risk of STS (Hansen et al., 1992), with a standardized morbidity ratio of 5.3 (CI 1.1-15.4). Individual

estimates of the level of herbicide exposure in the cohort were not available.

Case-Control Studies The first strong evidence for a carcinogenic effect of phenoxy acids in humans came from a case-control study conducted by Hardell and Sandstrom in 1979. This and four other STS case-control studies by Hardell and colleagues make a substantial contribution to the existing knowledge about soft tissue sarcoma and herbicides. These five studies used very similar methods and collectively address many of the weaknesses of case-control studies.

In the fall of 1976, three patients with soft tissue sarcoma were admitted to the Department of Oncology of the University Hospital in Umea, Sweden. Clinical observation suggested significant histories of phenoxy herbicide exposure in each case. A case-control study was conducted to pursue this hypothesis of association between phenoxy herbicide or chlorophenol exposure and soft tissue sarcoma (see Chapter 7). Phenoxy herbicides and chlorophenols have been shown to be contaminated with TCDD. Of histologically confirmed but not typed soft tissue sarcomas, 21 living and 31 deceased male cases were included in the matched-pairs analysis, and an odds ratio of 6.2 was observed for soft tissue sarcoma and exposure to either phenoxy herbicides or chlorophenols. When the analysis was conducted without regard to match status of the matched-pairs, the OR was similar, 5.7 (CI 2.9-11.3), so the unmatched analysis was used for all further investigations. Only two patients and two controls were exposed to compounds not containing dioxins. There was some difference in risk estimate among living versus dead subjects, although the numbers available for analysis were small in each group: living OR = 9.9, dead OR = 3.8. For phenoxy herbicide exposure alone, the OR was 5.3 (CI 2.4-11.5). For chlorophenol exposure, the OR was 6.6 (CI 1.8-25.1). When the three index cases were removed from the study, the OR for phenoxyacetic acid exposure fell slightly to 4.7 (CI 2.0-10.7).

Those reporting phenoxy herbicide and chlorophenol exposure tended to come from certain jobs in forestry and the paper industry. It was possible that another exposure in those jobs was actually responsible for some or all of the effect attributed to phenoxy acids and chlorophenols. Therefore the investigators estimated the risk of STS for those with no evidence of exposure, but who had reported work in jobs frequently associated with exposure; the odds ratio for such jobs was 0.6. Chain sawing, a common activity among those employed in forestry and one with an easily remembered exposure to fumes, also did not appear to carry an elevated risk; the odds ratio was 0.8. These latter two findings suggest that a general problem of over-reporting of all kinds of jobs or exposures was unlikely to have occurred in this study.

A second case-control study was conducted in the south of Sweden using almost identical methods (Eriksson et al., 1979, 1981). Results were similar to the previous study; with exposure to either phenoxy herbicides or chlorophenols, the odds ratio from the matched analysis was 5.1, and from the unmatched, 4.7 (CI 2.2-10.2). Further analyses used the unmatched method. For phenoxy exposure excluding those with chlorophenol exposure, the odds ratio was 6.8 (CI 2.6-17.3). When stratified by duration of exposure to phenoxy herbicides, the results listed in Table 8-12 were obtained.

The investigators excluded exposure to 2,4,5-T and found an odds ratio of 4.2 (CI 1.3-13.4) for exposure to all other types of phenoxy herbicides. Chlorophenol exposure excluding phenoxy herbicide exposure yielded an odds ratio of 3.3 (CI 1.3-8.1). No excess risk was observed for exposure to solvents, chain saws, DDT, mercury, or asbestos. The lack of association with the last exposure is noteworthy because at the time of the studies there was much public debate and concern over the carcinogenic risks of asbestos in Sweden. Thus one might have expected a recall bias to lead to an excess risk estimate for asbestos and STS.

Concern about potential recall and interviewer bias in the first two case-control studies led Hardell to gather additional data for further analyses of the earlier studies (Hardell, 1981). Although the telephone interviewers were blind to the case or control status of the subjects, it seemed possible that cases might have revealed their illness during the interview, raising the possibility that interviewers may have probed more fully for recollections of past exposure in cases than in controls. Hardell therefore reanalyzed the original case-control study results, using only the information on exposure obtained from mail questionnaires and found similar results.

It is important in case-control studies to avoid leading questions or a line of questioning that allows the subject to recognize the prior hypothesis of the investigators. In the Hardell studies the standard exposure questionnaire contained some 130 questions, of which only 10 were related to herbicide use, while for example 16 others asked about solvent use. It thus did not seem likely that the subjects would have noticed that the prime hypothesis

TABLE 8-12 Results of a Case-Control Study of STS by Eriksson and Colleagues (1979, 1981)

Duration of Exposure to Phenoxy Herbicides	Cases	Controls	Estimated Relative Risk (95% CI)
Unexposed	85	206	1.0
W30 days	7	3	5.7 (1.3-34.5)
> 30 days	7	2	8.5 (1.6-84.6)

NOTE: Test for trend: $^{2} = 15.3$, $p < .001$.

of the investigators was directed toward phenoxy herbicides and chlorophenols.

Despite these precautions, the considerable public debate in Sweden about the possible health risks of exposure to phenoxy herbicides still raised the possibility that cases might have selectively recalled exposures that controls might have failed to remember. As a further investigation of this possibility, Hardell selected a series of incident male colon cancer cases (Hardell, 1981) from the same region as the STS cases and gathered exposure information for them with methods identical to those used in the previous studies. These cases were studied simultaneously with the subjects, cases and controls, in a study of a nasopharyngeal cancer (Hardell et al., 1982). The investigators who conducted the telephone interviews to clarify exposure information did not know whether the subject was a colon cancer or nasopharyngeal cancer case or a control. After observing that colon cancer was not associated with exposure to phenoxy herbicides (OR = 1.3, CI 0.6-2.8) or chlorophenol (OR = 1.8, CI 0.6-5.3), Hardell reanalyzed the first case-control study, using the colon cancers as the control series. The results were quite similar to those in the original analysis of STS data: for phenoxy herbicide exposure, the odds ratio for STS was 5.5 (CI 2.2-13.8), and for chlorophenol exposure the OR was 5.4 (CI 1.3-22.5) (Hardell, 1981).

These additional analyses provide convincing evidence that recall bias and interviewer bias are unlikely to explain the observed associations in the first Swedish case-control studies.

Hardell and colleagues then conducted a third case-control study of STS in northern Sweden (Hardell and Eriksson, 1988). The most common histological types of STS were malignant fibrous histiocytoma (29 percent) and leiomyosarcoma (13 percent). No other type accounted for more than 10 percent of the cases.

The same exposure assessment procedure was performed with one exception. By the time of this study, Dr. Hardell's name had become associated with research on phenoxy herbicides, and it was suggested that perhaps his name on the return envelope for the mailed questionnaire might have biased the responses. Therefore one-half of the living population controls received an envelope with his name on it and the other half received the same questionnaire but with the return address listed as an independent statistical research center. The frequency of phenoxy herbicide use in the two halves was nearly identical (8.5 versus 8.8 percent).

The odds ratio for STS and exposure to phenoxy herbicides for at least one day more than five years before diagnosis was 3.3 (CI 1.4-8.1) by using the population controls. When the cancer controls were used, the odds ratio decreased somewhat to 2.2 (CI 0.9-5.3) (Hardell and Eriksson, 1988). This suggests either that some recall bias may have occurred or that an effect of herbicide exposure on some other cancer site increased the exposure prevalence

among controls. Based on results presented elsewhere in this chapter, the latter should be considered in interpreting the results. Unlike previous studies, no effect of chlorophenol exposure was observed, although the authors note that the prevalence of chlorophenol exposure was lower in this study than in previous ones.

In both of these studies (Hardell, 1981; Hardell and Eriksson, 1988), no increase in risk of STS was observed when exposure was defined by job titles thought to be linked to herbicide use. The authors argue that their exposure assessment allowed more precise identification of those exposed and not exposed than a procedure relying solely on job title information.

Hardell and colleagues conducted a fourth case-control study much like the previous three in an area of central Sweden around the city of Uppsala (Eriksson et al., 1990). Leiomyosarcoma accounted for 35 percent of the cases, malignant fibrous histiocytoma for 12 percent, and liposarcoma for another 12 percent. No other type occurred in more than 10 percent of the cases. There was again an elevated risk for exposure to phenoxy herbicides and chlorophenols, although not as strong as in previous studies. For phenoxy herbicide exposure of at least one day more than five years before diagnosis, or chlorophenol exposure for one week or more continuously or for a total of one month in all, the odds ratio was 1.8 (CI 1.0-3.2). For phenoxy herbicide exposure without chlorophenol exposure, the odds ratio was 1.4 (CI 0.7-2.6). This study was large enough that the investigators could separately estimate risks for those beginning exposure early (in the 1950s) and those with later dates of first exposure. For those with phenoxy herbicide exposure beginning before 1960, the OR was 2.4 (CI 1.0-5.4), whereas those with later first exposures had a lower risk (OR = 0.7, CI 0.2-2.3). Exposure to chlorophenols without phenoxy herbicides carried an OR of 5.3 (CI 1.7-16.3), a figure quite similar to the first two studies.

Viewed as a group, the four case-control studies of Hardell and colleagues on STS provide strong evidence for an association with phenoxy herbicide exposure and chlorophenol exposure (Figure 8-3). The pooled odds ratio for phenoxy herbicide exposure is 2.7 (CI 1.8-4.1). There is some suggestion of a diminution of the strength of the association as the studies progressed over time, although because the four studies were conducted in three different regions of Sweden, local variations in agricultural and industrial practices might also explain the pattern observed. It is also possible that varying levels of TCDD contamination of the chemicals studied might explain the apparent decrease in risk over time.

Collectively, these four studies include 435 cases of STS—a very large number for such a rare cancer. Nevertheless the published data on these cases provide only limited evidence with which to investigate the hypothesis that certain histologic types may be more or less associated with phenoxy herbicide exposure. Half of the cases come from a single study (Eriksson et

al., 1990), and the authors of this report note that they failed to find any evidence of association with any specific histologic types in that study population.

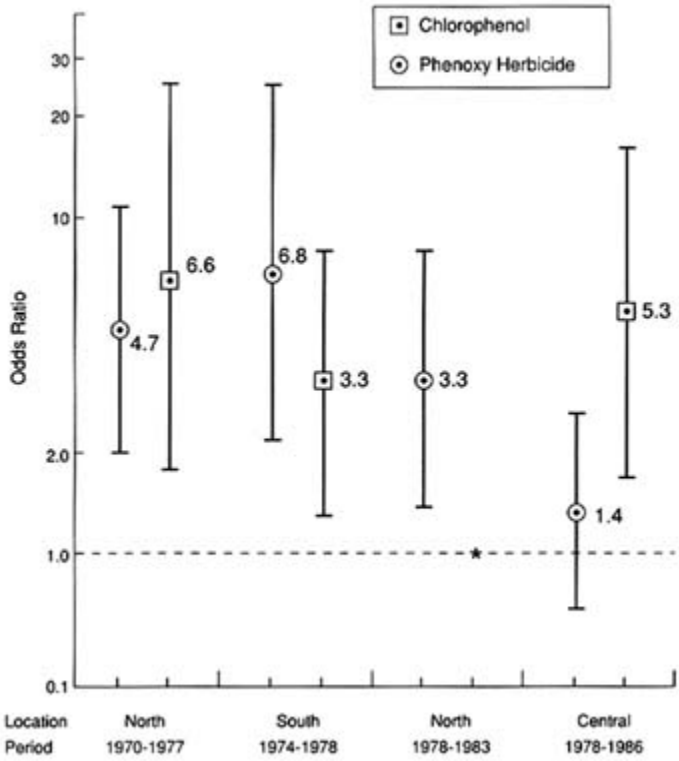

FIGURE 8-3 Odds ratios and confidence intervals for the four case-control studies of STS done by Hardell and colleagues done in different geographical regions of Sweden. References: North (1970-1977) Hardell and Sandstrom, 1979 (note: index cases excluded); South (1974-1978) Eriksson et al., 1981; North (1978-1983) Hardell and Eriksson, 1988; Central (1978-1986) Eriksson et al., 1990.

A fifth case-control study was conducted in southern Sweden by an essentially different group of investigators, with methods that seem to differ somewhat from those of the Hardell studies (Wingren et al., 1990). For cases of STS reported to the regional cancer registry, no separate histologic confirmation of the diagnoses was done, nor were the histologic types listed in the paper. Follow-up was through a somewhat different questionnaire assessment, with only 74 percent of the original cases and controls included in the final analyses because of the various exclusions and refusals.

The authors did not attempt to develop estimates of phenoxy herbicide and chlorophenol exposure as Hardell and colleagues did, but instead calculated risks for various occupations likely to entail exposure to these substances. For example, they found that gardeners and railroad workers were at increased risk of STS: by using population controls, the odds ratio for gardeners was 4.1 (CI 1.1-14.6), and for railroad workers 3.1 (CI 0.6-13.3). The differences in methods of this last study make it difficult to compare to the other four Swedish case-control studies.

A team of investigators in New Zealand conducted a case-control study of STS as a response to reports by Hardell and colleagues, with a subsequent update including two more years of cases (Smith et al., 1983, 1984; Smith and Pearce, 1986). For those reporting more than one day of exposure to herbicides ("probable or definite"), not during the five years prior to diagnosis, the odds ratio was 1.6 (CI 0.7-3.8; Smith et al., 1984). For those with a similar degree of exposure to chlorophenols, the odds ratio was also 1.5 (CI 0.4-8.0). Many of these exposures came from work in the pelt department of the "meat works" or in a pelt department in a tannery where chlorophenol exposure is quite likely in the process of treating sheepskin. An odds ratio for STS of 7.2 was associated with the latter processes. However, this estimate is based on six exposed cases and one exposed control, and so yields an exact CI of 0.8-334. The authors do not report a combined odds ratio for either chlorophenol or herbicide exposure, nor can one be estimated from the separate tables because of the possibility that some subjects may have reported both types of exposure. If there were no overlap in those reporting the two different types of exposures (for more than one day, more than five years before diagnosis), the odds ratio would be 1.7 (CI 0.8-3.6). The authors note that none of their exposed cases were commercial herbicide applicators; they tended to be farmers and other agricultural workers with some herbicide exposure. Thus exposure levels may have been fairly low compared to those in studies of workers with sustained and regular exposure.

When 51 additional cases were included with the previous series (Smith and Pearce, 1986), the risk of STS for those reporting herbicide spraying for more than one day not in the five years before diagnosis was not elevated: OR = 0.7 (CI 0.3-2.0). Again, there were no commercial herbicide applicators in the case series, and most of those judged to have been exposed were farmers with only occasional exposure.

An Italian case-control study on STS focused on exposures to phenoxy herbicides during rice weeding (Vineis et al., 1986). This population-based case-control study covered three rice-growing provinces and included all STS diagnosed in residents over the age of 19 years from 1981-1983. The final case series included 44 living and 24 deceased cases. Controls for living and dead cases were selected separately: living controls were chosen

from provincial electoral rosters, with a distribution of municipalities representative of the provincial populations from which the cases came, while dead controls were chosen from the municipalities of the deceased cases. "Certain exposure," which occurred almost entirely among rice weeders, was restricted primarily to women. Among all living women, the odds ratio for certain or possible exposure was 2.4 (CI 0.4-16.1), but was based on five cases and seven controls so that confidence intervals are quite wide and do not exclude the null. When living and dead women are combined in the analysis, the odds ratio for certain or possible exposure is 2.3 (CI 0.6-6.1). Restricting analyses to certain exposure does not materially increase the point estimate, but does increase the width of the confidence interval.

No single histologic type dominated the case series. The most common was Kaposi's sarcoma (21 percent of cases), and no other type represented more than 12 percent of cases. There was also no clear difference in the distribution of histological types between cases judged to have been exposed and those unexposed. The authors point out that 1981-1983 may have been a little late to observe an increase in STS from exposure to herbicides in the period 1950-1955, given a median latency in other studies of about 15 years.

Hoar and colleagues (1986) conducted a population-based case-control study of soft tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease in the state of Kansas, focusing on herbicide use. The authors observed no increase in risk of STS with increasing use of herbicides, when the latter was classified as "ever/never," or with either frequency or duration of use. For example, the odds ratio for STS comparing those reporting any farm use of herbicides was 0.9 (CI 0.5-1.6). There was, however, a weak elevation of risk in the category with the longest duration of herbicide use, 16 years or more (OR = 1.4, CI 0.6-3.1). Despite this, however, there was no clear evidence of a trend with increasing duration of use. This study started with specific hypotheses, based on the earlier Swedish studies, and used a large population base for identification of cases. Kansas has extensive wheat farming, which commonly involves herbicide use. Although uncertainties arising from multiple exposure and uncertainties of actual individual exposure levels exist, the study obtained more individual exposure information than many others. It should be noted that the phenoxy herbicide most used was 2,4-D, whereas 2,4,5-T use was less frequent.

A population-based case-control study was conducted in western Washington State where phenoxyacetic acid herbicides and chlorophenols are widely utilized by agricultural, forestry, and wood product industries (Woods et al., 1987). Independent histologic confirmation of the diagnosis of STS was performed by a single pathologist. There was generally good agreement when occupational exposures from self-reports were confirmed with employers. When all subjects were classified into high, medium, low, or no

exposure to phenoxy herbicides or chlorophenols, no association with STS was observed. For example, the odds ratio for high phenoxy exposure was 0.9 (CI 0.4-1.9) and for high chlorophenol exposure also 0.9 (CI 0.5-1.8). For jobs determined a priori to have chlorophenol exposure, those reporting work as "log-lumber inspectors" showed an odds ratio for STS of 4.8 (CI 0.6-38.2), and for "lumber grader" the odds ratio was 2.7 (CI 1.1-6.4). No such elevated risks of STS were found among jobs identified as being exposed to phenoxy herbicides. Further analyses either using duration of exposure or incorporating latency periods between exposure and diagnosis failed to identify any important trends. Those with self-reported chloracne had an elevated risk of STS (OR = 3.3, CI 0.8-14.0).

The authors use a pharmacokinetic model to estimate the dose of 2,4,5-T received by an herbicide applicator and predicted that in Sweden the dose might have been substantially higher than that in Washington State because of the shorter, more intense application season in Sweden. They also suggest that general environmental contamination with phenoxy acids and their contaminants in the study region may have biased any true exposure-risk association toward the null.

The authors also identify preliminary evidence suggesting that there may be important heterogeneity in human susceptibility to STS from phenoxy herbicides and chlorophenols. Because of the relatively high proportion of the population of the study area that was of Scandinavian heritage, the authors were able to separate the study group (blind to case or control status) into those with and without Scandinavian surnames. This distinction was found to have no direct association with STS risk, but among those with Scandinavian surnames, the risk of STS in those reporting high phenoxy herbicide or chlorophenol exposure was elevated. For high phenoxy exposure the odds ratio was 2.8 (CI 0.5-15.6), and for high chlorophenol exposure the odds ratio was 7.2 (CI 2.1-24.7). Among those without Scandinavian surnames there was no elevation in the risk estimates. If it is true that there is a heterogeneity in human susceptibility to STS risk from phenoxy herbicides and chlorophenols, the risk may not be limited to Scandinavians per se; some as yet unidentified metabolic trait may be more prevalent in this ethnic group. As noted in Chapter 4, there is ample evidence from laboratory animals to suggest genetic variability in the metabolism of TCDD.

A case-control study (30 cases) of STS and exposure to herbicides and chlorophenols was also conducted in Australia (Smith and Christophers, 1992). Of the cases studied, 30 percent were malignant fibrous histiocytomas, 17 percent were leiomyosarcomas, and the rest were distributed among many other types. For each case, one population control and one control with another type of cancer were selected. A five year latency period prior to diagnosis of the cases was applied when identifying exposures. There were no major differences between the population controls and the cancer controls

with respect to definite exposure or possible exposure; hence the two control groups were combined. No evidence of an association between exposure to phenoxy acids and STS was observed, but the sample size of the study was small, and hence there was little power to detect an association if one existed. The odds ratio for STS following at least one day of exposure to phenoxy herbicides or chlorophenols more than five years before diagnosis was 1.0 (CI 0.3-3.1). For more than 30 days of exposure, the risk estimate was 2.0, but based on very small numbers, yielding a CI from 0.5 to 8.0.

A case-control study of STS ($N = 14$) was conducted by investigators from the Dow Chemical Company among workers at the Midland, Michigan, facility (Sobel et al., 1987). This study failed to find an association between STS and any chemical exposure, although the paper describes the subject selection and exposure assessment procedures in insufficient detail to judge the validity of the study confidently. Fortunately, the study of Fingerhut and colleagues (1991) incorporates the TCDD-exposed workers at this facility and, as discussed previously, combines them with other production worker groups to improve study power.

Summary of Case-Control Studies The case-control studies are quite disparate in their results, but are particularly important because of their size. The first study, conducted by Hardell, involved 52 cases; in contrast, the large cohort of licensed pesticide applicators studied by Wiklund contained only 7 cases of STS. The detailed exposure data gathered in many of the case-control studies are both a strength and a weakness; if these data are accurate, better estimates of risk are possible than in many of the cohort studies, but at the same time these data may introduce biases into the study results through inaccurate recall by subjects.

The four case-control studies by Hardell and colleagues in Sweden (Hardell and Sandstrom, 1979; Eriksson et al., 1981; Hardell and Eriksson, 1988; Eriksson et al., 1990) show an association between STS and exposure to phenoxy herbicides, chlorophenols, or both (Figure 8-3). In the second study conducted in the south of Sweden, the relative risk rose strongly with increasing duration of exposure (Table 8-12) (Eriksson et al., 1979, 1981).

Because of the importance of these studies in judging the effects of herbicides on STS, certain key methodologic points common to the studies bear emphasis:

1. The questionnaire methods of exposure assessment used in these studies are well accepted in occupational epidemiology. Care was taken to avoid biases. Recall bias was a potentially serious problem but was exhaustively investigated by the authors (see below).
2. Control selection was excellent. The methods used are standard and well accepted. Follow-up by phone was appropriately blinded.

3. Similar methods were used in three different parts of Sweden and in two nonoverlapping time periods.
4. An identical study on colon cancer found no association with herbicides, and in using the colon cancer series as an alternative referent group to reanalyze the previous case series (STS, lymphoma), similar results were found. This makes serious recall bias unlikely, because it would have to be acting for certain cancers and not for others.
5. To investigate the possibility that recall could be influenced by Dr. Hardell's name on return envelopes, a study was done with identical methods, but 50 percent of the subjects received a letter with his name and 50 percent with no name. The results were identical in the two subgroups.
6. The strength of association with STS is higher in the earlier studies and tends to decrease over the series, but it remains positive overall. This may be because of a bias in the first studies, which was eliminated, or it could be explained by TCDD being a "true causal agent" and its concentration in herbicides having decreased over time; alternatively, the statistical bias called "regression to the mean" may cause the first study of an agent to result in a higher risk estimate than subsequent studies.

The study by Wingren and colleagues (1990), also in Sweden, does not show as strong an association, although it is difficult to compare with studies of Hardell and Eriksson because of different exposure assessment methods. It is not however without suggestions of phenoxy herbicide or chlorophenol effects: both gardeners and railroad workers have elevated risks of STS.

The two case-control studies from New Zealand (Smith et al., 1983, 1984; Smith and Pearce, 1986) show a suggestion of an effect of exposure to both phenoxy herbicides and chlorophenols, as also shown for those likely exposed to phenoxy herbicides during rice weeding in northern Italy in a case-control study of Vineis and colleagues (1986).

The large case-control study conducted in Kansas by Hoar and colleagues (1986) at the National Cancer Institute does not support the hypothesis of an association between exposure to phenoxy herbicides and STS. The odds ratio for ever having farm use of herbicides was 0.9 (CI 0.5-1.6). The large study size is reflected in the relatively narrow confidence interval around this estimate. Because the study results indicated 2,4-D was the primary herbicide used, rather than 2,4,5-T, the difference between these findings and the more positive results in production workers and other occupational cohorts suggest that TCDD, which is a contaminant of 2,4,5-T but not 2,4-D, may be responsible for the association with STS seen in this group of studies.

Another large American case-control study, conducted in western Washington State, is also generally supportive of no association, although several findings point in the direction of some herbicide-STS association (Woods et al., 1987). The overall odds ratio for high phenoxy herbicide use and risk

of STS was similar to that found by Hoar: 0.9 (CI 0.4-1.9); for estimated high chlorophenol exposure, the odds ratio was similar. However, the authors calculate that the likely body dose of 2,4,5-T for a typical herbicide sprayer in Sweden would be higher than for the same occupation in western Washington State because of differences in spraying practices. Spraying in Sweden is described as daily over a period of several weeks, but in western Washington, periods of a few days of spraying are separated by several weeks without spraying.

Herbicide Pesticide Applicators Most of the cohort studies of pesticide applicators are too small to be useful in studying risk of as rare a cancer as STS. The following studies observed no cases of STS, but because of their small size, considerably less than one case of STS was expected based on mortality rates in the comparison populations: Axelson and Sundell (1974); Axelson et al. (1980); Riihimaki et al. (1982, 1983); Blair et al. (1983); Green (1991); and Swaen et al. (1992). Bender and colleagues' (1989) study was slightly larger, with 1.4 expected deaths and none observed. Green (1991) notes that her study of Ontario forestry workers spraying utility rights of way had 80 percent power to detect a relative risk of 27, which means there would be almost no chance of detecting relative risks of the order of 4 or 5.

In a study of 20,245 Swedish pesticide applicators (Wiklund et al., 1988b, 1989a,b), 7 cases of STS were identified with no latency from the Swedish Cancer Registry, compared to 7.7 expected, based on total population rates (SIR = 0.9, CI 0.4-1.9). There was no effect of introducing latency times of 5 and 10 years. Individual exposure estimates were not available, but data from two surveys suggest that 72 percent of applicators used phenoxy herbicides for at least one day compared to 16 percent of forestry and agricultural workers.

Summary of Pesticide Applicator Studies Most of the pesticide applicator studies are too small to individually have power to detect STS, although presumably the degree of exposure for the average study subject was much higher in these studies than in, for example, the agricultural worker studies. The study by Wiklund (1988b, 1989a,b) was rather large and did detect 7 cases of STS, compared to 7.7 expected (SIR = 0.9, CI 0.4-1.9). There was no individual assessment of exposure for members of this cohort, and most of the members were agricultural workers who sprayed herbicides only occasionally, so that heavy or regular exposure cannot be assumed.

Environmental Studies

Studies of both morbidity and mortality in the 10 years following the accident at Seveso, Italy, have been published (Bertazzi et al., 1989a,b; Pesatori et al., 1992). Ten years after the accident, no cases of STS had been

observed in zones A and B, and less than one case was expected based on regional incidence rates. In the larger, but less exposed area, zone R, six incident cases and two deaths from STS were observed among males, and two incident cases and no deaths among females. These numbers exceed those expected based on regional rates: the relative risk for STS incidence in zone R males was 2.8 (CI 1.1-7.4) and in zone R females 1.4 (CI 0.3-6.6). The standardized mortality ratio in males corroborates this observation: SMR = 6.3 (CI 0.9-45.0). There were no deaths from STS in females (Bertazzi et al., 1989a,b; Pesatori et al., 1992).

A cohort incidence study of lymphomas and STS was conducted by Vineis and colleagues (1991) in the rice-growing Italian provinces of Novara and Vercelli, where no patterns of association were observed for STS.

A Finnish town found to have been exposed to high levels of chlorophenols through contamination of the drinking water supply (Lampi et al., 1992) was compared to two nearby towns. Six observed STS cases were found, which was more than expected (RR = 8.9, CI 1.8-44.0). When compared to the larger reference region, the risk was only moderately elevated (RR = 1.6, CI 0.7-3.5). The authors were unable to explain this large discrepancy. A nested case-control study was conducted to try to link the excess risk to particular sources of exposure (drinking water contamination, fish consumption, work in the sawmill that caused the contamination), but no individual risk factors could be identified. Despite the uncertainties in this study, it is difficult to fully discount the excess STS risk. Exposure to chlorophenols was fairly substantial—for example, levels of 70 to 140 µg/liter in drinking water, and 175 to 925 µg/kg in fish.

Summary of Environmental Studies The environmental contamination studies include two important findings. First, the incidence of STS in zone R in Seveso, Italy, was elevated for the 10 years following the accident; the smaller and more highly exposed zones A and B have experienced no cases, but less than one was expected.

The study of a Finnish town (Lampi et al., 1992) exposed to chlorophenols in drinking water and fish found an elevated incidence of STS. Despite uncertainties about the magnitude of the increased risk, which stem from differences among comparison populations, the increase cannot be ignored.

Vietnam Veterans Studies

Ranch Hand Study The Ranch Hand study is described in Chapter 7. In approximately 20 years of follow-up (through 1987), one Ranch Hand had died of STS. This case is noted by Michalek et al. (1990) without description or comment. A single death from STS also occurred in the approximately 15 times larger comparison cohort of other Air Force personnel

serving in Vietnam. Although it is impossible to draw conclusions based on only two cases, the incidence rate in the comparison population is roughly what would be expected based on national data. One case in the small Ranch Hand population is more than expected (the relative risk is about 15, with obviously very wide confidence limits—the paper does not provide this information or the data with which to make the calculations).

CDC The CDC Selected Cancers Study (1990b) was designed with service in or off the coast of Vietnam as the primary exposure variable, so it provides little information on exposure to herbicides. Case-control analysis for STS found no overall effect of service in Vietnam (OR = 1.0, CI 0.6-1.6). However, there are several suggestive findings that would seem to warrant further investigation. The authors report that a higher proportion of cases than controls had occupational exposure to chlorophenols ($p < .05$), and a higher proportion also reported work in a meat-packing or processing plant, where chlorophenol exposure sometimes occurs ($p < .05$). Odds ratios for these associations are not provided, but it is possible to calculate crude (i.e., without adjustment for age or other factors) odds ratios of 1.5 for each of these two exposures (CI 1.0-2.0 for the former and 0.9-2.3 for the latter). Other indicators of occupational exposure to phenoxy herbicides and chlorophenols show weaker associations.

When risk estimates are calculated for selected characteristics of Vietnam service, those who served in I Corps had an elevated risk of STS, although based on small numbers (OR = 1.6, CI 0.7-3.8). This was not the area of heaviest spraying (III Corps, near Saigon, received the heaviest aerial spraying, see Chapter 3) but was nevertheless subject to considerable amounts. All subjects reporting service in and off the coast of Vietnam were asked about possible contact with Agent Orange, and those who reported passing through a defoliated area had a slightly higher risk of STS than those not reporting such an event (OR = 1.6, CI 0.6-4.1).

DVA Studies As described elsewhere in this report, the Department of Veterans Affairs has conducted a proportionate mortality study among deceased veterans of the Vietnam era (Breslin et al., 1988; Watanabe et al., 1991). No evidence of an elevated risk of STS among either Army or Marine veterans serving in Vietnam is found in these studies, which are based on fairly large numbers of observed deaths. For example, the PMR comparing Army Vietnam veterans to all Vietnam era veterans was 1.0, based on 30 observed STS deaths. For the Marines the comparable PMR was 1.1 based on 8 observed STS deaths.

A related study focusing on Army veterans who served in the northernmost region of Vietnam (I Corps) used the same data base supplemented with additional deaths from Army veterans (Bullman et al., 1990). There

were 10 observed deaths from STS among Army veterans serving in I Corps, compared to 11.4 expected (PMR = 0.9, CI 0.4-1.6).

The DVA conducted analyses for the veterans from states in which state studies had already been performed (Breslin et al., 1986); individual state studies are discussed below. These were conducted in the same manner as the nationwide study, but were simply limited to veterans resident in a particular state. Interestingly, elevated PMRs for Wisconsin, Massachusetts, and West Virginia were observed, while no such excess was seen among New York State veterans. These findings are consistent with those reported from the individual states, in which quite different methods and data bases were used.

Two case-control studies of soft tissue sarcoma were conducted by DVA investigators (Kang et al., 1986, 1987), but it is likely that cases from the two studies overlapped. The first used data from DVA hospitals and found an odds ratio for STS comparing those serving in Vietnam to those without such service of 0.8 (CI 0.6-1.1) (Kang et al., 1986).

There are several weaknesses in this study. First, the period of case ascertainment was early for detecting STS, which probably has a latency of 15 to 20 years. The study relies on cases reported by 1983. This allows for a latency period of 15 years or less for personnel with service in Vietnam in the late 1960s or early 1970s. The authors note that 80 percent of the available cases were diagnosed within the first 10 years of exposure. Second, it seems possible that Veterans Administration (VA) hospitals do not treat a representative fraction of all veterans (Constable et al., 1987). Third, there is no individual measure of potential exposure to herbicides in this study.

The second case-control study (Kang et al., 1987) drew cases from the Armed Forces Institute of Pathology. It also found that occupational exposures to herbicides or chlorophenols did not appear to be more frequent among cases than among controls. The study was limited to cases diagnosed between 1975 and 1980. Thus, the maximum latency period from the beginning of herbicide spraying in 1962 is 18 years. The latency period is no more than 9 years for individuals with service in 1971 when spraying was ended. Service in Vietnam was similarly evenly distributed among cases and controls (OR = 0.8, CI 0.6-1.2). There was a weak positive association between combat and risk of STS. Among Army veterans who served in Vietnam, the risk of STS for those in combat occupations was 2.6 times the risk for those not in combat occupations (CI 0.7-9.4). When this analysis is restricted to those serving in Military Region III, where the bulk of aerial herbicide spraying occurred, the risk is higher, although less precise because of small numbers (OR = 8.6, CI 0.8-111.8). For Marine veterans, the relative risk for those in combat was not as high (OR = 1.3, CI 0.1-7.9).

The second study also has several important limitations. First, the choice of an appropriate control series for cases drawn from a highly specialized national referral center like the Armed Forces Institute of Pathology is a very difficult matter. Controls should be individuals who, had they developed the disease of interest, would have been eligible to enter the case series. A significant fraction (32 percent) of the hospitals from which the controls were chosen did not cooperate, and in those that did, it is not clear exactly what criteria guided the choice of controls. It is not clear, for example, whether veteran status was considered in this choice. There are also no reliable measures of exposure, and the few comparisons that might have a bearing on herbicide exposure (combat/noncombat, military region) are made with inadequate power to detect reasonable effects. For example, the authors calculated that the study had only a 23 percent chance of detecting a twofold excess risk of STS among combat versus noncombat Army veterans.

State Studies A case-control study of STS in New York State (Greenwald et al., 1984) showed that Vietnam service was not associated with an increased risk of STS (OR = 0.5, CI 0.2-1.3), nor was self-reported exposure to TCDD, Agent Orange, or 2,4,5-T (OR = 0.7, CI 0.2-2.9). Work in chemical manufacturing carried a slightly increased risk (OR = 1.8, CI 0.8-3.7).

Because of the timing of this study, there was little opportunity to detect an effect of any Vietnam-related activity. The maximum latency (time from first exposure to diagnosis) was 18 years, and for some cases it could have been less than a year. The age at diagnosis ranged from 27 to 47 years, and 66 percent were under age 45. In the Hardell case-control studies, the median latencies were 15 to 20 years and the mean age of cases was about 57 years (Hardell and Sandstrom, 1979; Eriksson et al., 1981). It was thus too early to expect to detect an effect of the sort seen in the Swedish studies, because a minimum latent period had not been covered.

Another New York State study had a short latency for detection of an STS risk (Lawrence et al., 1985). Of the 59 deaths coded as STS (ICD 171) in the entire study, 12 were among veterans, yielding an age- and race-adjusted mortality odds ratio of 1.2 (CI 0.6-2.2) compared to nonveterans. The adjusted mortality odds ratio for STS comparing Vietnam veterans to other veterans of the Vietnam era was 1.1 (CI 0.2-6.7).

The DVA study calculated a PMR of 0.7, based on one death, for STS among New York State veterans, using all U.S. veterans who did not serve in Vietnam as the comparison population (Breslin et al., 1986).

A report prepared for the Iowa State Department of Health (Wendt, 1985) showed among respondents (24 percent) two self-reported soft tissue cancers. The study does not make an attempt to calculate the expected number of cancer cases.

In the Wisconsin State studies of veterans mortality, weak evidence for an increase in STS among Vietnam veterans is shown, although again the latency may be insufficient, and no direct estimates of herbicide exposure for individuals were available (Anderson et al., 1986a,b). Five deaths from STS occurred among Wisconsin Vietnam veterans. There are several comparisons that can be made, including 2.8 deaths expected based on the experience of nonveterans (PMR = 1.8, CI 0.8-4.3) and 3.4 expected deaths based on Vietnam era veterans (PMR = 1.5, CI 0.6-3.5) (Anderson et al., 1986a).

The DVA study also calculated a PMR for STS among Wisconsin veterans and found an elevated risk compared to all U.S. non-Vietnam veterans, based on three observed deaths (PMR = 5.1, CI 1.1-14.9) (Breslin et al., 1986).

Studies in Massachusetts veterans found excess STS risk among those who served in Vietnam (Kogan and Clapp, 1988; Clapp et al., 1991). The age-standardized mortality odds ratio for STS comparing Vietnam veterans to non-Vietnam veterans was 5.2, based on nine deaths (CI 2.4-11.1). Using nonveterans as the comparison yielded a slightly higher risk estimate (Kogan and Clapp, 1988). Confirmation of the diagnosis of STS was obtained for eight of the nine cases from hospital or physician records.

As a follow-up (Clapp et al., 1991), eight incident cases of STS among Vietnam veterans and nine among other veterans of the Vietnam era were reported. The authors calculated an odds ratio as an estimate of the relative risk of STS among these two groups, using all other subjects in the Massachusetts Cancer Registry with cancers other than STS, non-Hodgkin's lymphoma (NHL), or kidney cancer as the comparison population. The risk of STS among Vietnam veterans was 3.1 times that among veterans who did not serve in Vietnam (CI 1.1-8.7).

In neither of these two studies was information available about the details of service in Vietnam or about other possible sources of chemical exposure. Thus various explanations for the observed association with Vietnam service are possible.

The DVA study (Breslin et al., 1986) provided an independent confirmation of this observation. The PMR for STS among Massachusetts veterans compared to all U.S. non-Vietnam veterans was 3.8, based on two observed cases (CI 0.5-13.8).

Holmes and colleagues (1986) report a slight elevation in STS risk among West Virginia Vietnam veterans based on only three cases (SMR = 4.3, CI 0.9-12.5). The DVA study found a PMR of 2.0 based on only one observed case.

Australian Vietnam Veteran Studies In the cohort study of Australian Vietnam veterans, the mortality experience of those who served in Vietnam was compared to that of veterans of the same era who did not leave Australia (Fett et al., 1987b). The interpretation of the study is made more difficult

by an unusual method of classifying causes of death in the cohort. Rather than using just death certificates, comprehensive cause of death data were reviewed by a panel of experienced physicians to produce a "mock death certificate," which was used for this study. In contrast, the comparison population mortality experience was drawn from published national statistics relying solely on the standard death certificate. There was one death from STS found among Vietnam veterans and one among the Vietnam era group, yielding an age-adjusted relative mortality rate of 1.3 (CI 0.1-20.0).

Summary of Veterans Studies The studies of Vietnam veterans are largely uninformative for STS because of the lack of exposure data. As discussed elsewhere in this volume, it is difficult to detect an increase in the incidence of a rare cancer among all Vietnam veterans because of exposure to herbicides in some fraction of them. Yet several studies hint at such a pattern for STS. The state studies comparing Vietnam veteran incidence or mortality to that expected based on veterans who did not go to Vietnam, do sometimes show an excess risk (Table 8-13). The DVA largely confirmed this pattern with its own, independent data. However, the large PMR study

TABLE 8-13 State Vietnam Veteran Studies of STS Mortality

Reference	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Massachusetts		
Kogan and Clapp, 1988	9	5.2 (2.4-11.1)
Breslin et al., 1986	2	3.8 (0.5-13.8)
New York		
Lawrence et al., 1985	2	1.1 (0.2-6.7)
Breslin et al., 1986	1	0.7
West Virginia		
Holmes et al., 1986[b]	3	4.3 (0.9-12.7)
Breslin et al., 1986	1	2.0 (0.1-10.9)
Wisconsin		
Anderson et al., 1986a	5	1.5 (0.6-3.5)
Breslin et al., 1986	3	5.1 (1.1-14.9)

NOTE: Each state's veterans were studied twice: once by investigators in the state and once by investigators from the U.S. Department of Veterans Affairs.

[a] Given when available.

[b] Comparison group is nonveterans.

from which the state-specific relative risks in Table 8-13 have been extracted fails to find an overall excess risk of STS among Vietnam veterans, compared to either nonveterans or veterans who did not serve in Vietnam (Watanabe et al., 1991).

Two possibly overlapping case-control studies by investigators at the DVA do not demonstrate adequate exposure measures for herbicides in Vietnam (Kang et al., 1986, 1987). There is a suggestion that Army veterans in combat occupations are at higher risk of STS than those in other occupations in Vietnam, and this tendency is particularly strong in Military Region III, where the bulk of the aerial herbicide spraying occurred.

The CDC (1990b) Selected Cancers Study was designed with service in or off the coast of Vietnam as the primary exposure variable, so it provides little information on exposure to herbicides per se. There is no increase in risk of STS for those serving in and off the coast of Vietnam versus those serving elsewhere during the same era. Those reporting an occupational (nonmilitary) history of chlorophenol exposure had a 50 percent excess risk of STS (OR = 1.5, CI 1.0-2.0), whereas Vietnam veterans who reported walking through a defoliated area had a 60 percent excess risk compared to Vietnam veterans who did not report this experience (OR = 1.6, CI 0.6-4.1).

Finally, the Ranch Hand study is of insufficient size to detect excess risk of STS (Michalek et al., 1990). One case of STS occurred in this heavily exposed group, while less than one-tenth of one case would have been expected based on the experience of the non-herbicide-exposed Air Force comparison population being followed.

Summary

There are at least three fundamental problems that make the interpretation of epidemiologic studies on soft tissue sarcoma and exposure to phenoxy herbicides and related compounds especially difficult. First, if there are several potential causative agents present in a particular environment, it may be difficult to determine which is associated with STS. This problem is, of course, not limited to STS studies. Using a job title as indicative of exposure to herbicides is not sufficient as a measure of exposure; the presence and level of contamination with TCDD may have varied over time. Given uncertainty about whether TCDD or herbicides themselves are associated with STS, even full knowledge of which herbicides were contaminated with TCDD will not clarify whether TCDD or the herbicides are the causative factor.

A second difficulty peculiar to soft tissue sarcomas is the diagnosis and classification of these cancers in a consistent and reliable manner. The available epidemiologic evidence for an association of STS risk with herbicides

and dioxins comes from studies including a wide variety of different histologic types. Each set of investigators chose to include or exclude various histologic types of this diverse class of tumors with various justifications. Consequently, the committee was unable to identify and particular types of soft tissue sarcoma as more or less likely to be associated with herbicide exposure. If certain tumors in the general category of STS are caused by exposure to herbicides, whereas others are not, a wide range of results is to be expected. The problems of the existing classification schemes have been discussed above, without clear conclusions about what may be the "correct" classification system. Additional refinement of the clinical and pathological definitions of soft tissue sarcomas in epidemiologic studies would also help to determine which of the specific cancers in this class are associated with herbicides and/or TCDD.

A third difficulty presented by soft tissue sarcomas is their rarity. This has been mentioned several times, but it cannot be overemphasized. The task of unequivocally detecting a positive association between STS and environmental exposure to (the worst case) a variable contaminant in an herbicide stretches the limits of the epidemiologic method. In a cohort of 10,000 middle-aged men not exposed to an agent that causes STS, followed for 10 years, one would not expect to observe a single death from STS. Even a sixfold elevation in risk due to some exposure (as first reported by Hardell and Sandstrom, 1979) would result in only a handful of cases. If, in addition, there is some misclassification of exposure and some misclassification of disease diagnosis, both of which are more than likely in these sorts of studies, then a serious elevation in risk could easily be missed even in a large study.

The strongest evidence for an association between STS and exposure to phenoxy herbicides comes from a series of case-control studies involving a total of 506 cases conducted by Hardell and colleagues in Sweden (Hardell and Sandstrom, 1979; Eriksson et al., 1981, 1990; Hardell and Eriksson, 1988; Wingren et al., 1990) that show an association between STS and exposure to phenoxy herbicides, chlorophenols, or both. Although these studies have been criticized, the committee feels that there is insufficient justification to discount the consistent pattern of elevated risks, and the clearly described and sound methods employed. These findings are supported by a significantly increased risk in the NIOSH study (SMR = 9.2, CI 1.9-27.0) for the production workers most highly exposed to TCDD (Fingerhut et al., 1991), and a similar increased risk in the IARC cohort (SMR = 6.1, CI 1.7-15.5) for deaths that occurred between 10 and 19 years after the first exposure (Saracci et al., 1991). These are the two largest, as well as the most highly exposed occupational cohorts. Some studies in other occupational, environmental, and veterans groups showed an increased risk for STS, but the results were commonly nonsignificant possibly because of

small sample sizes related to the relative rarity of STS in the population, except for males in zone R of Seveso. The risk in this group was significantly elevated and is consistent with the findings supporting an association.

Because of difficulties in diagnosing this group of tumors, the epidemiologic studies reviewed by the committee were inconsistent with regard to the specific types of tumors included in the analyses. The available data did not permit the committee to determine whether specific forms of STS were or were not associated with TCDD and/or herbicides. Therefore, the committee's findings relate to the class as a whole.

Conclusions

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and soft tissue sarcoma.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. TCDD administration increased fibrosarcoma formation in both rats and mice. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

SKIN CANCERS

Background

Skin cancers are generally divided into two broad categories, malignant melanomas and nonmelanotic skin cancers. According to the American Cancer Society, 32,000 new cases of melanoma (ICD-9 172.0-172.9) were diagnosed in the United States in 1992, and some 6,700 men and women died of this cancer (ACS, 1992). The incidence is similar in men and women, but men account for about 60 percent of deaths. Other skin cancers (basal cell and squamous cell carcinomas) led to about 600,000 new cases and 2,100 deaths. According to the committee's calculations, 486 cases of melanoma are expected among male Vietnam veterans and 1.1 among female veterans in the year 1995. In the year 2000, the expected numbers are 632 cases in male veterans and 1.3 in female veterans. No calculations were made for the very common and highly curable nonmelanotic skin cancers.

Malignant melanoma arises in melanocytes that are located throughout the skin and in other areas of the body, including the eye and nervous system. Regardless of location, these cells all derive from the embryonic neural crest. Four pathologic types of melanoma are recognized: superficial spreading (70 percent), nodular (15 percent), acral lentiginous (10 percent), and lentigo maligna (5 percent) (Sober and Koh, 1991). The preponderance of superficial spreading melanomas and their occurrence in middle years make them the most common lesion for epidemiologic study. More advanced melanomas are much more likely to be fatal than those identified at earlier stages. In contrast, nearly all nonmelanotic skin cancers are treatable, which means that mortality studies can provide little information about them.

The incidence of malignant melanoma in both males and females has increased steadily during the past three decades; between 1973 and 1989, the rate of increase was higher than for any other cancer (Miller et al., 1992). Mortality has increased as well. During 1985-1989, however, the incidence rates stabilized. In contrast to incidence, mortality is continuing to increase, although at a slower rate than in the 1970s. Increases in both incidence and mortality have been greatest for white males age 65 and older. Improved case finding, including those treated in physicians' offices, contributed to an increase in incidence of more than 10 percent between 1984 and 1985. Clinical diagnostic criteria have, however, remained generally consistent over investigators and over the time period (van der Esch et al., 1991). The change in case-finding procedures can explain only a small part of the increased incidence.

Because of the apparent association between melanoma and exposure to

ultraviolet (UV) light, increases in voluntary sun exposure (Armstrong, 1988; Glass and Hoover, 1989), the use of artificial tanning devices (Walters et al., 1990; Husain et al., 1991), and migration to southern states may be contributors to the increased incidence of melanoma. Certain skin types (those that sunburn easily with little tanning) may be especially vulnerable (Fitzpatrick, 1986). Higher melanoma rates among Caucasian populations living nearer the equator than among those living at higher latitudes seem to support the association with UV exposure. A small percentage of these cancers are hereditary.

The recent stabilization of the incidence of melanoma may be due in part to missed cases among those diagnosed and treated in nonhospital settings (Karagas et al., 1991; Koh et al., 1991), but this factor cannot account for the similar stabilization of mortality rates. Although some may think it is too soon to see a significant effect, the slower increase in melanoma incidence may also be related to greater use of sunscreen or other protective behaviors. Adult use of sunscreens may be beneficial if ultraviolet light acts as a promoter of melanoma, as has been suggested by recent work (Husain et al., 1991).

Basal cell carcinomas are the more common nonmelanoma skin cancers. They arise in the layer of cells between the epidermis and dermis. They generally grow slowly and rarely prove invasive. Squamous cell carcinomas develop in the outermost layer of skin. They grow somewhat faster than basal cell cancers and are more likely to become invasive. Both of these cancers are found most frequently (but not exclusively) in older whites and on parts of the body most frequently exposed to the sun (face, ears, scalp). More frequent basal cell cancers among younger people may reflect greater recreational sun exposure. In addition to sun exposure, risk factors may include chronic irritation and scarring (Emmett, 1975) and exposures to polycyclic hydrocarbons and arsenic (Everall and Dowd, 1978).

Epidemiologic Studies

Skin cancers are of particular interest with regard to TCDD exposure because of its known association with chloracne, a dermatologic condition (see Chapter 11).

On the whole, most of the epidemiologic studies reviewed by the committee did not find an excess risk of skin cancer among TCDD-exposed workers or veterans. These included studies of chemical production workers in the United States and other countries (Suskind and Hertzberg, 1984; Lynge, 1985; Coggon et al., 1986; Bond et al., 1988; Zober et al., 1990; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991), agricultural workers (Burmeister, 1981; Alavanja et al., 1988; Wigle et al., 1990; Hansen et al., 1992; Ronco et al., 1992), pesticide applicators (Blair et al., 1983;

Swaen et al., 1992), Seveso residents (Pesatori et al., 1992), and Vietnam veterans (Lawrence et al., 1985; Boyle et al., 1987; Breslin et al., 1988; CDC, 1988; Anderson et al., 1986a,b). The lack of association includes a study in which the cohort observed consisted of those with chloracne (Moses et al., 1984). One exception is melanoma mortality following the Seveso accident. Bertazzi and colleagues (1989a,b) found an elevated risk in males from zones B and R, but this was based on one and two melanoma deaths, respectively. In addition, the Ranch Hand study (Wolfe et al., 1990) found a relative risk of 1.5 (CI 1.1-2.0) for nonmelanomic skin cancer. One study of agricultural workers in Sweden (Wiklund, 1983) found an elevated risk for skin cancer excluding melanoma (RR = 1.1, 99% CI 1.0-1.2), but these results may be confounded by sun exposure in these groups.

Summary

Some of the studies have utilized melanoma as the end point of interest, whereas others have utilized skin cancer, which primarily reflects melanoma. On the whole, the studies are fairly evenly distributed around the null and, for a number of studies, the confidence intervals were relatively narrow. The only study with a significant excess risk is from the Seveso area, which found an SMR of 3.3 for men, based on only three cases. Results are summarized in Table 8-14.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of no association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and skin cancer.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

TABLE 8-14 Selected Epidemiologic Studies—Skin Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Fingerhut et al., 1991	NIOSH cohort	4	0.8 (0.2-2.1)
Bond et al., 1988	Dow 2,4-D production workers	0	0.0 (0-6.8)
Suskind and Hertzberg, 1984	Monsanto production workers	8	1.6
Coggon et al., 1986	British MCPA chemical workers—other skin cancers	3	3.1 (0.6-9.0)
	Melanoma	1	0.5 (0.0-2.8)
Lynge, 1985	Danish production workers—men	14	0.7
Saracci et al., 1991	IARC cohort	3	0.3 (0.1-0.9)
Zober et al., 1990	BASF production workers	0	0 (0-42.0)
Burmeister, 1981	Farmers in Iowa	105	1.1 (NS)
Wiklund, 1983	Swedish agricultural workers	708	1.1 (1.0-1.2)[b]
	Melanoma	268	0.8 (0.7-1.0)[b]
Swaen et al., 1992	Dutch herbicide applicators	2	4.8 (0.5-17.4)
Hansen et al., 1992	Danish gardeners—melanoma	32	1.1 (0.8-1.6)
Ronco et al., 1992	Danish self-employed farmers—other skin cancers	493	0.7 ($p < .05$)
	Melanoma	72	0.7 ($p < .05$)
Alavanja et al., 1988	USDA agricultural extension agents	5	1.1 (0.5-2.6)
Blair et al., 1983	Florida pesticide applicators	2	1.3
Wigle et al., 1990	Saskatchewan farmers	24	1.1 (0.7-1.6)
Environmental			
Bertazzi et al., 1989	Seveso residents—zones A, B, R	3	3.3 (0.8-13.9)
Pesatori et al., 1992	Seveso male residents—zones A and B	1	2.1 (0.3-16.0)
	Female residents—zones A and B	0	—
Vietnam veterans			
Wolfe et al., 1990	Air Force Ranch Hand veterans		
	Sun exposure-related skin cancers	88	1.5 (1.1-2.0)
	Melanoma	4	1.3 (0.3-5.2)
CDC, 1988	Army enlisted Vietnam veterans	15	0.8 (0.4-1.7)
Breslin et al., 1988	Army Vietnam veterans—melanoma	145	1.0 (0.9-1.1)
	Marine Vietnam veterans—melanoma	36	0.9 (0.6-1.5)
Lawrence et al., 1985	New York Vietnam veterans—melanoma	2	0.7 (0.1-3.5)
Anderson et al., 1986a	Wisconsin Vietnam veterans	6	0.9 (0.4-2.0)
Anderson et al., 1986b	Wisconsin Vietnam veterans	5	1.3 (0.4-3.1)

NOTE: NS = not significant.

[a]Given when available.
[b]99% CI.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

CANCERS OF THE FEMALE REPRODUCTIVE SYSTEM AND BREAST

Background

Considered as a group, cancers of the reproductive organs, including the breast (ICD-9 174.0-174.9), the ovary (ICD-9 183.0), and the uterus (cervix and endometrium; ICD-9 179, 180.0-180.9, 182.0-182.1, 182.8), account for 45 percent of new cases and 29 percent of cancer deaths in women. According to the American Cancer Society, new cases and deaths in 1992 for each of these cancers were as follows (ACS, 1992):

Site	New Cases	Deaths
Breast	181,000*	46,300
Cervix	13,500*	4,400
Corpus uteri	32,000	5,600
Ovary	21,000	13,000
Other genital	5,000	1,000

* Excludes carcinoma in situ (about 20,000 cases for breast cancer and 55,000 cases for cervical cancer).

According to the committee's calculations, 13.2 cases of breast cancer and 1.6 cases of uterine cancer are expected among female Vietnam veterans in 1995. In 2000, the expected numbers are 15.5 and 2.8 cases, respectively.

Histopathology

Breast By far the most common histological type of breast cancer is adenocarcinoma, derived from the epithelium of breast ducts. Lobular carcinoma derived from gland lobule epithelium is a separate category (less than 5 percent of breast cancer). Lobular cancers are usually bilateral and grow very aggressively. Other malignant, although somewhat less invasive,

cancers include medullary, mucinous, and tubular carcinomas. Noninvasive carcinomas are found in breast ducts (e.g., comedocarcinoma) and lobules. Intraductal papillomas, another histological variant, are nearly always benign and do not appreciably alter the overall statistics.

Ovary Carcinomas of the ovary, although most commonly derived from the ovarian epithelium or stroma, are morphologically and clinically heterogeneous. Three major groups can be identified. The largest group is the epithelial tumors, which constitute more than half of ovarian tumors. They appear to originate from the surface epithelium, and nearly all are malignant. Sex cord or stromal tumors are probably derived from ovarian mesenchyme (theca cells, granulosa cells, etc.). Tumors derived from germ cells form a small but diverse group including dysgerminoma (resembling testicular seminoma), choriocarcinomas (resembling placental tissue), and teratomas (resembling embryonic tissue). Other ovarian cancers include tumors of nonspecialized tissues of the ovary, unclassified tumors, and metastatic tumors, which are rare. This diverse array of forms has tended to impede the study of risk factors. However, the predominance of epithelial tumors, their relative uniformity, and their malignancy have enabled epidemiologists to gather useful data.

Uterus Epidermoid carcinoma of the uterine cervix is very uniform in appearance and site (epithelial junction) and represents a well-defined group for epidemiologic studies. Other malignancies of the cervix and endocervical canal are rare. Uterine cancers usually appear in the form of adenocarcinoma of the endometrium. Although there are some endometrial carcinoma variants, the overwhelming majority of uterine cancers are endometrial adenocarcinoma.

Epidemiology

Common Risk Factors Except for cervical cancer, the risk of these reproductive cancers is dominated by markers of cumulative hormonal exposure. Early age at menarche and late age at menopause are associated with increased risk as are late or no childbearing.

Breast Cancer Among U.S. women, 40-55 years of age, breast cancer is the leading cause of death (U.S. DHHS, 1987). Rates of breast cancer increase rapidly up to the time of menopause. After menopause, incidence rates continue to increase with age but more slowly than before. Long-term increases in incidence rates have been observed. An analysis of SEER data indicates that the incidence of breast cancer increased 4-6 percent annually between 1980 and 1987. Only some of the increase can be attributed to more extensive screening and earlier diagnosis (Miller et al., 1991; Harris et

al., 1992). Mortality patterns vary by age and race, with decreases seen among white women under age 65 and increases among older white women and black women of all ages (Miller et al., 1992). Earlier detection of tumors and improved treatments have kept increases in mortality lower than increases in incidence.

Risk factors for breast cancer include early age at menarche, late age at first birth and low parity (or nulliparity), late age at menopause, and in addition, family history of breast cancer and personal history of benign cystic breast disease (Henney and DeVita, 1987). Women living in the United States who are of northern European heritage have four to five times more breast cancer than women of Asian heritage living in Asian countries. Dietary factors have been postulated to modify risk, but only alcohol intake is consistently related to increased risk of breast cancer (Henderson, 1991). Investigations into the relationship between stress and breast cancer have not been conclusive. Age is an important modifier of risk such that exposure to radiation between the onset of menses and first pregnancy creates a greater risk than a similar exposure at older ages.

Ovarian Cancer New cases of ovarian cancer account for 4 percent of all cancers among women. Although only half as many ovarian cancers as uterine cancers are diagnosed, a greater number of deaths are caused by ovarian cancer. The relatively high mortality rate is generally due to late diagnosis. The risk of ovarian cancer increases with age, with the highest rates for women over 60. Over the past 20 years, incidence has shown little change; small declines in mortality have been seen primarily in women under age 50. Women who have never had children are twice as likely to develop ovarian cancer as those who have had children. Risk of ovarian cancer is also higher among women who have had breast or endometrial cancer. Early age at first pregnancy, early menopause, and the use of oral contraceptives, which reduces the frequency of ovulation, are protective against ovarian cancer.

Uterine Cancer The incidence of invasive cervical cancer and mortality from cervical cancer have dramatically decreased during the past 50 years with the development of screening methods and improved therapy. Endometrial cancer of the uterus, once less common than cervical cancer, is now the most common invasive reproductive cancer in women. It occurs most often in mature women and diagnosis is usually made after age 50. Following an earlier increase, the incidence and mortality of uterine cancer have declined over the past 20 years. Mortality has declined more among younger women (under age 50) than older women. The more recent decreases in the incidence of uterine cancer may reflect more limited postmenopausal use of estrogens following warnings in the mid-1970s of their association with endometrial cancer.

Factors increasing the risk for cervical cancer include early sexual activity, multiple sex partners, and some sexually transmitted diseases. Because the risk of cervical cancer is higher among women of low socioeconomic status, apparent racial differences in incidence and mortality may, in fact, reflect socioeconomic factors (Brinton and Fraumeni, 1986). Low socioeconomic status has been associated with various sexual, behavioral, and dietary practices that have been either documented or postulated as risk factors for cancer of the cervix (Brinton and Fraumeni, 1986).

Risk for uterine (i.e., endometrial) cancer has been related to excessive exposure to estrogen, to which nulliparity, early age at menarche, and late age at menopause contribute (Elwood et al., 1977), as does estrogen replacement therapy. High socioeconomic status (Elwood et al., 1977) and obesity (McDonald et al., 1977) have been associated with increased risk as well. Use of oral contraceptives, however, appears to confer a protective effect (CDC, 1987b).

Epidemiologic Studies

The data relating exposure to herbicides to cancer among women are extremely limited. The committee attempted to examine cancer among women separately from men; however, compared with the sparse data available for men, data for women are almost non-existent. Therefore, the focus of the epidemiologic studies in this section is on breast and reproductive cancers (Table 8-15), as these cancers are examined in most studies which do in fact include women, whereas not all other tumor sites are consistently evaluated. Cancers of other organs in women are noted and discussed elsewhere in the chapter along with studies of the tumors occurring in men.

Occupational Studies

Production Workers Many studies have excluded women from analysis because of their small numbers in the groups under study. For example, Fingerhut and colleagues (1991) in their follow-up of workers from 12 companies, identified 67 women who were then excluded from the report of the follow-up study. Likewise, Moses and colleagues (1984) excluded three women from analysis of their follow-up of workers, and Zack and Suskind (1980) excluded the one woman who was living at the end of the study. Among the studies that were based on follow-up of workers, women contribute a minor portion of the data, and the results are accordingly even less stable than those reported for men.

Manz and colleagues (1991) describe a retrospective cohort of chemical workers employed in an herbicide plant in Hamburg, Germany. The standardized

mortality ratio for breast cancer was elevated at 2.2 (CI 1.0-4.1). This SMR, however, was based on only nine deaths. Only 7 percent of the women in this study worked in high-exposure departments, and the small number of women precluded separate examination of those with high exposure.

TABLE 8-15 Selected Epidemiologic Studies—Female Reproductive Cancers and Breast Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Ronco et al., 1992	Danish family farm workers	429 breast	0.8 (p 8 .05)
Wiklund, 1983	Swedish economically active agricultural workers	444 breast	0.8 (0.7-0.9)[b]
Case-control studies			
Donna et al., 1984	Female residents near		
	Alessandria, Italy	18 ovarian	4.4 (1.9-16.1)
Lynge, 1985	Danish production workers	13 breast	0.9
		9 cervical	1.3
		2 endometrial	0.7
Manz et al., 1991	German production workers	9 breast	2.2 (1.0-4.1)
Saracci et al., 1991	IARC cohort	1 breast	0.3 (0.01-1.7)
		3 female genital organs	0.9 (0.2-2.7)
Environmental			
Bertazzi et al., 1989b	Seveso residents—zone B	5 breast	0.9 (0.4-2.1)
Vietnam Veterans			
Thomas et al., 1991	Women Vietnam veterans	17 breast	1.2 (0.6-2.5)
		4 uterine	0.9

[a] Given when available.
[b] 99% CI.

In a study focusing on all persons employed in the manufacture of phenoxy herbicides in Denmark before 1982, Lynge (1985) linked employment records for 1,069 women with the National Cancer Register contributing 17,624 person-years of follow-up: 13 cases of breast cancer were diagnosed, giving an SMR of 0.9; 9 cases of cervical cancer, SMR = 1.3; and 2 cases of cancer of the endometrium, SMR = 0.7.

As described elsewhere in this report, Saracci and colleagues (1991)

have established a study population comprised of members of 20 cohorts from nine countries other than the United States who were likely to have had exposure to phenoxy herbicides or TCDD. Among the more than 18,000 workers included in this cohort, 1,527 were women. Follow-up continued for an average of 17 years. It is assumed that the follow-up rates were similar for women and for men, although details are not reported. Among the exposed women there were one death from breast cancer and three from cancers of the genital organs (ovary and uterus). For both of these groups the mortality was lower than expected. Among nonexposed women, four deaths were observed due to breast cancer and three due to ovarian and uterine cancer combined. This mortality was not significantly different from that expected. Overall, the relatively small number of women and the apparent lack of exposure among these women result in little information from this study.

Agricultural/Forestry Workers Among women farm workers in Denmark the standardized incidence ratios for breast cancer, ovarian cancer, and uterine cancer were all less than 1 (Ronco et al., 1992). There were 429 cases of breast cancer diagnosed among Danish family workers, and the standardized incidence ratio of 0.8 was significantly less than unity. In this group, the standardized ratios for cervical cancer, uterine cancer, and ovarian cancer were all based on 100 or more cases, and all were significantly less than 1.

The results from Italy in the Ronco et al. study (1992) are based on far fewer cases but parallel those from Denmark. The mortality ratios for breast cancer, uterine cancer, and ovarian cancer were less than 1, but only breast cancer among self-employed women was based on more than 10 cases. It is of note that the actual level of exposure of these women to herbicides is not defined, and it remains possible that the reduced incidence of reproductive cancers reflects general patterns of female cancers seen elsewhere, in which rates are lower for rural than for urban populations.

In a similar occupational study based on census data including economically active women from Sweden (Wiklund, 1983), the standardized incidence ratio was 0.8 for breast cancer, 0.6 for cervical cancer, 0.9 for uterine cancer. These results, comparable to those reported by Ronco, are not adjusted for reproductive risk factors for these cancers, and the actual exposures of interest are not defined.

In a study specifically designed to address the relation between herbicide exposure and risk of ovarian cancer, Donna and colleagues (1984) compared exposure histories among 60 women with ovarian cancer to controls (women with cancers at other sites including breast, endometrium, cervix, and other organs). Exposure information is detailed in Chapter 7. Overall, 18 women with ovarian cancer were classified as definitely and probably exposed compared to 14 controls, giving an odds ratio of 4.4 (CI

1.9-16.1). These data provide the most direct evidence of an association between herbicides and ovarian cancer.

Environmental Studies

In Seveso, one study includes cancer mortality among women (Bertazzi et al., 1989b). The 10 year mortality follow-up provides limited information for women in the high- and medium-exposure groups. Person-years of follow-up were 2,490 in zone A (high exposure), 16,707 in zone B, 114,558 in zone R, and 726,014 in the reference area. There were only three deaths due to any cancer in females in zone A; therefore, no conclusions regarding reproductive cancers are possible. Among the 14 deaths of zone B women, 5 were due to breast cancer, resulting in a mortality ratio of 0.9 (CI 0.4-2.1). In zone R, 28 women died from breast cancer, giving a significantly reduced estimated relative risk of 0.6 (CI 0.4-0.9). There were six deaths due to ovarian cancer and four due to cancer of the uterus. Cancer incidence for breast and uterus among those in zones A and B was not significantly elevated (Pesatori et al., 1992).

The Bertazzi et al. (1989b) study follows the Seveso population until 10 years after the accident. If the TCDD released in 1976 did initiate cancers of female reproductive organs, this time is insufficient for these tumors to have come to clinical attention. In particular, women exposed to TCDD at a young age, during adolescence, may be at increased risk for cancers that could not be detected for 20 years or more after the exposure. Thus, additional follow-up is needed before the impact of the accident on female cancer incidence can be assessed with confidence.

Vietnam Veterans Studies

Thomas and colleagues (1991) assembled a list of female Vietnam veterans and followed them from 1973 to 1987. Cause-specific estimates of mortality risk among women Vietnam veterans relative to that among Vietnam era veterans were derived from proportional hazards multivariate models adjusted for rank (officer, enlisted), military occupation (nurse, non-nurse), duration of military service (at least 10 years), age at entry to follow-up, and race. Of these women, 80 percent were classified as officers/nurses, and the majority served between 3 and 19 years.

Slightly more than one-fourth of the cancer deaths were due to breast cancer among the Vietnam veterans; compared to the other Vietnam era veterans, the relative risk was not significantly elevated (RR = 1.2, CI 0.6-2.5). The small numbers of deaths within the cohort of women who served in Vietnam preclude conclusions at this time regarding Vietnam experience and cause-specific mortality; additional follow-up is essential to determine whether

risk of cancer at any specific site is truly different from that expected. Additional details on reproductive factors would facilitate this comparison.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and female reproductive (cervical, uterine, ovarian, and breast) cancers.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides.

While animal data suggest TCDD may act as an antiestrogen, and it has been shown to inhibit growth of breast cancer cell lines in tissue culture, the extrapolation to prevention of reproductive cancers is plausible but has not been demonstrated.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

GENITOURINARY CANCERS

Background

Genitourinary cancers include renal (kidney), bladder, prostate, and testicular cancer. Cancers of the female reproductive organs are discussed in the section on female reproductive cancers.

According to the American Cancer Society, 51,600 new cases of bladder cancer (ICD-9 188.0-188.9) and 26,500 new cases of kidney and other urinary cancers (ICD-9 189.0, 189.1) were diagnosed in the United States in 1992, and some 9,500 men and 10,700 women, died of these cancers (ACS, 1992). These cases are slightly more common in men than in women. Unlike other cancers, in situ bladder cancers are included in these numbers (Miller et al., 1992). According to the committee's calculations, 374 cases of bladder cancer and 307 cases of renal cancer are expected among male Vietnam veterans and 0.4 and 0.3, respectively, among female veterans in 1995. In 2000, the expected numbers are 777 bladder cancers and 497 renal cancers in male veterans and 0.7 and 0.6, respectively, in female veterans.

The American Cancer Society figures for 1992 also showed 132,000 new cases of prostate cancer (ICD-9 185) and 7,600 cases of testicular (ICD-9 186.0-186.9) and other male genital cancers diagnosed, and 34,000 and 550 deaths, respectively, due to these cancers. According to the committee's calculations, 179 cases of prostate cancer and 117 cases of testicular cancer are expected among male Vietnam veterans in 1995. In 2000, the expected numbers are 855 and 86 cases, respectively.

Histopathology

Most tumors of the kidney take the form of adenocarcinoma, which arises from the interior parenchymal tissue. Cancer of the bladder originates in the cells lining the interior of the bladder. Bladder cancer generally involves transitional cell carcinomas, histologically similar to squamous cells.

Generally, prostate cancer has an adenocarcinoma histology. Onset is associated with nonspecific changes in urine flow similar to benign conditions. Testicular cancers generally arise in sperm-producing cells. They are grouped into seminomas and nonseminomas, depending on cell histology. This is a very heterogeneous group of neoplasms ranging from placental type cells to diverse endocrine and germinal cells and mixtures of cell types. The various nonseminomatous tumors are generally more aggressive than the seminomas, and some occur at younger ages.

Epidemiology

Bladder cancer accounts for about 3 percent of all cancer deaths and has a strong male predominance. The last characteristic presumably reflects two of its known etiologic factors, cigarette smoking and occupational exposure. Smoking doubles the risk for bladder cancer (ACS, 1992), and occupational exposures to dyes, rubber, leather, paint, and specific chemicals

have been shown to increase the risk of bladder cancer. The sensitivity of the bladder mucosa to occupational carcinogens is known, perhaps reflecting their concentration in the urinary excretory system.

Leather tanners, shoe workers, and those occupationally exposed to asbestos appear to have an increased risk for cancer of the kidney. Exposure to cadmium, thorosate, and petroleum products is also associated with increased mortality from kidney cancer (Linehan et al., 1989). In addition, smoking, obesity (particularly in women) (Yu et al., 1986), and high doses of analgesics (particularly those containing phenacetin) (McLaughlin et al., 1984) increase the risk, as does cystic disease among hemodialysis patients (MacDougal et al., 1987). Cancer of the renal pelvis has been linked to analgesics (especially those with phenacetin), phenazone, and caffeine (IARC, 1987), and workers in the aniline dye, textile, plastics, and rubber industries appear to be at greater risk (Linehan et al., 1989).

The incidence of cancers of the kidney and renal pelvis increased at an average rate of 2 percent per year from 1973 to 1989, with a somewhat higher rate among women than men. Mortality rates have increased more slowly. These cancers commonly occur in middle-aged adults. Separate from these are a much smaller number of carcinomas of the renal pelvis, which are found most often after age 60. Also seen at older ages are tumors of the ureter. The incidence rates peak among 75 to 79 year olds. Bladder cancer increased at less than 1 percent per year between 1973 and 1989, but mortality rates declined by nearly 2 percent per year during that period. After age 40, incidence increases rapidly with age.

One in every 11 men develops prostate cancer, and it is the most common cancer in men (excluding skin cancers) and the second leading cause of death (Pienta and Esper, 1993). Increased age is the major risk factor; more than 80 percent occurs in men over 65. The incidence of prostate cancer increases sharply at about age 40. Among men age 65 and older, it occurs at higher rates than any other cancer. With advancing age the incidence of noninvasive prostate cancer increases. The percentage of these that undergo invasive transformation remains unknown. Prostate cancer occurs with about double the incidence in black as compared to white men. Incidence has increased since 1973 at an annual rate of about 3 percent for whites and about 2 percent for blacks. In the period 1985-1989, the rate of increase for whites had reached 6 percent per year. Specific causes of prostate cancer are unknown, but associations have been observed with family history of prostate cancer, having had a vasectomy, hormonal factors, a high-fat diet, a history of untreated venereal diseases, multiple sex partners, cigarette smoking, some occupations, and possibly exposure to ionizing radiation or cadmium (Nomura and Kolonel, 1991; Pienta and Esper, 1993). Improved detection accounts for some of the increase in incidence, but mortality rates are increasing as well. Early detection is the most important

consideration for a cure. Hormonal treatment, radiation, and/or surgery remain the methods of choice.

One major risk factor for testicular cancer is cryptorchidism (undescended testis). About 10 percent of new cases are associated with this condition (Schottenfeld and Warshauer, 1982). Other risk factors include high socioeconomic status, gonadal dysgenesis, genetic abnormalities, and specific exogenous factors that produce testicular atrophy and dysfunction (Schottenfeld and Warshauer, 1982).

Cancer of the testis is of particular interest in studying the Vietnam veterans because the cohort is mostly male and they have had an opportunity to pass through the period of peak incidence, between the ages of 20 and 45. The incidence of testicular cancer is much lower for black men than white, and between 1973 and 1989, it increased by 3 percent per year among whites but showed little change among blacks.

Epidemiologic Studies of Renal Cancer

For renal cancer, Alavanja and colleagues (1988, 1989) found excess mortality in studies of both USDA agricultural extension agents (PMR = 2.0, CI 1.2-3.3) and USDA forest and soil conservationists (PMR = 2.1, CI 1.2-3.3). Subsequent case-control studies of excess renal cancer mortality were conducted among the extension agents and forest and soil conservationists. Comparing ever versus never being an extension agent resulted in an OR of 1.7 (CI 0.9-3.3). The relative risk for being a soil conservationist was 2.4 (CI 1.0-5.9), and for being a forest conservationist RR = 1.7 (CI 0.5-5.5). Other studies of renal cancer have generally produced inconclusive results, in some cases because of small sample sizes. These include studies of chemical production workers in the United States and other countries (Lynge, 1985; Coggon et al., 1986; Bond et al., 1988; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991), agricultural workers (Burmeister, 1981; Wiklund, 1983; Ronco et al., 1992), pesticide applicators (Blair et al., 1983), paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989), the Seveso population (Pesatori et al., 1992), and Vietnam veterans (Anderson et al., 1986a,b; Breslin et al., 1988; Kogan and Clapp, 1985, 1988; Clapp et al., 1991). Results are summarized in Table 8-16.

Epidemiologic Studies of Bladder Cancer

For bladder cancer, Fingerhut and colleagues found an excess mortality in studies of TCDD production workers. In the total cohort of 5,172 workers there was an SMR = 1.6 (CI 0.7-3.0) based on 9 cases. In workers with at least 1 year of employment and 20 years latency there were 4 cases (SMR = 1.9, CI 0.5-4.8).

TABLE 8-16 Selected Epidemiologic Studies—Renal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Fingerhut et al., 1991	NIOSH cohort	8	1.4 (0.6-2.8)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0-6.2)
Lynge, 1985	Danish production workers—men	3	0.6
Coggon et al., 1986	British MCPA production workers	5	1.0 (0.3-2.3)
Manz et al., 1991	German production workers	3	1.6 (0.3-4.6)
Saracci et al., 1991	IARC cohort	11	1.0 (0.5-1.7)
Burmeister, 1981	Farmers in Iowa	178	1.1 (NS)
Wiklund, 1983	Swedish agricultural workers	775	0.8 (0.7-0.9)[b]
Ronco et al., 1992	Danish male self-employed farm workers	141	0.6 ($p < .05$)
Alavanja et al., 1988	USDA agricultural extension agents		1.7 (0.9-3.3)
Alavanja et al., 1989	USDA forest conservationists		1.7 (0.5-5.5)
	Soil conservationists		2.4 (1.0-5.9)
Blair et al., 1983	Florida pesticide applicators	1	0.5
Robinson et al., 1986	Paper and pulp workers	6	1.2 (0.5-3.0)
Henneberger et al., 1989	Paper and pulp workers	3	1.5 (0.3-4.4)
Environmental			
Pesatori et al., 1992	Seveso male residents zones A and B	0	—
	Female residents zones A and B	1	1.1 (0.2-8.1)
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	55	0.9 (0.5-1.5)
	Marine Vietnam veterans	13	0.9 (0.5-1.5)
Anderson et al., 1986a	Wisconsin Vietnam veterans	1	—
Anderson et al, 1986b	Wisconsin Vietnam veterans	2	—
Kogan and Clapp, 1988	Massachusetts Vietnam veterans	9	1.8 (1.0-3.5)

NOTE: NS = not significant.

[a]Given when available.
[b]99% CI.

Other studies of bladder cancer have produced inconclusive results. Occupational studies include studies of chemical production workers in the United States and other countries (Lynge, 1985; Coggon et al., 1986; Bond et al., 1988; Zober et al., 1990; Saracci et al., 1991), agricultural and forestry workers (Burmeister, 1981; Alavanja et al., 1988, 1989; Green, 1991; Ronco et al., 1992), pesticide applicators (Blair et al., 1983), and paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989). Environmental

studies of bladder cancer and herbicide or TCDD exposure include the Pesatori et al. (1992) study of Seveso residents and the Lampi et al. (1992) study of a Finnish community exposed to chlorophenols. Studies in Vietnam veterans examining bladder cancer include the Breslin et al. (1988) study of Army and Marine Corps Vietnam veterans and the Wisconsin State study (Anderson et al., 1986a,b). Results are summarized in Table 8-17.

TABLE 8-17 Selected Epidemiologic Studies—Bladder Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Fingerhut et al., 1991	NIOSH cohort	9	1.6 (0.7-3.0)
	20 year latency, ≥1 year exposure	4	1.9 (0.5-4.8)
Saracci et al., 1991	IARC cohort	13	0.8 (0.2-1.4)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0-7.2)
Lynge, 1985	Danish production workers—men	11	0.8
Coggon et al., 1986	British MCPA production workers	8	0.9 (0.4-1.7)
Zober et al., 1990	BASF production workers	0	— (0.0-15.0)
Burmeister, 1981	Farmers in Iowa	274	0.9 (NS)
Ronco et al., 1992	Danish male self-employed farmers	300	0.6 ($p < .05$)
Green, 1991	Canadian forestry workers	1	1.0 (0.01-5.6)
Alavanja et al., 1988	USDA agricultural extension agents	8	0.7 (0.4-1.4)
Alavanja et al., 1989	USDA forest/soil conservationists	8	0.8 (0.3-1.6)
Blair et al., 1983	Florida pesticide applicators	3	1.6
Robinson et al., 1986	Paper and pulp workers	8	1.2 (0.6-2.6)
Henneberger et al., 1989	Mortality amg paper and pulp workers	4	1.2 (0.3-3.2)
Environmental			
Pesatori et al., 1992	Seveso male residents—zones A and B	10	1.6 (0.9-3.1)
	Female residents—zones A and B	1	0.9 (0.1-6.8)
Lampi et al., 1992	Finnish community exposed to chlorophenols		1.0 (0.6-1.9)
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	9	0.6 (0.3-1.2)
	Marine Vietnam veterans	4	2.4 (0.1-66.4)
Anderson et al., 1986a	Wisconsin Vietnam veterans	0	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	1	—

NOTE: NS = not significant.

[a]Given when available.

Epidemiologic Studies of Prostate Cancer

For prostate cancer, several studies have shown elevated risk in agricultural or forestry workers. Mortality was raised in studies of USDA agricultural extension agents (PMR = 1.5, CI 1.1-2.0) and forest and soil conservationists (PMR = 1.6, CI 1.1-2.0) (Alavanja et al., 1988, 1989). However, subsequent case-control analysis of these deaths showed no increased risk of prostate cancer for ever being an extension agent (OR = 1.0, CI 0.7-1.5) or a soil conservationist (OR = 1.0, CI 0.6-1.8). The risk was elevated for forest conservationists (OR = 1.6, CI 0.9-3.0). A case-control study of white male Iowans who died of prostate cancer (Burmeister et al., 1983) found a significant association (OR = 1.2) with farming, which was not connected to a specific agricultural exposure.

In a large cohort study of Canadian farmers, Morrison et al. (1993) found that an increased risk of prostate cancer was associated with herbicide spraying, and increasing risk was shown with increasing number of acres sprayed. For the entire cohort, the relative risk for prostate cancer and spraying at least 250 acres was 1.2 (CI 1.0-1.5). Adjustment for potential confounders in the analysis showed no evidence of confounding for the association. Additionally, the analysis was restricted to a one-third sample of farmers most likely to be exposed to phenoxy herbicides or other herbicides (RR = 1.3, CI 1.0-1.8 for $\geq$ 250 acres sprayed) and was further restricted for analysis by those with no employees (RR = 1.4, CI 1.0-1.9 for $\geq$ 250 acres sprayed), no custom expenses for assisting in work and which may include spraying (RR = 1.6, CI 1.1-2.2 for $\geq$ 250 acres sprayed), age between 45-69 years (RR = 1.7, CI 1.1-2.8 for $\geq$ 250 acres sprayed), and a combination of the three restrictions (RR = 2.2, CI 1.3-3.8 for $\geq$ 250 acres sprayed). For each of these restricted comparisons, a statistical test for trend over increasing number of acres sprayed was significant.

Other occupational and environmental studies of prostate cancer generally have been consistent. These include studies of chemical production workers in the United States and other countries (Bond et al., 1988; Lynge, 1985; Coggon et al., 1986; Zober et al., 1990), agricultural workers (Burmeister, 1981; Wiklund, 1983; Ronco et al., 1992), pesticide applicators (Blair et al., 1983; Swaen et al., 1992), paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989; Solet et al., 1989), the Seveso population (Bertazzi et al., 1989a,b; Pesatori et al., 1992), and Vietnam veterans (Anderson et al., 1986a,b; Breslin et al., 1988).

Studies of prostate cancer among Vietnam veterans or following environmental exposures have not consistently shown an association. However, prostate cancer is generally a disease of older men, and the risk among Vietnam veterans would not yet be detectable in currently published epidemiologic studies.

Summary for Prostate Cancer

Most of the agricultural studies indicate some elevation in risk of prostate cancer. One large well-done study in farmers showed an increased risk, and subanalyses in this study indicate that the increased risk is specifically associated with herbicide exposure (OR = 2.2, CI 1.3-3.8; Morrison et al., 1993). The three major production worker studies (Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991) all show a small, but not statistically significant, elevation in risk. In the subcohort with at least 20 years latency and at least 1 year of exposure, the SMR increased slightly in the NIOSH cohort (SMR = 1.5, CI 0.7-2.9). Most of the studies use mortality as an outcome, so detection bias is not likely to explain these results. It should be noted, however, that most of the associations are relatively weak (<1.5). Vietnam veterans have not yet reached the age when this cancer tends to appear. Results are summarized in Table 8-18.

Epidemiologic Studies of Testicular Cancer

A case-control study of 137 testicular cancer cases and 130 hospital controls (Tarone et al., 1991) found an odds ratio of 2.3 (CI 1.0-5.5) for service in Vietnam. Risk was not significantly elevated for testicular cancer by service branch. In general, the other veteran studies and all the occupational and environmental studies have shown no association between exposure and outcome, but the sample size of some of these studies may have been too small to detect an elevated risk. Other studies of testicular cancer have generally been inconsistent. These include studies of chemical production workers in the United States and other countries (Coggon et al., 1986; Bond et al., 1988; Saracci et al., 1991), agricultural workers (Wiklund, 1983; Ronco et al., 1992), residents of Seveso (Pesatori et al., 1992), and Vietnam veterans (Anderson et al., 1986a,b; Breslin et al., 1988; Watanabe et al., 1991).

Summary for Testicular Cancer

One case-control study showed a significant elevation in risk of testicular cancer for Vietnam service, but there is no information regarding herbicide exposure in Vietnam. The Saracci study also showed an elevated risk for production workers, based on seven cases, while the study of Dow workers (Bond et al., 1988) showed an elevated risk based on only one case. Results are summarized in Table 8-19.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure

TABLE 8-18 Selected Epidemiologic Studies—Prostate Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Fingerhut et al., 1991	NIOSH cohort	17	1.2 (0.7-2.0)
	≥20 year latency, ≥1 year exposure	9	1.5 (0.7-2.9)
Bond et al., 1988	Dow 2,4-D production workers	1	1.0 (0.0-5.8)
Coggon et al., 1986	British MCPA production workers	18	1.3 (0.8-2.1)
Lynge, 1985	Danish production workers	9	0.8
Manz et al., 1991	German production workers	7	1.4 (0.6-2.9)
Zober et al., 1990	BASF production workers	0	— (0-7.5)
Saracci et al., 1991	IARC cohort	30	1.1 (0.8-1.6)
Burmeister, 1981	Iowa farmers	1,138	1.1 ($p < .01$)
Morrison et al., 1993	Canadian farmers		
	Age 45-69 years, no employees or custom workers, sprayed ≥ 250 acres	20	2.2 (1.3-3.8)
Ronco et al., 1992	Danish self-employed farm workers	399	0.9 ($p < .05$)
Wiklund, 1983	Swedish agricultural workers	3,890	1.0 (0.9-1.0)[b]
Blair et al., 1983	Florida pesticide applicators	2	0.5
Swaen et al., 1992	Dutch herbicide applicators	1	1.3 (0.0-7.3)
Solet et al., 1989	Paper and pulp workers	4	1.1 (0.3-2.9)
Robinson et al., 1986	Paper and pulp workers	17	1.2 (0.7-2.0)
Henneberger et al., 1989	Paper and pulp workers	9	1.0 (0.7-2.0)
Case-control studies			
Burmeister et al., 1983	Iowa residents		1.2 ($p < .05$)
Alavanja et al., 1988	USDA agricultural extension agents		1.0 (0.7-1.5)
Alavanja et al., 1989	USDA forest conservationists		1.6 (0.9-3.0)
	Soil conservationists		1.0 (0.6-1.8)
Environmental			
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	19	1.6 (1.0-2.7)
Bertazzi et al., 1989b	Seveso male residents—zone B	3	2.2 (0.7-6.9)
Pesatori et al., 1992	Seveso residents—zones A and B	4	1.4 (0.5-3.9)
	Zone R	17	0.9 (0.6-1.5)
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	30	0.9 (0.6-1.2)
	Marine Vietnam veterans	5	1.3 (0.2-10.3)
Anderson et al., 1986b	Wisconsin Vietnam veterans	2	—

[a]Given when available.
[b]99% CI.

to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and prostate cancer.

TABLE 8-19 Selected Epidemiologic Studies—Testicular Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Bond et al., 1988	Dow 2,4-D production workers	1	4.6 (0.0-25.7)
Coggon et al., 1986	British MCPA production workers	4	2.2 (0.6-5.7)
Saracci et al., 1991	IARC cohort	7	2.3 (0.9-4.6)
Wiklund, 1983	Swedish agricultural workers	101	1.0 (0.7-1.2)[b]
Ronco et al., 1992	Danish self-employed farm workers	74	0.9
Environmental			
Pesatori et al., 1992	Seveso residents—zones A and B	1	0.9 (0.1-6.7)
	Residents—zone R	9	1.5 (0.7-3.0)
Vietnam veterans			
Cohort studies			
Breslin et al., 1988	Army Vietnam veterans	90	1.1 (0.8-1.5)
	Marine Vietnam veterans	26	1.3 (0.5-3.6)
Watanabe et al., 1991	Army Vietnam veterans	109	1.2
	Marine Vietnam veterans	28	0.8
Anderson et al., 1986b	Wisconsin Vietnam veterans	9	1.0 (0.5-1.9)
Anderson et al., 1986a	Wisconsin Vietnam veterans	11	1.0 (0.5-1.7)
Case-control studies			
Tarone et al., 1991	Patients at three Washington, D.C., area hospitals		2.3 (1.0-5.5)

[a]Given when available.
[b]99% CI.

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* and renal cancer or testicular cancer.

There is limited/suggestive evidence of no association between exposure to herbicides* and urinary bladder cancer.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD

has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

BRAIN TUMORS

Background

According to the American Cancer Society, 16,900 new cases of brain and other nervous system cancers (ICD-9 191.0-191.9, 192.0-192.3, 192.8-192.9) were diagnosed in the United States in 1992, and some 11,800 men and women died of these cancers (ACS, 1992). These cases are slightly more common in men than in women. According to the committee's calculations, 226 cases of cancers of brain and nervous system are expected among male Vietnam veterans and 0.4 among female veterans in 1995. In 2000, the expected numbers are 268 cases in male veterans and 0.4 in female veterans.

The most common cancer arising in the brain is known as a glioma. Several subtypes have been established on the basis of cellular origin and clinical characteristics; these include glioblastoma multiforme (generally the most invasive), astrocytomas, ependymoma, medulloblastoma, and oligodendrocytoma (Shapiro, 1986). Malignant tumors may also involve the spinal cord. Brain cancers also occur as metastases of cancers elsewhere in the body, but only primary brain cancers are considered here.

Compared to other cancers, brain and nervous system cancers occur at relatively high rates among the young; incidence increases only moderately at older ages. The incidence of brain and nervous system cancers has been increasing steadily since 1973, particularly among those over age 65. Mortality has increased at older ages as well but has declined slightly among

those under age 65. Some of the increase in incidence may be due to improved diagnostic abilities with the development of noninvasive scanning technologies, but the continued increase after widespread adoption of these techniques suggests that improved diagnosis does not account for all of the change.

Little is known about the causes of primary brain tumors. Epidemiologic studies in the past have occasionally suggested an excess risk for chemical workers exposed to glues and other types of chemicals.

Epidemiologic Studies

A case-control study of gliomas and occupational exposure to chemical carcinogens was conducted in Italy (Musicco et al., 1988). It was found that farmers had an increased risk of gliomas (RR = 1.6, CI 1.1-2.4) compared to all controls; this was found to be associated with the use of chemicals by these farmers, including insecticides and herbicides. Another occupational study (Alavanja et al., 1988) found a PMR of 2.1 (CI 1.2-3.7) among USDA agricultural extension agents. A subsequent case-control analysis comparing ever versus never being an extension agent resulted in an OR = 1.0 (CI 0.4-2.4). A study of Wisconsin veterans (Anderson et la., 1986a) showed an excess risk (RR = 1.6, CI 0.9-2.7).

On the other hand, no excess risk of central nervous system (CNS) tumors has been found among other occupational groups or in other studies. Other than the study of Wisconsin veterans, other studies have not shown a consistent increase in CNS tumors (see Table 8-20).

The studies that had inconsistent findings with regard to brain cancers included studies of chemical production workers in the United States and other countries (Lynge, 1985; Coggon et al., 1986; Bond et al., 1988; Fingerhut et al., 1991; Saracci et al., 1991), agricultural workers (Burmeister, 1981; Wigle et al., 1990; Morrison et al., 1992; Ronco et al., 1992), pesticide applicators (Blair et al., 1983; Swaen et al., 1992), paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989), the Seveso population (Bertazzi et al., 1989a; Pesatori et al., 1992), and Vietnam veterans (Lawrence et al., 1985; Anderson et al., 1986a,b; Breslin et al., 1988; Thomas and Kang, 1990).

Summary

Although the number of cases of brain tumors is small in many studies, it is apparent that the risks associated with herbicide exposure are fairly evenly distributed around the null, and the confidence intervals are relatively narrow. Table 8-20 summarizes the results.

TABLE 8-20 Selected Epidemiologic Studies—Brain Tumors

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Occupational			
Cohort studies			
Fingerhut et al., 1991	NIOSH cohort	5	0.7 (0.2-1.6)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0-4.1)
Coggon et al., 1986	British MCPA production workers	11	1.2 (0.6-2.2)
Saracci et al., 1991	IARC cohort	6	0.4 (0.1-0.8)
Lynge, 1985	Danish production workers—men	4	0.7
Burmeister, 1981	Farmers in Iowa	111	1.1 (NS)
Wigle et al., 1990	Saskatchewan farmers	96	1.0 (0.8-1.3)
Morrison et al., 1992	Canadian prairie farmers		
	250+ acres sprayed with herbicides	24	0.8 (0.5-1.2)
Ronco et al., 1992	Danish male self-employed		
	farm workers	194	1.1
Blair et al., 1983	Florida pesticide applicators	5	2.0
Swaen et al., 1992	Dutch herbicide applicators	3	3.2 (0.6-9.3)
Robinson et al., 1986	Paper and pulp workers	4	0.6 (0.2-2.1)
Henneberger et al., 1989	Paper and pulp workers	2	1.2 (0.1-4.2)
Case-control studies			
Musicco et al., 1988	Men and women in the Milan,		
	Italy, area	61	1.6 (1.1-2.4)
Alavanja et al., 1988	USDA agricultural extension agents		1.0 (0.4-2.4)
Alavanja et al., 1989	USDA forest/soil conservationists	6	1.7 (0.6-3.7)
Environmental			
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	5	1.2 (0.4-3.1)
	Female residents—zones A, B, R	5	2.1 (0.8-5.9)
Pesatori et al., 1992	Seveso male residents—zones A and B	0	—
	Female residents—zones A and B	1	1.5 (0.2-11.3)
Vietnam veterans			
Breslin et al., 1988	Army Vietnam veterans	116	1.0 (0.3-3.2)
	Marine Vietnam veterans	25	1.1 (0.2-7.1)
Thomas and Kang, 1990	Army Chemical Corps Vietnam		
	veterans	2	5.0
Anderson et al., 1986a	Wisconsin Vietnam veterans	13	1.6 (0.9-2.7)
Anderson et al., 1986b	Wisconsin Vietnam veterans	8	0.8 (0.3-1.5)
Lawrence et al., 1985	New York Vietnam veterans	4	0.5 (0.2-1.5)

NOTE: NS = not significant.

[a]Given when available.
[b]99% CI.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of no association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and brain tumors.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. if TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantity the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

MALIGNANT LYMPHOMAS

Background

According to the American Cancer Society, 60,900 new cases of lymphomas and myelomas were diagnosed in the United States in 1992, and some 30,100 men and women died from these cancers (ACS, 1992). These diseases are slightly more common in men than in women.

The malignant lymphomas are a group of morphologically related neoplasms derived from lymphoreticular cells in lymph nodes, bone marrow, spleen, liver, or other sites in the body such as the skin, intestine, and lung. The common stem cell origin in lymphoreticular tissue in lymph nodes and

extranodular tissues underscores their unity despite a plethora of histologic and immunologic cell subtypes. Significant microscopic characteristics important for treatment and prognosis are the basis for separating the lymphomas into Hodgkin's disease (HD; ICD 201.0-201.9), non-Hodgkin's lymphomas (NHL; ICD-9 200.0-200.8, 202.0-202.2, 202.8-202.9), and multiple myeloma (MM; ICD-9 203.0, 203.2-203.8).

In 1992, the annual incidence and deaths for each cancer were as follows:

	Males		Females	
Cancer	New Cases	Deaths	New Cases	Deaths
Hodgkin's disease	4,200	900	3,200	600
Non-Hodgkin's lymphomas	23,000	10,000	18,000	9,400
Multiple myeloma	6,300	4,700	6,200	4,500

According to the committee's calculations, 94 new cases of Hodgkin's disease, 380 new cases of non-Hodgkin's lymphoma, and 57 new cases of multiple myeloma are expected among male Vietnam veterans and a total of 0.8 of all of these cancers among female veterans in 1995. In the year 2000, the expected numbers are 109, 494, and 133 cases, respectively, in male veterans and a total of 1.1 cases in female veterans.

Histopathology

Non-Hodgkin's Lymphoma About 60 percent of lymphomas in Europe and North America are NHL. Although NHL and HD show similarities in clinical and pathological features, they are sufficiently distinct to warrant the subclassification. There are at least 10 subgroups of NHL, the majority being derived from B cells. The widely used Rappaport (McGee et al., 1992) classification identifies five types, which in turn are described as nodular or diffuse: (1) lymphocytic, well differentiated; (2) lymphocytic, poorly differentiated; (3) mixed lymphocytic-histiocytic; (4) histiocytic; and (5) undifferentiated. The best prognosis occurs with a follicular structure and small lymphocytes. The more recent Kiel (McGee et al., 1992) classification divides NHL into 9 types and 19 subtypes on the basis of morphology.

Hodgkin's Disease The presence of Reed-Sternberg cells in biopsied lymph nodes distinguishes HD from other lymphomas (Lacher, 1986). The nodes also show characteristic structural abnormalities. Four types of HD have been identified: lymphocyte predominant, nodular sclerosis, mixed cellularity, and lymphocyte depleted. Prognosis shows some relationship to type but more to the stage of the disease.

Multiple Myeloma Multiple myeloma is characterized by proliferation of bone marrow stem cells resulting in an excess of neoplastic plasma cells with the production of excess abnormal proteins, usually immunoglobulins. Identification of these proteins in the blood or urine (Bence-Jones protein) represents the best diagnostic feature of this disease. Multifocal aggregates of plasma cells in bone result in destructive "punched-out" bone lesions. Renal deposits of myeloma cells (interstitial infiltrates) occur in about 75 percent of cases.

Epidemiology

Both viral infections and chemical exposures have been postulated as risk factors for this group of diseases, but the cause(s) remain unknown. The incidence of NHL has increased by more than 3 percent per year since 1973. Among men under age 65, the increase has been 4 percent per year and may reflect an association of NHL with human immunodeficiency virus (HIV) infection and AIDS. Mortality rates have also increased, particularly at older ages. The incidence of NHL begins increasing at younger ages than many cancers and generally shows a steady increase with age. NHL has been associated with the Epstein-Barr virus (EBV) and with altered immune system function as observed in HIV infection or from medications administered to organ transplant patients (Fraumeni and Hoover, 1977). Exposure to industrial solvents, vinyl chloride, or herbicides (Zahm et al., 1990) has also been linked to NHL (Hartge and Devesa, 1991).

The immunodeficiency associated with AIDS has led to substantial increases in the incidence of NHL, particularly among single males, aged 20-49, in metropolitan areas (Gail et al., 1991; Rabkin et al., 1991). However, the increase has been evident in U.S. cancer registries only since 1983, when the disease increased rapidly and when treatments increased the survival of patients with AIDS (Gail et al., 1991). The Selected Cancers Study of the CDC excluded individuals with AIDS or a related illness. This was not done in other studies. However, virtually all studies of agricultural or forestry use of herbicides covered periods prior to the AIDS-related increase in NHL. This was especially true for those studies in Sweden and the United States showing strong positive effects. Of four case-control studies with a high percentage of cases after 1983, three obtained information on and took account of AIDS. Finally, the agricultural/forestry groups are populations not known to be at high risk for AIDS. Thus, AIDS will not play an important role in the following analyses relating NHL to herbicide usage.

A decrease over the past 20 years in the incidence of HD has been greatest at older ages. The decline in the mortality rate during that period has been similar at younger and older ages. Unlike many cancers, incidence

rates show two peaks: among 20 to 24 year olds of both sexes and among men age 80 to 84. Individuals with infectious mononucleosis infections, caused by the Epstein-Barr virus (EBV), have three times the risk of developing HD as the nonexposed population. Patients with HD also have an increased incidence and titer of antibodies to EBV (Herbst et al., 1992). No significant environmental risk factors have been identified for HD (Page and Asire, 1985), but studies indicate that exposure to wood dust might increase the risk for HD (Grufferman and Delzell, 1984). Some studies have reported a clustering or aggregation of HD cases, suggesting person-to-person transmission (Vianna and Polan, 1973), but such clustering has not been observed in other studies (Grufferman et al., 1979). HD appears to occur more frequently among the well educated and has been associated with ataxia telangiectasia, a rare genetic immune deficiency disease (Miller et al., 1992).

The incidence of MM has changed little since 1973 and has shown no consistent trend upward or downward during that period. The incidence of MM is very low at ages under 40 years. Incidence reaches a peak at age 70 and older. Mortality rates have shown a small but steady increase of about 1.5 percent per year. Rates for blacks are about twice those for whites. Risk factors for MM remain unclear. Links to exposure to ionizing radiation have been suggested by studies of Japanese atomic bomb survivors (Higami et al., 1990), U.S. radiologists (Matanoski et al., 1975), and radium dial workers (Stebbings et al., 1984). Increases in multiple myeloma deaths have also been seen in those engaged in farming and agricultural work and for nonspecific occupational exposure to metals, rubber, leather, paint, and petroleum (Riedel et al., 1991). The evidence for an association with immune suppressive diseases is mixed. Some studies indicate an increased risk in association with such conditions as allergies, rheumatoid arthritis, and rheumatic fever (Riedel et al., 1991). New research suggests the possibility of genetic factors as well (Riedel et al., 1991).

Epidemiologic Studies of Non-Hodgkin's Lymphoma

Several classes of epidemiologic studies provide information concerning the association of non-Hodgkin's lymphoma with herbicide exposure or exposure to TCDD. Concern that herbicide or TCDD exposure may be associated with NHL dates from the study of Hardell in 1979. Stimulated by having a patient with a histiocytic lymphoma and heavy exposure to phenoxyacetic acids, Hardell (1979) collected information on subsequent cases of non-Hodgkin's lymphoma of the histiocytic type admitted to the University Hospital in Umea, Sweden. Of 17 cases identified over a nine month period, 14 had occupations compatible with possible exposure to phenoxy herbicides or chlorophenols, and 11 cases reported such exposures.

A later case-control analysis was published by Hardell and colleagues (Hardell et al., 1981) of 168 cases of NHL and HD that suggested an association with herbicide exposure. As with Hardell and Sandstrom's (1979) earlier work in Sweden suggesting a similar association of soft tissue sarcoma with herbicide exposure, many of the exposed workers were in forestry and agriculture industries using 2,4,5-T, which contains trace amounts of TCDD. It was noted early, however, that some herbicides used extensively in Sweden (2,4-D and MCPA) were highly unlikely to contain TCDD. These Swedish findings prompted further studies in Sweden and many studies of the health effects of herbicide use in other countries, including the United States.

Occupational Studies

Production Workers Two studies dominate the evidence relating exposure to TCDD and a risk of NHL, in that their results include virtually all studies providing exposure information in plants producing phenoxy herbicides or chemicals potentially contaminated with TCDD. Thus, the focus is mainly on these two studies, and reference to information from studies of individual plants is made only as needed.

The study by Fingerhut and colleagues (1991) is one of the two comprehensive mortality studies of production workers published: 10 non-Hodgkin's lymphoma deaths occurred and 7.3 were expected (SMR = 1.4, CI 0.7-2.5). Those with unknown vital status were assumed to be alive, which may slightly dilute some of the observed SMRs. No SMR for any specific site was significantly elevated. An analysis of mortality according to duration of exposure was unrevealing with respect to NHL. It is noteworthy that among employees of one of the companies, Dow Chemical Company, 6 cases of NHL were seen, compared to 2.9 expected (Bond et al., 1989). This implies an SMR of 0.9 for the remaining 11 companies, comprising 58 percent of the study population. For the group with 20 year latency there were 4 deaths, 3.6 expected.

Bond and colleagues (1988) have published data on the mortality experience of 878 employees of the Dow Chemical Company involved in the production of 2,4-D: 2 deaths from lymphosarcoma and reticulosarcoma occurred; 0.5 was expected.

A study by Saracci and colleagues (1991) assembled multinational cohorts in a manner similar to Fingerhut et al. (1991). In contrast to the U.S. study, exposed workers included both production workers and herbicide sprayers, and some cohort members may have had little, if any, exposure to TCDD. Among the non-Hodgkin's lymphomas, the overall number of deaths for exposed and probably exposed was similar to that expected (11 observed versus 11.6 expected). There was an excess among production workers

(SMR = 1.5, eight deaths) and a deficit among sprayers (SMR = 0.5, three deaths). It is likely that most workers in this latter group were not exposed to compounds with TCDD contamination. There was no evident trend in analysis by years since first exposure.

Additional workers exposed to TCDD in production accidents have been reported separately. Zober and colleagues (1990) studies 247 employees of BASF who were exposed during and after an accident in 1953. Of 78 deaths that occurred through 1987, none were from NHL (less than 0.5 were expected).

A cohort mortality study was conducted (Manz et al., 1991) of the work force of a plant in Hamburg, Germany, "heavily contaminated with TCDD" during trichlorophenol (TCP) and 2,4,5-T production. Based on measured TCDD levels in 48 workers, an average TCDD level of 150 parts per trillion (ppt) can be estimated for the group. In comparison with gas workers, cancers of the lymphatic and hematopoietic system (9 observed, 3.4 expected) were significantly increased (SMR = 2.7, CI 1.2-5.0). Three of the nine were non-Hodgkin's lymphoma, but expected numbers for NHL were not available.

Summary of Production Worker Studies The production studies suggest an increased risk of NHL from exposure to TCDD-contaminated chemicals, but not of a degree that would allow a definitive statement to be made. The data on NHL deaths among 2,4-D manufacturing workers were too limited for estimation of a risk because of the few deaths that occurred. One conclusion from these studies is that exposure to phenoxy herbicides per se is a more important risk factor than is any TCDD contaminant.

Agricultural/Forestry Workers The first epidemiologic study of NHL and HD in relation to occupation and exposures to various chemicals, including phenoxy acids and chlorophenols, was a case-control study by Hardell and colleagues (1980, 1981). Results of Swedish studies are shown in Table 8-21. An odds ratio of 6.0 (CI 3.7-9.7) was found for exposure to phenoxy acids or chlorophenols. By considering only exposure to phenoxy acids and dissolving explicit case-control matching, odds ratios of 7.0 and 4.3 were found for exposure to phenoxy acids for 90 or more days and less than 90 days, respectively. Only exposures five or more years prior to case or control identification were considered. The odds ratios were 4.8 (CI 2.9-8.1) for any exposure to phenoxy acids and 4.3 (CI 2.7-6.9) for any exposure to chlorophenols. Although no data were given, it was stated that there was no noticeable difference between NHL and HD in the excess risks from exposures to phenoxy acids or chlorophenols (Hardell et al., 1981).

A later study by Hardell (Hardell, 1981) investigated the validity of exposure assessment in the above study and the possibility of bias contributing to the findings. The results of case-control analyses, using questionnaire

response exposure data, indicated a risk ratio of 4.1 for exposure to phenoxy acids within five years prior to diagnosis, versus 4.8 when both questionnaire and interview data were used (Hardell et al., 1980), which suggests that interview data introduced little bias. Analyses within forestry or agricultural worker groups, according to continuous or limited employment since 1950, showed, respectively, odds ratios of 0.8 and 1.0 for workers unexposed to phenoxy acids versus 5.1 and 2.4, respectively, for continuous and limited forestry/agriculture employment with exposure to phenoxy acids. The odds ratios of about 1.0 for unexposed individuals suggest that inappropriate attribution of unexposed cases to an exposed category was unlikely to have occurred.

TABLE 8-21 Non-Hodgkin's Lymphoma Results From Swedish Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Case-control studies			
Hardell et al., 1980	Umea Hospital patients		
	Exposed to phenoxy acids	41	4.8 (2.9-8.1)[b]
	Exposed to chlorophenols	50	4.3 (2.7-6.9)[b]
Persson et al., 1989	Orebro Hospital		
	Exposed to phenoxy acids	6	4.9 (1.0-27.0)
Olsson and Brandt, 1988	Lund Hospital patients		
	Exposed to herbicides		1.3 (0.8-2.1)
	Exposed to chlorophenols		1.2 (0.7-2.0)
Cancer registry studies			
Wiklund, 1983	Swedish agricultural workers		1.1 (0.9-1.2)
Wiklund et al., 1989b	Swedish pesticide applicators	27	1.1 (0.7-1.6)
Wiklund et al., 1988a	Swedish agricultural and forestry workers		
	Workers in land/animal husbandry		1.0 (0.9-1.1)
	Timber cutters		0.9 (0.7-1.1)

[a]Given when available.
[b]Includes both non-Hodgkin's lymphoma and Hodgkin's disease.

A more recent study (Eriksson et al., 1992) investigated the incidence of NHL in relation to specific occupations using cases from the Swedish Cancer Registry. A significant increased SIR of 1.2 for carpenters, but not for farmers, forestry workers, or horticultural workers, was found. Specific exposures of individuals to phenoxy acids or TCDD were not elicited in this study.

Another Swedish study (Persson et al., 1989) investigated the relationship

of NHL to occupational exposure among 106 cases from the register of the Department of Oncology of the Orebro Hospital. By using logistic regression, an OR of 4.9 (CI 1.0-27.0) was found for exposure to phenoxy acids. Other chemicals showing significantly increased risks of disease included solvents and creosote. Despite the high odds ratio for herbicide exposure, the study showed a significantly reduced OR = 0.3 (CI 0.1-1.0) for farming, based on seven cases.

One study by Olsson and Brandt (1988) investigated the relationship between exposure to phenoxy herbicides, chlorophenols, or solvents and NHL among 167 cases admitted to the University Hospital in Lund. The adjusted odd ratios for all cases compared to controls were 2.0 (CI 1.5-2.6), 1.3 (CI 0.8-2.1), and 1.2 (CI 0.7-2.0) for exposures to solvents, herbicides, and chlorophenols, respectively. For NHL the odds ratios were 3.4 (CI 2.3-5.2) and 1.7 (CI 0.7-4.2) for exposure to solvents and chlorophenols, respectively; these estimates were adjusted for age and other risk factors. No important interactions were found between risk factors. A weakness of the study was that one of two control groups came from all of Sweden rather than the catchment area of the hospital. The second group, which was geographically similar to cases, was on average 10 years younger and might involve different activities, and interviewers were aware of the case-control status of the subject, which may have biased the interview.

In contrast to the studies of Hardell and colleagues, those of Wiklund (1983) and Wiklund et al. (1988a) used disease data from the Swedish Cancer Registry and employment data from the decennial census, and do not demonstrate a relation of NHL with occupations that might have involved exposure to phenoxy acids. An initial study (Wiklund, 1983) focusing on agricultural workers showed an SIR of 1.1 for NHL from 1961 to 1973. No information on the degree or frequency of exposure to agricultural chemicals was obtained.

NHL incidence was further investigated (Wiklund et al., 1987, 1989a) in a cohort of 20,245 pesticide applicators licensed between 1965 and 1976, and followed through 1982; the SIR = 1.0 (CI 0.6-1.5) for NHL (Wiklund et al., 1987). Results from a mail questionnaire of 273 cohort members indicated that 72 percent of the respondents had exposure to phenoxy acids for 1 day or more per year and 10 percent had exposure for more than 20 days per year. An extension of this study through 1984 (Wiklund et al., 1989b) did not significantly change the overall SIR, although a nonsignificant trend according to years since license was observed.

A cohort study (Wiklund et al., 1988a) of agricultural and forestry workers also showed no increased risk of NHL compared to other employed Swedish males during the years 1961-1979. The relative risk for NHL was not significantly increased in any occupational subcohort, did not differ significantly between subcohorts, and showed no trends with calendar year

in any group. However, it was mentioned in the Wiklund et al. (1989b) study that only 16 percent of forestry workers ever used phenoxy acid herbicides.

After the publications by Hardell and his colleagues, a number of studies were undertaken in the United States, most under the aegis of the National Cancer Institute (NCI), to assess the association of NHL in agricultural circumstances in the United States with actual or potential exposure to phenoxy acids (Table 8-22).

Some studies provide information on NHL odds ratios only in relation to farm employment but had hypothesized that herbicide exposure was a risk factor of interest. A case-control study for NHL deaths in relation to farming for 1958-1983 in Hancock County, Ohio, found an overall odds

TABLE 8-22 Non-Hodgkin's Lymphoma Results from U.S. Case-Control Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Alavanja et al., 1988	USDA extension agents		1.2 (0.7-2.3)
Alavanja et al., 1989	USDA soil conservationists		1.8 (0.7-4.1)
	USDA forest conservationists		2.5 (1.0-6.3)
Burmeister et al., 1983	Iowa residents		
	Farmers		1.3
	Farmers in 33 counties with highest herbicide use		
	Born before 1890		3.4
	Born 1890-1900		2.2
	Born after 1900		1.3
Cantor, 1982	Wisconsin residents	175	1.2 (1.0-1.5)
Dubrow et al., 1988	Ohio residents	15	1.6 (0.8-3.4)
Hoar et al., 1986	Kansas residents		
	Farmers compared to nonfarmers	133	1.4 (0.9-2.1)
	Farmers using herbicides > 20 days/year	7	6.0 (1.9-19.5)
Woods et al., 1987	Male residents of Washington State		
	Phenoxy herbicide use		1.1 (0.8-1.4)
	Chlorophenol use		1.0 (0.8-1.2)
	Farming occupations		1.3 (1.0-1.7)
	Forestry herbicide applicators		4.8 (1.2-19.4)
Zahm et al., 1990	White male residents of Nebraska		
	Ever done farm work	147	0.9 (0.6-1.4)
	Ever mixed or applied 2,4-D	43	1.5 (0.9-2.5)

[a]Given when available.

ratio of 1.6 (CI 0.8-3.4) (Dubrow et al., 1988). No specific analysis with respect to herbicide usage was undertaken.

A mortality study of Iowa farmers, using death certificate classification of occupation, was conducted by Burmeister (1981) for 1971-1978. An SMR of 1.3 and a PMR of 1.1 were found for NHL among farmers or farm workers. The SMR was significantly elevated at the 99 percent level. This study was followed by a case-control study of selected cancers, including NHL, conducted by Burmeister and colleagues (1983) for the years 1964-1978. This later study, however, took into account various farm practices, including herbicide use. An overall significantly elevated OR = 1.3 for NHL in relation to farming was found. The odds ratio was strongly related to birth cohort, and consequently to calendar year and age of death. The odds ratios for farm work were 3.4, 2.2, and 1.3, respectively, for those born before 1890, from 1890 to 1900, and after 1900, and farming in the 33 counties with highest herbicide use. For deaths over age 65, the odds ratio was 1.8, compared to 0.7 for farmers' deaths at age 65 or less. Highly significant elevations in odds ratios were found for herbicide usage, but also for other farm characteristics unassociated with herbicide usage, such as presence of egg-laying chickens, amount of milk products sold, and hog production.

A study by Cantor (1982) of NHL mortality in Wisconsin found an odds ratio of 1.2 for NHL in farmers (CI 1.0-1.5) compared to nonfarmers. In contrast to Burmeister et al., Cantor found a higher odds ratio (1.7, CI 1.1-2.5) for deaths under age 65, versus OR = 1.0 (CI 0.8-1.4) for those 65 and over. Recent birth cohorts had an increasing odds ratio. Among the types of NHL, reticulum cell sarcoma was found to have the highest OR = 1.4 (CI 1.0-2.0) in farmers, which was consistent in subsequent analyses. Elevated odds ratios, especially for reticulum cell sarcoma, were found for herbicide use according to acres treated (OR = 2.9, CI 1.0-8.2), insecticide use (OR = 4.6, CI 1.6-13.1), and especially residence in a county with high small-grain acreage. In this last category, the reticulum cell sarcoma odds ratios for farmers in the 15 highest counties with respect to small-grain acreage was 5.0 (CI 2.1-11.7).

A well-conducted study, particularly with respect to herbicide use, was that by Hoar and colleagues (1986). One hundred and seventy interviewed cases of NHL were studied in relation to potential herbicide exposure. An odds ratio of 1.6 (CI 0.9-2.6) was found for any farm use of herbicides. Detailed analyses in terms of frequency of use (days per year) showed a significant trend with number of days; the odds ratio rose to 6.0 (CI 1.9-19.5) for more than 20 days per year. Similarly, an important trend was found for years of herbicide use, but in this analysis the highest odds ratio was 2.0 (CI 1.0-4.0) for more than 15 years of use. An important feature of this study was that the researchers sought information on the herbicides

used on a particular farm not only from the owner, but also from the suppliers of 110 subjects. The most commonly used herbicides were phenoxy acids, with uracils second. In a hierarchical analysis, odds ratios of 2.2 (CI 1.2-4.1) were found for phenoxy acids, 1.0 (CI 0.5-2.1) for uracil usage and no use of phenoxy acids, and 2.2 (CI 0.4-9.1) for triazines, based on three cases. The phenoxy herbicide was usually 2,4-D; only three patients and 18 controls had used 2,4,5-T. Analyses by 2,4-D usage show odd ratios increasing with duration and frequency of use. There was no association with increasing years of insecticide use, but an inconsistently increased risk was seen with days per year of insecticide use.

To further investigate the specific relationship between NHL and exposure to 2,4-D, Zahm and colleagues (1990) conducted a case-control study in Nebraska. An OR = 1.5 (CI 0.9-2.5) was found for men who mixed or applied 2,4-D, which increased to 3.3 (CI 0.5-22.1) for those exposed 21 or more days per year. Excluding 2,4,5-T users did not change the odd ratios. An analysis for organophosphate use, adjusted for 2,4-D use, showed an odds ratio of 2.4. This is an independent association with NHL. The risks of 2,4-D were little affected by deletion of cases and controls who had used organophosphates. The authors concluded that the various possible confounding exposures in this group could not explain the 2,4-D-associated odds ratios. There was no consistent increase in risk with the number of years of 2,4-D usage or with the first year of 2,4-D use.

A case-control study of NHL in Washington State (Woods et al., 1987; Woods and Polissar, 1989) estimated that risks were 1.3 (CI 1.0-1.7) for farmers and 4.8 (CI 1.2-19.4) for forestry herbicide applicators. A limited analysis according to latency was undertaken, in which odds ratios were calculated for groups having 15 or more years of potential exposure prior to a specified latency period. The odds ratios were 1.3, 1.7, and 2.5, respectively, for latency periods of 5, 15, and 25 years. In this analysis, exposures that may have occurred in a latency period received no consideration. However, there was no increased risk of NHL among farmers who reported using 2,4-D, 2,4,5-T, or phenoxy herbicides per se. The overall odds ratios for any past occupational exposure to phenoxy herbicides were 1.1 (CI 0.8-1.4) and 1.0 (CI 0.8-1.2) for exposure to chlorophenols. This anomaly led the authors to suggest that herbicide exposure in combination with other cancer risk factors may contribute to an elevated NHL risk.

The principal results of additional case-control studies of effects from exposure to phenoxy acids or TCDD in agriculture or forestry conducted in other countries are listed in Table 8-23. The exposure circumstances and study protocols are more varied than among the above U.S. studies.

One of the earliest sets of studies following the Swedish series is that of Pearce and colleagues (1985, 1986b, 1987) in New Zealand. In the first study (Pearce et al., 1985) for all lymphomas, including Hodgkin's disease,

multiple myeloma, and NHL, an OR = 1.3 (CI 1.0-1.6) was found for the occupational classification of agriculture/forestry/fishing, adjusting for age. When social class and age were controlled, the odds ratio decreased to 1.2 (CI 0.9-1.6). The occupations of 95 of 118 cases in this category were general or unspecified farm work, with little information on specific exposure. The odds ratio for NHL for the agriculture category was 1.4 (CI 0.9-2.0) in the age group 20-64 years, which was largely influenced by a significant odds ratio of 1.8 (CI 1.0-3.0) for ICD 202, NHL other than reticulosarcoma or lymphosarcoma. Analyses of incidence and mortality rates in New Zealand for the years 1955-1979 indicate a relatively flat trend for ICD 200, but a steeply rising trend for ICD 202.

TABLE 8-23 Non-Hodgkin's Lymphoma Results from Non-U.S. Case-Control Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
LaVecchia et al., 1989	Residents of the Milan, Italy, area		
	Agricultural occupations		2.1 (1.3-3.4)
Pearce et al., 1985	Male residents of New Zealand		
	Agricultural occupations, ages 20-64		1.4 (0.9-2.0)
Pearce et al., 1987	Male residents of New Zealand		
	Farming occupations		1.0 (0.7-1.5)
	Fencing work		1.4 (0.9-2.2)
Pearce et al., 1986b	Male residents of New Zealand		
	Agricultural sprayers	19[b]	1.5 (0.7-3.3)
Smith and Christophers, 1992	Male residents of Australia		
	Exposure ≥ 1 day	15	1.5 (0.6-3.7)
	Exposure > 30 days	7	2.7 (0.7-9.6)

[a]Given when available.
[b]Only cases of non-Hodgkin's lymphoma other than lymphosarcoma and reticulosarcoma (ICD 202).

A follow-up to the above study (Pearce et al., 1986b) resulted in odds ratios, based on cancer controls, of 1.5 (CI 0.7-3.3) and 1.3 (CI 0.6-3.2), respectively, for any use of agricultural spray and for likely use of phenoxy herbicides. An odds ratio of 1.9 (CI 1.0-3.6) was obtained for farmers who did fence work, and for meat workers the OR = 1.9 (CI 0.9-4.1); both groups may have been exposed to chlorophenols. The excess associated with fencing among farmers is sufficient to account for any excess cases in this group. A further analysis (Pearce et al., 1987) combined ICD 200 and 202; the odds ratios were 1.4 (CI 0.9-2.2) for fencing work and 1.8 (CI 1.1-2.9)

for employment at meat works. It was noted that many individuals employed at meat works did prior or subsequent fencing work with potential exposure to chlorophenols.

A case-control study conducted in the Australian state of Victoria by Smith and Christophers (1992) showed no significant association for NHL and exposures to phenoxy herbicides or chlorophenols. The odds ratios increased from 1.5 (CI 0.6-3.7) for exposures of 1 day or more to 2.7 (CI 0.7-9.6) for exposures of more than 30 days. One interesting feature of the study was an analysis of NHL risk in relation to smoking habits. They found an odds ratio of 2.2 (CI 0.7-7.0) for both current and ex-smokers.

A case-control study in Italy by LaVecchia and colleagues (1989) found an odds ratio for NHL, adjusted for age and gender, of 2.1 (CI 1.3-3.4) associated with agricultural occupations. A multivariate regression analysis with statistical adjustment for age, gender, area of residence, and smoking gave a relative risk of 1.9 (CI 1.2-3.0) for agriculture.

A very limited study of NHL was undertaken in Yorkshire, England (Cartwright et al., 1988). A crude odds ratio for fertilizer/herbicide use was 1.3 (CI 1.0-1.8); however, two-thirds of diagnosed cases from 1979 to 1984 were unable to be interviewed.

Four studies of European agriculture workers considered groups likely to have used herbicides, but did not elicit direct information on herbicide exposure (Table 8-24).

A study of Danish gardeners (Hansen et al., 1992) found an SMbR of 1.7 (CI 0.6-3.8) for male gardeners who are likely to work outside and use herbicides; the SMbR for women who work in greenhouses and do not use herbicides was 3.6 (CI 0.4-13.1), based on two cases. A study of Danish and Italian farmers (Ronco et al., 1992) found no increase in cancer incidence among Danish workers, but a nonsignificant increased mortality (OR = 1.3) among Italian men who were self-employed or farm employees. It was noted that herbicides are usually sprayed by professionals in Denmark and by the farmers themselves in Italy. A study of 19,481 hospitalized licensed pesticide users and nonusers in the Piedmont region of Italy (Corrao et al., 1989) had an incidence rate for lymphomas 1.4 times expected (CI 1.0-1.9; 45 cases, 31.8 expected). The data for men from villages situated within predominantly arable land had the highest SIR (1.8, CI 1.2-2.5), based on 31 cases.

A study of the population in two northern, rice-growing provinces of Italy by Vineis and colleagues (1991) showed a significant association of NHL with 2,4-D and 2,4,5-T concentrations in community water 10 years previously. The ratio of standardized incidence rates for NHL in males between the 30 highest communities and the 199 communities with levels below the detection limit was 2.2 (CI 1.4-3.5).

A cohort mortality study in Canada (Wigle et al., 1990) utilized a detailed

multivariate analysis of the risk of death from non-Hodgkin's lymphoma; results showed an increasing relative risk according to acres sprayed with herbicides and dollars spent on herbicides. The increase in non-Hodgkin's lymphoma was found to be largely confined to farms of less than 1,000 acres. On such farms it is likely that the farmer would be directly involved in herbicide application, whereas the application on large farms might be done more often by aircraft. For farms of less than 1,000 acres, the RRs for NHL are 1.0, 1.3, 1.9, and 2.2 for 0, 1-99, 100-249, and ≥ 250 acres sprayed, respectively. In these analyses, other variables including the expenditure on fuel were controlled. 2,4-D constituted 90 percent and 75 percent by weight of all agricultural herbicide use in the 1960s and 1970s, respectively. The herbicide 2,4,5-T was used infrequently, although it was in regular use for brush control on noncrop land. Use of insecticide was uncommon; only two lymphoma deaths occurred in the group reporting spraying of insecticides.

TABLE 8-24 Non-Hodgkin's Lymphoma Results From Non-U.S. Cohort Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Corrao et al., 1989	Italian farmers licensed to apply pesticides		
	Licensed pesticide users and nonusers	45[b]	1.4 (1.0-1.9)
	Farmers in arable land areas	31	1.8 (1.2-2.5)
Hansen et al., 1992	Danish gardeners—men and women	8	2.0 (0.9-3.9)
Riihimiki et al., 1983	Finnish herbicide applicators	0	—
Ronco et al., 1992	Danish farm workers—self-employed and employees	147	1.0
	Italian farm workers—self-employed and employees	14	1.3
Swaen et al., 1992	Dutch herbicide applicators	0	—
Vineis et al., 1991	Residents of selected Italian provinces		
	Male residents of contaminated areas		2.2 (1.4-3.5)
Wigle et al., 1990	Canadian farmers		
	All farmers	103	0.9 (0.8-1.1)
	Farmers spraying herbicides on ≥250 acres	10	2.2 (1.0-4.6)

[a]Given when available.
[b]Includes cases of both non-Hodgkin's lymphoma and Hodgkin's disease.

Three small studies showing no association between exposure and NHL were conducted in Canada and Finland. A Canadian study by Green (1991)

was of 1,222 forest workers at a public utility who routinely sprayed 2,4-D and 2,4,5-T for brush control. No lymphoma deaths were observed, but very few deaths of any type occurred during the follow-up period. A similar study in Finland (Riihimaki et al., 1983) found no deaths from lymphoma in 105 deaths, nor did a Dutch study (Swaen et al., 1992) among 63 deaths of herbicide applicators.

Summary of Agricultural/Forestry Worker Studies Hardell's data appear to have been carefully collected, and the effects of possible biases and confounding factors were seriously considered. This study demonstrates a high risk among individuals who have sprayed phenoxy herbicides for 90 or more days, the risk being related to the degree of exposure. The risk in unexposed individuals was not increased, which suggests an absence of exposure misclassification. Finally, the risk for a disease, colon cancer, unassociated with herbicide exposure increased with asbestos exposure, but not with phenoxy acid exposure. The study must be given strong weight in the consideration of whether an association exists between the development of NHL and phenoxy acid exposure. Studies of Persson et al. (1989) and Olsson and Brandt (1988) also showed large positive odds ratios for herbicide exposures, although each of these studies had methodological limitations.

The lack of association in cohort studies of Wiklund and colleagues, using the Swedish Cancer Registry data base, must be considered with reference to potential exposure. Exposure was elicited by Wiklund et al. only among a small sample of pesticide applicators in one study. The SIR of NHL could not be analyzed separately by degree or fact of exposure. Absence of control for smoking in these registry studies may have affected NHL risk estimates.

Overall, the Swedish studies demonstrate an association between NHL and exposure to phenoxy herbicides.

Of eight U.S. case-control studies of NHL in relation to farming, all show an increased risk of NHL in relation to some aspect of farming, forestry, or herbicide use. Several of the studies show increased odds ratios that are significant at the 95 percent level forming highly exposed population subgroups. The evidence is very strong that an increased NHL risk is associated with farming. This risk would appear to be associated with several factors in the farm work environment, including exposure to herbicides, particularly 2,4-D. Increasing odds ratios were usually found in analyses where there was likelihood of herbicide exposure. In studies that controlled for other possible NHL risk factors the herbicide-related risk generally remained. To the extent that NHL is related to smoking, studies of farmers that do not control for smoking, such as those using population data files, could seriously underestimate NHL risks. A notable feature of the studies of production workers exposed to TCDD-contaminated chemicals

is the much lower relative risk observed, compared to individuals spraying phenoxy acids in agriculture or forestry, despite the likelihood of many production groups having had much higher exposure to TCDD. Much agricultural spraying involved herbicides other than 2,4,5-T, and TCDD exposures would have been low.

Studies other than in the United States and Sweden are more limited, but significantly increased risks for NHL in relation to herbicide use are seen, adding strong support to the conclusions of the Swedish and U.S. studies. Thus, taken as a group, the studies of agricultural and forestry workers suggest that there is an association between exposure to herbicides (including 2,4-D) and NHL.

Paper/Pulp Workers Exposures in pulp and paper mills include chlorophenols used in wood preservation and dioxins produced during the pulp bleaching process. Robinson and colleagues (1986) studied five pulp and paper mills on the West Coast of the U.S.. Among 3,572 study subjects with at least one year of work, 915 deaths occurred, 10 of which were of lymphosarcoma or reticulosarcoma (5.9 expected, SMR = 1.7, CI 0.8-3.5). A higher SMR (2.1, CI 0.9-5.4) was observed among the three sulfate mill employees than among workers at sulfite mills (1.3, CI 0.4-4.2). Among members of the United Paperworkers International Union studied by Solet and colleagues (1989), 201 deaths occurred in white men, of which 3 were lymphosarcoma or reticulosarcoma (0.7 expected). The members of this union work largely in the eastern, southern, and midwestern United States. Finally, a study of Henneberger and colleagues (1989) of a New Hampshire pulp and paper mill had one lymphosarcoma or reticulosarcoma death, with 1.5 expected. Neither of the above increased SMRs is significant, nor is an SMR combining the results of all three studies.

Environmental Studies

Two environmental studies are based on the experience of residents of Seveso, Italy, during the 10 years following contamination of the community with TCDD. Bertazzi and colleagues (1989b) examined cancer mortality, and Pesatori and colleagues (1992) investigated the incidence of cancer. Bertazzi and colleagues (1989b) provide limited data on NHL deaths. Among women in zone B, 2 lymphatic cancers were found against an expected 1.9 cases. Among residents of zone R, 3 lymphomas (ICD 202) were reported for men; 2.9 were expected. Among women, 4 cases were found with 2.5 expected. Pesatori and colleagues (1992), who reported on the incidence of cancer, found 3 cases of NHL among men in zones A and B compared to an expected 1.6 cases. Among women in zones A and B, there was 1 case when 1.6 were expected. In zone R, they found 13 cases of NHL among

men when 9.6 were expected. There were 10 observed cases in women compared to 8.8 cases expected. Both studies are limited by the short time of follow-up since the contamination incident.

A second environmental study is that of Lampi and colleagues (1992) who investigated the mortality of residents of a community in Finland whose water became contaminated by chlorophenols from a nearby sawmill. Relative risks for NHL in the contaminated village were 2.8 (CI 1.4-5.6) compared to two uncontaminated neighboring municipalities and 2.1 (CI 1.3-3.4) compared to the larger cancer control region. A case-control regression analysis showed increased risks associated with contamination of drinking water and fish.

Summary of Environmental Studies The time of follow-up of the study of most interest, the cancer morbidity and mortality follow-up among Seveso residents, is too short to draw any conclusion. Data on NHL are only suggestive of an increased risk, but further follow-up must continue. The other studies are consistent with a TCDD-related increased risk of NHL in pulp and paper work or from chlorophenol contamination of drinking water. However, the number of deaths is small, and only the water contamination study achieves statistical significance.

Vietnam Veterans Studies

Ranch Hands The mortality experience of the Air Force veterans of Operation Ranch Hand has been observed by Michalek and colleagues (1990) in conjunction with a 20 year health study. No deaths from NHL have occurred among 74 deaths of 1,261 members of this group. However, during the conduct of the morbidity study described by Wolfe and colleagues (1990), one case of NHL was found among the Ranch Hands versus none in the comparison examination group of Air Force veterans primarily involved in air cargo operations in Southeast Asia.

CDC The Selected Cancers Study (CDC, 1990a) included NHL in one of the case-control studies. There was virtually no overlap with cases or deaths in the various DVA studies described below since case accrual was after 1984. The adjusted odds ratio for NHL compared to male veterans who did not serve in Vietnam was 1.5 (CI 1.1-2.0); compared to Vietnam era veterans with no service in Vietnam, the odds ratio was also 1.5 (CI 1.0-2.3). Various confounders were considered in regression analyses, including registry, age, race, ethnicity, education and potential exposure to herbicides, pesticides, or chlorophenols outside Vietnam. Cases with AIDS or that could reasonably be thought to have HIV infection were not included in the analysis because of the relationship between AIDS and NHL. The overall odds ratio was little affected by any of these other variables. The

odds ratios for specific service branches were 1.2, 1.8, 1.0, and 1.9, respectively, for Army, Marines, Air Force, and Navy, compared to men with no military service. No cases occurred among naval personnel stationed on river and near-shore ships, and only four among shore personnel. No explanation is known for the high blue water Navy odds ratio (OR = 2.2, CI 1.2-3.9). The study's authors feel that it is unlikely to be the result of occupational exposures aboard ship. Of interest here is a study of NHL incidence among Navy enlisted personnel (Garland et al., 1988) during 1974-1983. Vietnam service was not considered in the study, but the NHL standardized incidence ratio of active duty enlisted naval personnel, compared with SEER, was 0.7.

Of these results, it is notable that the NHL odds ratios for Marines was higher than that for Army troops, as was observed in the DVA study (see below). On the other hand, this study showed lower odds ratios for combat (OR = 1.3) than for support forces (OR = 1.5), and no association with self-reported exposure to Agent Orange (OR = 1.1). I Corps personnel had a high odds ratio (2.3), some but not all of which could be attributed to the high risk among Marines; odds ratios for the other military regions were 0.9 for III Corps, 1.2 for II Corps, and 0.9 for IV Corps.

Overall the study shows a statistically significant risk of NHL among Vietnam veterans. Great care was taken in this study to control for confounders, and their role was found to be minimal. Further, it is unlikely that bias is an explanation of the elevated risks since six other cancers considered showed no elevated odds ratios. The authors conclude that Vietnam veterans have approximately a 50 percent increased risk of developing NHL, but state that Agent Orange or dioxin would appear not to be responsible for the increase, based on the above negative associations of service in sprayed areas and the low levels of TCDD found among 646 Vietnam veterans with service in III Corps (CDC, 1989).

A cohort mortality analysis was conducted by Boyle and colleagues (1987) of Vietnam veterans enrolled in the Vietnam Experience Study. From 1965 through 1983, 12 deaths of cancer occurred among 9,324 Vietnam veterans. Of these, one was certified as NHL, but two additional deaths were found to be from NHL, based on a review of hospital records. In a comparison cohort of 8,989 Vietnam era veterans who served elsewhere, one NHL death occurred. Based on U.S. population rates, one would expect 0.6 NHL death to have occurred in the Vietnam cohort. A later publication by O'Brien and colleagues (1991) mentions that four individuals still alive in the cohort had NHL, bringing the total cases to seven, although one of the three deaths listed by Boyle and colleagues (1987) above may have been an acute lymphoblastic leukemia. Only one case of NHL was found in the non-Vietnam veterans. The expected number of cases of NHL in the Vietnam cohort was calculated to be 3.9.

TABLE 8-25 Non-Hodgkin's Lymphoma Results from Centers for Disease Control Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)
Selected Cancers Study			
CDC, 1990a	U.S. men born between 1921 and 1953		
	Vietnam veterans	99	1.5 (1.1-2.0)
	Army Vietnam veterans	45	1.2 (0.8-1.8)
	Marine Vietnam veterans	10	1.8 (0.8-4.3)
	Air Force Vietnam veterans	12	1.0 (0.5-2.2)
	Navy Vietnam veterans	32	1.9 (1.1-3.2)
	Blue-water Navy Vietnam veterans	28	2.2 (1.2-3.9)
Vietnam Experience Study			
O'Brien et al., 1991	Army enlisted Vietnam veterans	7[b]	1.8

[a] Given when available.
[b] NHL, 4 living cases and 3 deaths listed by Boyle et al., 1987.

Results for the Selected Cancers Study and the Vietnam Experience Study are summarized in Table 8-25.

DVA Studies The largest and most comprehensive group of veterans studies on NHL are those of the Department of Veterans Affairs (Burt et al., 1987; Breslin et al., 1988; Bullman et al., 1990; Dalager et al., 1991; Thomas et al., 1991; Watanabe et al., 1991), which analyzed the causes of death of 24,235 Vietnam veterans compared with 26,685 Vietnam era veterans who served between 1965 and 1974. Summary data from these studies are presented in Table 8-26.

The first DVA study (Breslin et al., 1988) yielded a PMR of 1.0 for NHL for all Vietnam Army and Marine veterans (143 NHL cases). However, the PMR for Marines largely stationed in I Corps area was 2.1 (CI 1.2-3.8) compared with 0.8 (0.6-1.0) for Army personnel stationed throughout Vietnam. It was noted that the most intense spraying of Agent Orange was not in I Corps area but in the III Corps area near Saigon (Kang et al., 1987). An analysis by Bullman and colleagues (1990) using deaths from two additional years showed a PMR for Army personnel in I Corps of 0.8 (CI 0.6-1.1), based on 35 cases, no different from that of Army personnel throughout Vietnam in the Breslin study. The data for Army ground troops do not

TABLE 8-26 Non-Hodgkin's Lymphoma Results From Department of Veterans Affairs Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Proportionate mortality studies			
Breslin et al., 1988	Army Vietnam veterans compared to Army Vietnam era veterans	108	0.8 (0.6-1.0)
	Marine Vietnam veterans compared to Marine Vietnam era veterans	35	2.1 (1.2-3.8)
Watanabe et al., 1991	Army Vietnam veterans compared to Army Vietnam era veterans	140	0.8
	Army Vietnam veterans compared to combined Army and Marine Vietnam era veterans	140	0.9
	Marine Vietnam veterans compared to Vietnam era veterans	42	1.8
	Marine Vietnam veterans compared to combined Army and Marine Vietnam era veterans	42	1.2
Case-control mortality studies			
Burt et al., 1987	Army combat Vietnam veterans compared to Army Vietnam era veterans	39	1.1 (0.7-1.5)
	Marine combat Vietnam veterans compared to Marine Vietnam era veterans	17	3.2 (1.4-7.4)
	Army Vietnam veterans (service 1967-1969) compared to Army Vietnam era veterans	64	0.9 (0.7-1.3)
	Marine Vietnam veterans (service 1967-1969) compared to Marine Vietnam era veterans	17	2.5 (1.1-5.8)
Dalager et al., 1991	Vietnam era veterans diagnosed with NHL	100	1.0 (0.7-1.8)
Cohort mortality and morbidity studies			
Thomas et al., 1991	Women Vietnam veterans	3	1.3 (0.3-1.8)

[a]Given when available.

suggest any association with the amount of Agent Orange sprayed in an area or the presence of a unique situation for I Corps Army personnel. However, descriptions of active duty procedures given to the committee indicated that Marines tended to stay in the countryside for long periods (weeks to months), in contrast to Army personnel who were stationed at bases from which forays would be made and to which the troops would shortly return. Thus, Marines may have had more opportunity for contact with herbicides, even though less was sprayed in the area.

Case-control analyses according to combat status by Burt and colleagues (1987) showed that men with combat specialties had higher mortality odds ratios (MORs) for NHL than men in support specialties. In the Army, the OR was 1.1 for combat troops versus 0.8 and 0.8, respectively, for direct and indirect support troops; in the Marines the OR was 3.2 for combat veterans versus 1.6 and 1.4, respectively, for direct and indirect support personnel. Additionally, somewhat higher mortality odd ratios were seen for service during the years 1967-1969, during which the spraying of herbicides was the most intense: OR = 0.9 for Army and 2.5 for Marines versus 0.7 and 1.7, respectively, for all other years. Although both of these findings and the high PMR for Marines are suggestive of an herbicide contribution to the risk of death from NHL, a later DVA analysis weakens the association.

In this later analysis, Watanabe et al. (1991) included deaths through 1984. They calculated PMRs for Army and Marine Vietnam veterans using as comparison groups same service Vietnam era veterans and combined Army and Marine Vietnam era veterans. Comparisons were also made with the U.S. male population. But unlike Burt et al. (1987), they did no analyses according to potential combat status or specific calendar year. For Marine Vietnam veterans the PMR for NHL was 1.8 in comparison to Marines without Vietnam service; it dropped to 1.2 when the comparison group was changed to the combined Army and Marine Vietnam era veterans and to 1.1 for the comparison with U.S. males. They also found that the Marine Vietnam era comparison group had a particularly low PMR (0.5) for NHL, in comparison to U.S. males. The reason for the low-risk for NHL among Marines without Vietnam service is unknown at this time. It may have occurred by chance, but this group showed a similarly low PMR (0.5) for Hodgkin's disease.

A hospital-based case-control study for 1969-1985 (Dalager et al., 1991) showed no relation of NHL to Vietnam service. An OR = 1.0 (CI 0.7-1.8) for ever being in Vietnam, adjusted for branch of service, was found for NHL among Vietnam era veterans.

Thomas and colleagues (1991) studied all female military personnel who could be identified with service in Vietnam. Three deaths occurred from NHL, compared with 2.3 expected, based on rates for U.S. women.

Although these various DVA studies do not indicate a statistically significant increased risk in relation to herbicide spraying in Vietnam, the pattern is consistent with an increased risk related to herbicides. The higher PMR for Marines compared to Army veterans in the I Corps area, higher PMRs for combat versus support troops PMRs for both Army and Marine personnel, and higher PMRs for both Army and Marine personnel who served in Vietnam during 1967-1969, coupled with the numerous civilian studies suggesting an association of NHL with herbicide exposure, must be considered.

State Studies Personnel of state departments of health have conducted studies of Vietnam veterans in their respective jurisdictions. Their principal outcomes are summarized in Table 8-27. The largest and most thorough of the state studies is that by Anderson and colleagues (1986a,b) of Wisconsin veterans. The proportionate mortality analyses of 923 deaths of Vietnam veterans, through 1979, yielded a PMR = 1.0 for NHL (13 cases) compared with other Wisconsin Vietnam era veterans and a PMR = 0.7 compared to Wisconsin nonveterans. In the cohort mortality study (Anderson et al., 1986b), which extended through 1984, the SMRs from NHL were 1.1 and 0.8 when using comparison rates derived, respectively, from Wisconsin Vietnam era veterans and Wisconsin general population data.

Three other state studies have been conducted (Lawrence et al., 1985; Holmes et al., 1986; Clapp et al., 1991). Two are case-control studies and involve fewer cases than the Wisconsin studies. The results are presented in Table 8-27, and none suggest any significant risk of NHL associated with Vietnam service. The third is a proportionate mortality study of West Virginia Vietnam veterans (Holmes et al., 1986). It demonstrates a significant increase in all lymphomas (7 observed, 2.5 expected) in Vietnam veterans compared to Vietnam era veterans who did not serve in Vietnam. Five of the seven lymphomas were Hodgkin's disease. The two remaining lymphomas are to be compared with 1.9 expected.

Australian Vietnam Veterans Fett and colleagues (1987b) have conducted a study of 19,205 Australian Vietnam veterans and 25,677 Vietnam era veterans. Four NHL deaths occurred among the Vietnam veterans and three among the Vietnam era veterans, for a relative death rate for NHL of 1.8 (CI 0.4-8.0).

Summary of Veterans Studies The two most significant groups of studies are those of the Department of Veterans Affairs and the Centers for Disease Control. The DVA studies investigated several different potential exposure circumstances. A high odds ratio was found for Marines who served in the I Corps area, but was attributed to a low-risk in the control population. This attribution can be questioned. Although associations of

NHL risks with exposure to herbicides could not be made, the risks of Marine troops were greater among combat forces compared to support troops and during the time of highest herbicide use compared to other years. Further, in CDC studies, a high risk was also found for a different population of Marines and using a general population control. Both the DVA and the CDC studies generally found only small nonsignificant increases in odds ratios for Army personnel. The remaining studies, generally by state agencies, were largely negative. All, however, had few cases of NHL, and there

TABLE 8-27 Non-Hodgkin's Lymphoma Results From State Veterans Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Proportionate mortality studies			
Anderson et al., 1986a	Wisconsin Vietnam veterans		
	Wisconsin Vietnam veterans Wisconsin nonveterans	13	0.7
	Wisconsin Vietnam veterans compared to non-Vietnam era veterans	13	0.6
	Wisconsin Vietnam veterans compared to Vietnam era veterans	13	1.0
Holmes et al., 1986	West Virginia Vietnam veterans compared to West Virginia Vietnam era veterans	2	1.1
Case-control mortality studies			
Clapp et al., 1991	Massachusetts Vietnam veterans compared to Vietnam era veterans		1.2 (0.6-2.4)
Lawrence et al., 1985	New York Vietnam veterans	10[b]	1.0 (0.4-2.2)
Cohort mortality and morbidity studies			
Anderson et al., 1986b	Wisconsin Vietnam veterans compared to general population	24	0.8
	Wisconsin Vietnam veterans compared to Wisconsin Vietnam era veterans	24	1.1

[a]Given when available.
[b]Includes both non-Hodgkin's lymphoma and Hodgkin's disease.

was no attempt to group the study subjects by any measure of potential exposure to herbicides.

Although no single study shows definite associations between NHL and exposure to herbicides since individual exposures were not determined except for Ranch Hands, none rules out the possibility that an herbicide-related risk of NHL existed for some military personnel during service in Vietnam.

Summary for Non-Hodgkin's Lymphoma

One large, well-conducted case-control study in Sweden by Hardell and colleagues (1981) examined NHL and Hodgkin's disease together and found an odds ratio of 6.0 (CI 3.7-9.7) based on 105 cases for exposure to phenoxy acids or chlorophenols, and these results were replicated under further investigation of the validity of exposure assessment and other potential biases (Hardell, 1981). A more recent case-control study by Persson and colleagues (1989) showed increased risk for NHL in those exposed to phenoxy acids (OR = 4.9, CI 1.0-27.0), based on a logistic regression analysis of 106 cases, and other studies of farmers and agricultural workers (Tables 8-22, 8-23, 8-24) are generally positive for an association between NHL and herbicides or TCDD; however, only some are statistically significant. All of the studies of U.S. agricultural workers reviewed showed elevated relative risks (although none were statistically significant), and two NCI studies of farmers in Kansas and Nebraska (Hoar et al., 1986; Zahm et al., 1990) show patterns of increased risk linked to use of 2,4-D. The CDC Selected Cancers Study (CDC, 1990a) found an increased risk of NHL in association with service in Vietnam; other studies of veterans, largely with small sample sizes, are consistent with an association (Tables 8-25, 8-26, 8-27). In contrast, studies of production workers, including the largest, most heavily exposed cohorts (Zober et al., 1990; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991) indicate no increased risk. Thus, unlike most of the other cancers studied by the committee for which the data do not distinguish between the effects of herbicides and TCDD, the available epidemiologic data suggest that the phenoxy herbicides, including 2,4-D, rather than TCDD may be associated with non-Hodgkin's lymphomas.

Conclusions for Non-Hodgkin's Lymphoma

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and non-Hodgkin's lymphoma.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

Epidemiologic Studies of Hodgkin's Disease

Occupational Studies

Production Workers In the study of Fingerhut and colleagues (1991), three deaths from HD occurred, with 2.5 expected (SMR = 1.2, CI 0.3-3.5); of those in the group observed 20 years or more from onset of exposure there was one death from HD and 0.4 expected. The few deaths at this time limit the significance of an otherwise well-conducted study of all U.S. plants producing chemicals with potential TCDD contamination. Indeed, serum TCDD measurements on a sample of 253 workers demonstrate a current average lipid-based exposure of 233 ppt (range 2-3,400), levels much higher than those present in workers spraying herbicides, most of which did not contain TCDD. One relevant study not included in that of Fingerhut is the study of Bond and colleagues (1988) of 878 2,4-D production workers at the Dow Chemical Corporation. One HD death occurred; 0.4 was expected.

The study by Saracci and colleagues (1991) included workers engaged in the production and spraying of both phenoxy herbicides and compounds contaminated with TCDD. In this study of 18,390 workers, 1,870 deaths

occurred in persons classified as exposed, 2 from HD; 5 deaths were expected, resulting in an SMR of 0.4 (CI 0.1-1.4). An additional study cohort, not included in the Saracci review, was that of 247 workers of a BASF plant in which TCDD exposure occurred following an accident during trichlorophenol production. No HD cases occurred, approximately 0.1 was expected over the follow-up period, 1954-1987 (Zober et al., 1990).

Agricultural/Forestry Workers Table 8-28 summarizes the principal results of the studies undertaken to assess the association between HD and actual or potential agricultural exposures to phenoxy herbicides in the United States.

Of the four studies using only occupation as an indication of potential exposure, one is the study by Alavanja and colleagues (1988) of U.S. Department of Agriculture extension agents in which preliminary analysis gave a significantly elevated PMR for HD of 2.7 (CI 1.2-6.3), based on six deaths. To reduce possible effects of selection bias in the study population, a case-control analysis of significant findings including HD was undertaken using non-extension agent deaths as controls. An odds ratio of 1.1 (CI 0.3-3.5) was obtained for ever versus never an extension agent and having HD. A similar analysis was conducted by Alavanja and colleagues (1989) among forest and soil conservationists, demonstrating a PMR = 2.2 (CI 0.6-5.6) for HD, based on four deaths. Since this PMR was not significant, a case-control analysis was not undertaken.

A study was conducted by Dubrow and colleagues (1988) of HD deaths in relation to farming for the years 1958-1983 in Hancock County, Ohio. An odds ratio of 2.7 was found, based on three cases among farmers that occurred in a cluster during 1960-1962. No analysis was made in relation to herbicide use. A study of deaths in Iowa by Burmeister (1981) showed a

TABLE 8-28 Hodgkin's Disease Results From U.S. Case-Control Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Burmeister et al., 1983	Iowa residents		1.4
Dubrow et al., 1988	Ohio residents	3	2.7
Hoar et al., 1986	Kansas residents		
	All farmers	71	0.8 (0.5-1.2)
	Farmers using herbicides		
	> 20 days/year	3	1.0 (0.2-4.1)
Alavanja et al., 1988	USDA agricultural extension agents		1.1 (0.3-3.5)

[a] Given when available.

nonsignificant PMR of 1.2 for HD. In neither of the above two studies was a specific analysis undertaken with respect to herbicide usage.

The final U.S. agriculture study was that by Hoar and colleagues (1986) who investigated herbicide use by individual study subjects in Kansas. An overall odds ratio of 0.9 (CI 0.5-1.5) was found for any farm use of herbicides (phenoxy acids and others) and an odds ratio of 1.2 (CI 0.5-2.6) for farmers who used herbicides for more than 15 years. This study is one of the best with respect to exposure information; the researchers sought information on the herbicides used on a particular farm not only from the owner, but also from the suppliers of study subjects. This negative result for HD is to be contrasted with a positive finding in the same study for NHL in relation to herbicide use, particularly 2,4-D. It also contrasts with a very strong positive study of Hardell and Bengtsson (1983) in Sweden (see below).

Table 8-29 lists the principal results of the non-U.S. case-control studies of HD in relation to exposure to phenoxy herbicides or TCDD.

Studies from Sweden provide the most comprehensive information on the association between HD and exposure to phenoxy herbicides (2,4-D, 2,4,5-T) and picloram or chlorophenols. The first study to do so was by Hardell and colleagues (1980, 1981) in which NHL and HD were considered together. Later Hardell and Bengtsson (1983) considered the HD cases separately. In this latter study, 60 HD cases from the University Hospital in Umea and 335 general population controls were utilized in multiple analyses.

TABLE 8-29 Hodgkin's Disease Results From Non-U.S. Case-Control Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Hardell and Bengtsson, 1983	Umea Hospital patients		
	Exposed to phenoxy acids	6	5.0 (2.4-10.2)
	Exposed to high-grade chlorophenols	9	6.5 (2.7-19.0)
Persson et al., 1989	Orebro Hospital patients		
	Exposed to phenoxy acids	4	3.8 (0.5-35.2)
LaVecchia et al., 1989	Residents of the Milan, Italy, area		
	Agricultural occupations		2.1 (1.0-3.8)
Pearce et al., 1985	Male residents of New Zealand		
	Agricultural occupations, ages 20-64		1.0 (0.6-2.0)

[a] Given when available.

An OR = 5.0 (CI 2.4-10.2) was found for exposure to phenoxy acids after excluding those with exposure to chlorophenols. Odds ratios of 2.4 (CI 0.9-6.5) and 6.5 (CI 2.7-19.0) were found, respectively, for low-grade and high-grade exposures to chlorophenols. Great care was taken in establishing exposure by questionnaire and interview, and avoiding bias as previously discussed.

A study by Persson and colleagues (1989) investigated the relation of phenoxy acid exposures to HD among 54 cases from the register of the Department of Oncology of the Orebro Hospital in southern Sweden. An odds ratio of 3.8 (CI 0.5-35.2) was found for exposure to phenoxy acids by using logistic regression to control for other variables. Other exposures showing significantly increased risks of HD included welding and creosote. Despite the high odds ratio for herbicide exposure, the study showed only a slightly increased odds ratio of 1.2 (CI 0.4-3.5) for farming, based on six cases.

A case-control study of the Milan area in Italy by LaVecchia and colleagues (1989), using multivariate regression analysis with terms for age, gender, area of residence, and smoking, gave a relative risk of 2.1 (CI 1.0-3.8) for HD among those with agricultural employment. An analysis in relation to chemical industry employment also showed an odds ratio of 4.3 (CI 1.4-10.2).

In New Zealand, Pearce and colleagues (1985) utilized cancer registry cases to investigate the association of HD and other malignancies with exposure to phenoxy acids or TCDD-contaminated chemicals. The overall odds ratio for HD was less than 1.0 for agricultural occupations; it was 1.0 (CI 0.6-2.0) for cases identified between ages 20 and 64 and 0.2 (CI 0.0-1.4) for cases identified at older ages. No detailed exposure information was provided.

Wiklund and colleagues (1983, 1988a) used data from the Swedish Cancer Registry and employment data from the decennial census to study the relation between HD and occupations that might have involved exposure to phenoxy acids. An initial study (Wiklund, 1983), focused on agricultural workers, showed an SIR of 1.0 (99% CI 0.9-1.2) for HD during the years 1961-1973. No information on degree or frequency of exposure to agricultural chemicals was obtained.

HD incidence was investigated further by Wiklund and colleagues (1989b) in a cohort of 20,245 pesticide applicators; an SIR of 1.5 (CI 0.8-2.4) was found, based on 15 cases. From a mail questionnaire of 273 cohort members, it was estimated that 72 percent of the cohort had exposure to phenoxy acids for one day or more per year; 10 percent had exposure for more than 20 days a year. A further study (Wiklund et al., 1988a) of agricultural and forestry workers also showed no increased risk of HD during 1961-1979.

The relative risk, compared with Swedish males in other occupations, was significantly elevated only in the subgroups engaged in silviculture, where the odds ratio was 2.3 (CI 1.3-3.7), or in "other agricultural occupations," where the odds ratio was 1.7 (CI 1.1-2.6). Exposure to phenoxy herbicides is likely in silviculture, but uncertain in the other agricultural category, which consisted largely of animal husbandry other than livestock.

Eriksson and colleagues (1992) also investigated the incidence of HD in relation to specific occupations using cases from the Swedish Cancer Registry. They found a significantly increased SIR of 2.2 for sawmill workers, based on 10 cases, and a nonsignificant increase in SIR of 1.2 for farmers, forestry workers, and horticultural workers. Specific exposures of individuals to phenoxy acids or TCDD were not elicited in this study.

A study by Ronco and colleagues (1992) found an SIR for HD of 0.6 for Danish male, self-employed farmers based on 27 cases. In a study of male Italian farmers, an odds ratio of 2.9 was found, based on 10 cases, for self-employed farmers, and an odds ratio of 0.4, based on 1 case, for farm employees. The odds ratio of 1.9 for self-employed Italian women farmers was based on 1 case. A Dutch study by Swaen and colleagues (1992) observed 1 death due to HD among 63 deaths of herbicide applicators. Two small studies with negative results were conducted in Canada and Finland. In a Canadian study by Green (1991) of 1,222 electrical workers who routinely sprayed 2,4-D and 2,4,5-T for brush control no lymphoma deaths were observed, but only 80 deaths overall occurred during the follow-up period. A similar study in Finland (Riihimaki et al., 1983) with 105 deaths had no deaths from any lymphoma.

Table 8-30 summarizes the results of studies conducted outside the United States.

Summary of Agricultural Worker Studies The data of Hardell are noteworthy. They appear to have been carefully collected, and the effects of possible biases and confounding factors were considered seriously. Their studies demonstrate a high, significant risk among individuals who have sprayed phenoxy herbicides. These studies must be given strong weight in the consideration of whether an association exists between the development of HD and phenoxy acid exposure. The study of Persson and colleagues (1989) also showed a large positive odds ratio for herbicide exposures, although not statistically significant. On the other hand, an equally carefully conducted study in the United States by Hoar and colleagues (1986), with good exposure information, did not show any increased risk of HD in relation to herbicide exposure. Other studies were generally positive, although not statistically significantly; however, explicit herbicide exposure information was not obtained.

TABLE 8-30 Hodgkin's Disease Results From Cancer Registry or Cohort Mortality and Morbidity Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Wiklund, 1983	Swedish agricultural workers	226	1.0 (0.9-1.2)[b]
Wiklund et al., 1989b	Swedish pesticide applicators	15	1.5 (0.8-2.4)
Wiklund et al., 1988a	Swedish agricultural and forestry workers		
	Workers in land/animal husbandry	242	1.0 (0.9-1.2)
	Workers in silviculture	15	2.3 (1.3-3.7)
Ronco et al., 1992	Danish and Italian farm workers		
	Male Danish farmers—self-employed	27	0.6
	Male Italian farmers—self-employed and employees	11	1.9
Swaen et al., 1992	Dutch herbicide applicators	1	3.3

[a]Given when available.
[b]99% CI.

Vietnam Veterans Studies

In contrast to the extensive studies of NHL and soft tissue sarcoma among U.S. veterans by both federal and state agencies, there are relatively few studies of HD among Vietnam veterans. The major findings are shown in Table 8-31 for the data available.

The proportionate mortality study by Breslin and colleagues (1988) yielded HD PMRs of 1.2 (CI 0.7-1.9) for Army veterans and 1.3 (0.7-2.6) for Marine veterans. Vietnam era veteran rates served as the comparison.

A study of the same groups with two additional years of follow-up (Watanabe et al., 1991) found PMRs of 1.0 and 1.9, respectively, for Army and Marine Vietnam veterans in comparison with Vietnam era veterans. The value for the Marine veterans is significant at the 95 percent confidence level. However, in a comparison with all Vietnam era veterans and the U.S. male general population, the Marine veteran PMRs drop to 1.0 and 0.8, respectively. This is the same phenomenon that occurred with the DVA analysis of NHL; the comparison rates of HD mortality for Vietnam era Marines are unusually low, approximately 50 percent that of the U. S. male population. Such a finding for two diseases suggests that chance is an unlikely explanation and that either bias or causal factors may be playing a role.

The Selected Cancers Study (CDC, 1990c) shows odds ratios for HD among Vietnam veterans in comparison to other men in eight U.S. cancer registries during 1984-1988. The odds ratios for association with HD and service during Vietnam were 1.2 (CI 0.7-2.4) compared to Vietnam era

TABLE 8-31 Principal Hodgkin's Disease Results From Vietnam Veteran Studies

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
DVA proportionate mortality studies			
Breslin et al., 1988	Army Vietnam veterans compared to Army Vietnam era veterans	92	1.2 (0.7-1.9)
	Marine Vietnam veterans compared to Marine Vietnam era veterans	22	1.3 (0.7-2.6)
Watanabe et al., 1991	Army Vietnam veterans compared to Army Vietnam era veterans	116	1.0
	Marine Vietnam veterans compared to Vietnam era veterans	25	1.9
	Army Vietnam veterans compared to Vietnam era veterans	116	1.1
	Marine Vietnam veterans compared to Vietnam era veterans	25	1.0
CDC case-control mortality studies			
CDC, 1990c	U.S. men born between 1921 and 1953 Vietnam veterans	28	1.2 (0.7-2.4)
	Army Vietnam veterans	12	1.0 (0.5-2.0)
	Marine Vietnam veterans	4	1.7 (0.5-5.9)
	Air Force Vietnam veterans	5	1.7 (0.6-4.9)
	Navy Vietnam veterans	7	1.1 (0.4-2.6)
State proportionate mortality studies			
Anderson et al., 1986a	Wisconsin Vietnam veterans compared to Wisconsin nonveterans	6	0.5 (0.2-1.2)
	Wisconsin Vietnam veterans compared to non-Vietnam era veterans	6	1.0 (0.4-2.2)
	Wisconsin Vietnam veterans compared to Vietnam era veterans	6	1.0 (0.4-2.1)
Holmes et al., 1986	West Virginia Vietnam veterans compared to West Virginia Vietnam era veterans	5	8.3 (2.7-19.5)
Lawrence et al., 1985	New York Vietnam veterans compared to New York Vietnam era veterans	10[b]	1.0 (0.4-2.2)

[a]Given when available.
[b]Includes both non-Hodgkin's lymphoma and Hodgkin's disease.

service, and 1.2 (CI 0.7-1.9) compared to men with no military service. The odds ratios for HD according to service branch, compared to men with no military service, are 1.0 (CI 0.5-2.0), 1.7 (CI 0.6-4.9), 1.7 (CI 0.5-5.9), and 1.1 (CI 0.4-2.6), for Army, Air Force, Marine, and Navy Vietnam veterans, respectively. The large confidence limits are the result of very few HD cases in each category.

Six cases of HD were observed in a proportionate mortality study of Wisconsin Vietnam veterans by Anderson and colleagues (1986a). This led to the calculation of proportionate mortality ratios comparing Wisconsin Vietnam veterans to Wisconsin nonveterans, Vietnam era veterans and other veterans, respectively. A cohort mortality study by Anderson and colleagues (1986b) had only four deaths, and expected numbers of deaths were not reported.

No deaths from HD have occurred among the 9,324 Vietnam veterans enrolled in the VES (Boyle et al., 1987), where approximately 0.2 might be expected. Similarly no cases or deaths of HD have been reported among Ranch Hand veterans observed in Air Force studies (Michalek et al., 1990; Wolfe et al., 1990), or among Australian veterans studied by Fett and colleagues (1987b). In each of these last two studies, the expected number of HD deaths would appear to be less than one.

Summary for Hodgkin's Disease

Fewer studies have been conducted of HD in relation to exposure to herbicides or TCDD than have been conducted of STS or NHL, but the pattern of results is notably consistent. The 60 HD cases in the study by Hardell and colleagues (1981) were later examined by Hardell and Bengtsson (1983) who found odds ratios of 2.4 (CI 0.9-6.5) for low-grade exposure to chlorophenols and 6.5 (CI 2.7-19.0) for high-grade exposures. A more recent study by Persson and colleagues (1989) of 54 HD cases showed a large, but not statistically significant OR = 3.8 (CI 0.5-35.2) for exposure to phenoxy acids. Furthermore, nearly all of the 13 case-control and occupational cohort studies summarized in Tables 8-28, 8-29, and 8-30 show increased risk for HD, although only a few of these results are statistically significant. As with NHL, even the largest studies of production workers exposed to TCDD do not indicate an increased risk. The few studies of HD in Vietnam veterans tend to show elevated risks, all but one are not statistically significant (Table 8-31).

Conclusions for Hodgkin's Disease

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association

between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and Hodgkin's disease.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

Epidemiologic Studies of Multiple Myeloma

A substantial number of studies have investigated associations between multiple myeloma and occupation or exposure to specific agents. A consistent general finding from many studies is an association with farming. Unfortunately, most such studies did not further investigate specific farm exposures. Among those studies that did, an association with herbicide use, exceeding that of the category of farming, was found in some, but not all cases. Other farm-related associations were also found. These findings, some of which are mentioned below, preclude ascribing an observed increased risk of MM among farmers to phenoxy herbicide use. Thus, studies of farmers that do not provide further information on specific exposures to herbicides, or pesticides/herbicides, are not considered in this section.

Occupational Studies

Production Workers Two studies dominate the data on the effects of

production worker exposures to phenoxy acids and TCDD, those of Fingerhut and colleagues (1991) in the United States and of Saracci and colleagues (1991) elsewhere. In the U.S. cohort (Fingerhut et al., 1991), 1,052 deaths occurred, 5 from MM; 3.0 were expected (SMR = 1.6, CI 0.5-3.9). Three occurred in the group with 20 years or more latency and with more than one year of exposure, yielding an SMR of 2.6 (CI 0.5-7.7). No MM deaths occurred in a separate study by Bond et al. (1988) of 878 2,4-D production workers at Dow Chemical Corporation. The expected number of MM deaths, however, would have been less than 0.4.

Four MM deaths were reported in the Saracci et al. (1991) analysis of non-U.S. cohorts of workers engaged in the production and spraying of phenoxy herbicides or of compounds contaminated with TCDD. The SMR was 0.7 (CI 0.2-1.8). However, data on two of the 20 cohorts, comprising 3,544 of the 13,482 workers studied by Saracci et al., were published separately by Coggon et al. (1986). For this work force that manufactured or sprayed the phenoxy herbicide MCPA, Coggon and colleagues listed 5 deaths from MM, compared to 3.1 expected. One of these deaths was for an individual with only background exposure. The overall SMR was 1.6 (CI 0.5-3.8). The SMR for greater than 10 years latency was 2.3 and for the longest duration category, more than six months exposure, the SMR was 2.7. The apparent absence of MM deaths in the remainder of the Saracci group has no immediate explanation but may reflect a long latency for a cancer that occurs most commonly among the elderly (age 70 or older).

No MM deaths were observed in a group of workers exposed to TCDD from an accident that occurred in a BASF plant (Zober et al., 1990). However, fewer than 0.2 would be expected. A group of phenoxy herbicide production workers in the Netherlands was studied by Bueno de Mesquita et al. (1993) No MM deaths were observed; 0.8 was expected.

Summary of Production Worker Studies There is some limited evidence in the Fingerhut and colleagues (1991) study of a relationship between exposure to TCDD and development of MM, which is not present in the Saracci et al. (1991) study. However, only seven or eight deaths from MM are known to have occurred among all U.S. and foreign chemical production workers. Further, uncertainties regarding exposure to TCDD exist in the study of Saracci and colleagues.

Agricultural/Forestry Workers Table 8-32 summarizes the principal results of studies of agricultural and forestry workers. Multiple myeloma was investigated in analyses of the mortality of U.S. Department of Agriculture forest and soil conservationists by Alavanja and colleagues (1989). The analyses gave a PMR of 1.3 (CI 0.5-2.8). There was a nonsignificant trend in increased risk of MM associated with duration of work as a conservationist. A similar analysis was conducted by Alavanja and colleagues

(1988) of extension agents, but the uncertainty introduced by other possible MM farm-related risk factors would apply; other potentially confounding risk factors are less likely with the forestry/soil conservationists.

TABLE 8-32 Selected Epidemiologic Studies—Multiple Myeloma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Agricultural/forestry workers			
Cohort studies			
Alavanja et al., 1989	USDA forest/soil conservationists		1.3 (0.5-2.8)
Swaen et al., 1992	Dutch herbicide applicators	3	8.2 (1.6-23.8)
Riihimaki et al., 1983	Finnish herbicide applicators	1	2.5 (0.3-14.0)
Case-control studies			
Boffetta et al., 1989	ACS Prevention Study II subjects	12	2.1 (1.0-4.2)
	Farmers using herbicides or pesticides	8	4.3 (1.7-10.9)
Burmeister et al., 1983	Iowa residents		
	Farmers in counties with highest herbicide usage		
	Born 1890-1900		2.7 ($p < .05$)
	Born after 1900		2.4 ($p < .05$)
Cantor and Blair, 1984	Wisconsin residents		
	Farmers in counties with highest herbicide usage		1.4 (0.8-2.3)
Morris et al., 1986a	Residents of four SEER areas		2.9 (1.5-5.5)
Eriksson and Karlsson, 1992	Residents of northern Sweden	20	2.2 (1.0-5.7)
Pearce et al., 1986a	Male residents of New Zealand		
	Use of agricultural spray	16	1.3 (0.7-2.5)
	Likely sprayed 2,4,5-T	14	1.6 (0.8-3.1)
LaVecchia et al., 1989	Residents of the Milan, Italy, area		
	Agricultural employment		2.0 (1.1-3.5)

[a]Given when available.

Among studies of farming populations with information on potential herbicide use was a nested case-control analysis of MM among subjects enrolled in the prospective, nationwide Cancer Prevention Study of the American Cancer Society (Boffetta et al., 1989). From an analysis of 282 MM cases and 1,128 controls, an odds ratio of 2.1 (CI 1.0-4.2) was obtained for any exposure to pesticides and herbicides. For pesticide or herbicide use among farmers, the odds ratio was 4.3 (CI 1.7-10.9); farmers reporting no exposure to pesticides or herbicides had an odds ratio of 1.7 (CI 0.8-4.0). A

logistic regression analysis with six occupations and 15 risk factors gave an odds ratio of 1.6 (CI 0.7-3.7) for pesticides/herbicides.

Burmeister (1981) found an increased SMR of 1.5 ($p < .01$) and an increased PMR of 1.3 ($p < .01$) among white male farmers in Iowa. The PMR for deaths under age 65 was substantially greater than for those 65 or older (1.7 versus 1.2). Potential contributions to this difference could include a greater exposure of younger farmers to carcinogenic agents and a "healthy worker effect" for nonmalignant deaths among younger farmers. A case-control analysis was then undertaken (Burmeister et al., 1983), by overall odds ratio for farmers with MM was 1.5. The results showed odds ratios of 1.8, 2.7, and 2.4, respectively, for deaths of farmers born before 1890, from 1890 to 1900, and after 1900 in the 33 counties with highest herbicide use. The odds ratios for birth cohorts 1890-1900 and after 1900 were statistically significant and lend support to an herbicide-related association because younger farmers are more likely to have used herbicides than older farmers. Analyses also showed significantly increased odds ratios for counties high in egg-laying chickens, hog production, and insecticide use.

Cantor and Blair (1984) investigated the association of MM with farming and herbicide use in Wisconsin. Farmers had an odds ratio of 1.4 (CI 1.0-1.8) compared to nonfarmers. Among the 15 counties with greatest acreage use of herbicides, the odds ratio for farmers was also 1.4 (CI 0.8-2.3), suggesting no special herbicide-related risk.

A final U.S. study relating farm use of herbicides to the risk of cancer was that of Morris et al. (1986) who used data from cancer registries in Washington, Utah, metropolitan Detroit, and metropolitan Atlanta. Results indicated an adjusted odds ratio of 2.6 (CI 1.5-4.6) for exposure to pesticides when data from all cases and controls were used. When only self-respondent interviews were used, the adjusted odds ratio was 2.9 (CI 1.5-5.5); the only other exposures with odds ratios in which the confidence intervals did not include 1.0 were to paints or solvents, metals, and carbon monoxide. The odds ratio for having lived on a farm was 1.3 (CI 1.0-1.6). Explicit exposure to herbicides gave an odds ratio of 4.8 but was based on only four cases.

In New Zealand, two studies by Pearce and colleagues (1985, 1986a) did not provide evidence of an association between MM and herbicide exposure. In the initial study (Pearce et al., 1985), odds ratios for agricultural employment were 2.2 (CI 1.3-3.8) for MM cases identified at 20-64 years of age and 1.3 (CI 0.8-2.0) for cases identified over age 64 and compared with correspondingly aged cases of other cancer in the New Zealand cancer registry during the period 1977-1981. These odds ratios were the highest found in this study, which also considered NHL and HD, but no individual

data on exposure to herbicides were given. However, in a follow-up case-control study (Pearce et al., 1986a), interviews and a detailed questionnaire on use of herbicides were completed. An odds ratio of 1.7 (CI 1.0-2.9) was obtained for MM in relation to farming, an odds ratio of 1.3 (CI 0.7-2.5) for any agricultural use of chemical spray, and an odds ratio of 1.6 (CI 0.8-3.1) for spraying of specific plants with which phenoxy herbicides (specifically 2,4,5-T) are generally used. Potential exposure to chlorophenols found in wood preservatives used in fencing work gave an odds ratio of 1.6 (CI 0.9-2.7), and potential exposure in a sawmill gave an odds ratio of 1.4 (CI 0.5-3.9) for sawmill workers or timber merchants, although the odds ratio for explicit exposure to chlorophenols was 1.1 (CI 0.4-2.7) based on six cases.

Eriksson and Karlsson (1992) undertook a case-control study of MM in relation to occupation and exposures in northern Sweden during 1982-1986. An odds ratio of 1.7 (CI 1.2-2.6) was found for farmers and an odds ratio of 2.2 (CI 1.0-5.7) for exposure to phenoxy herbicides. An analysis by days of phenoxyacetic acid use showed no clear trend with exposure; the odds ratio for each of three exposure categories was 2.0 or greater. In a multivariate analysis involving 22 exposure factors, the odds ratio for phenoxyacetic acids was 1.9 (0.7-5.7). The multivariate analysis eliminated sheep, hogs, and poultry as risk factors and decreased those of horses, cattle, and goats.

A case-control study of MM was undertaken in the region surrounding Milan, Italy, by LaVecchia et al. (1989). A multivariate regression analysis with terms for age, gender, area of residence, and smoking gave a relative risk of 2.0 (CI 1.1-3.5) for MM among those with agricultural employment.

A Dutch study by Swaen et al. (1992) observed 3 deaths from MM among licensed herbicide applicators, 0.4 was expected, yielding an SMR of 8.2 (CI 1.6-23.8). A small study of herbicide applicators in Finland by Riihimaki et al. (1983) reported 1 death from MM, with 0.2 expected allowing for a 10 year latency period; one incident case of MM with 0.4 expected was identified. Another small study of herbicide applicators, with no deaths from MM, was conducted in Canada by Green (1991). However, only about 0.3 would have been expected.

Summary of Agricultural/Forestry Worker Studies Ten studies of agricultural and forestry workers provide information on MM risk in relation to herbicide or pesticide exposure. All demonstrated an odds ratio or SMR greater than 1.0, seven did so at a statistically significant level. Additional information linking this increased risk to herbicide exposure can be found in four of the case-control studies (Burmeister et al., 1983; Cantor and Blair, 1984; Alavanja et al., 1989; Boffetta et al., 1989) and is implicit in the positive findings in herbicide applicators (Riihimaki et al., 1983; Swaen et al., 1992).

Paper/Pulp Workers Three studies of pulp and paper workers mentioned that some cohort members may have had exposure to low levels of

dioxins (Robinson et al., 1986; Henneberger et al., 1989; Solet et al., 1989). Grouped cases of MM from these studies with lymphomas other than lymphosarcoma, reticulum cell sarcoma, and Hodgkin's disease resulted in a combined number of cases ($N = 4$); 4.4 were expected.

Environmental Studies

A report of the cancer morbidity of the population exposed to TCDD after the Seveso accident has been published by Pesatori et al. (1992). In a 10 year follow-up of males, there were two cases among residents of zones A and B and one among residents of zone R. The respective RRs were 2.7 (CI 0.6-11.3) and 0.2 (CI 0.0-1.5). The corresponding data for females were two observed in zones A and B and three in zone R, with RRs, respectively, of 4.4 (CI 1.0-18.7) and 0.9 (CI 0.3-3.1). The combined male and female RRs were 3.3 (CI 0.8-8.5) in zones A and B and 0.5 (CI 0.1-1.3) in zone R.

Vietnam Veterans Studies

The major study of multiple myeloma among veterans is the proportionate mortality study of Breslin et al. (1988) of the Department of Veterans Affairs. They found a PMR of 0.8 (CI 0.2-2.5) for Army Vietnam veterans and a PMR of 0.5 (CI 0.0-17.1) for Marine veterans, the latter based on two cases. Each group was compared to Vietnam era veterans of the same service. No MMs were noted in studies describing observations of the CDC Vietnam Experience Study (Boyle et al., 1987) or the Air Force Ranch Hand study (Wolfe et al., 1990). Goun and Kuller (1986) reported that the odds ratio for MM among Pennsylvania Vietnam veterans was less than 1.0. The veteran studies are particularly limited, first, because of the small numbers of MM deaths in the few analyses that have been conducted, and second, because of a broad exposure category, service in Vietnam. With the exception of the Ranch Hands, no definitively exposed groups were categorized.

Summary for Multiple Myeloma

Multiple myeloma has been less extensively studied than other lymphomas, but a consistent pattern of elevated risks appears in the studies that have been conducted, as can be seen in Table 8-32. Ten studies of agricultural and forestry workers provide information on MM risk in relation to herbicide or pesticide exposure. All demonstrated an odds ratio or SMR greater than 1.0; seven did so at a statistically significant level. However, two did not demonstrate an increase over the odds ratio for farming when specification

of herbicide or pesticide use was added, and two demonstrated a relatively flat exposure-response relation. Such limitations in some studies are to be expected, however, given the small number of MM cases with herbicide exposure. The committee determined that the evidence for this association was limited/suggestive because the individuals in the existing studies—mostly farmers—have, by the nature of their occupation, probably been exposed to a range of potentially carcinogenic agents other than herbicides and TCDD.

Conclusions for Multiple Myeloma

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and multiple myeloma.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides, although they have not been studied as extensively as TCDD.

The finding of an association between exposure to phenoxy acids or TCDD and Hodgkin's disease and non-Hodgkin's lymphoma in humans strengthens the suggestive evidence for an association between multiple myeloma and exposure to phenoxy acids.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

LEUKEMIA

Background

According to the American Cancer Society, 28,200 new cases of leukemia (ICD-9 202.4, 203.1, 204.0-204.9, 205.0-205.9, 206.0-206.9, 207.0-207.2, 207.8, 208.0-208.9) were diagnosed in the United States in 1992, and some 18,200 men and women died of this cancer (ACS, 1992). It is somewhat more common among men than women. According to the committee's calculations, 205 cases of leukemia are expected among male Vietnam veterans and 0.4 among female veterans in 1995. In 2000, the expected numbers are 359 cases among male and 0.5 among female veterans.

Leukemia encompasses several malignant disorders of the blood-forming cells in the bone marrow and lymph system. The two principal types of leukemia —lymphocytic and myeloid (or granulocytic)—each occur in acute and chronic forms. Acute myeloid leukemia (AML) accounts for about 45 percent of cases, chronic lymphocytic (CLL) for 30 percent, chronic myeloid (CML) for 15 percent, and acute lymphocytic (ALL) for 10 percent. Misclassification of subtypes of acute leukemia has been noted, particularly for the rarer forms.

CLL occurs largely in adults over the age of 60. The other leukemias are seen in children and adults. The incidence rates of AML and CML increase with age; ALL is concentrated among very young children and the incidence rates increase again among adults over age 65. For leukemias as a group, incidence has declined at less than 1 percent per year since 1973, with most of the decline at older ages. Mortality, however, has declined at younger ages due to treatment, while rising slightly at older ages.

Major epidemiologic studies of atomic bomb survivors and chemical workers have found dose-response related increases in leukemia from radiation and chemical exposures. Chemicals such as benzene and other aromatic hydrocarbons have been shown to be associated with development of AML (Champlin and Golde, 1987). Other specific compounds common to occupational and environmental exposures that have been implicated, but not proven, as human leukemogens include ethylene oxide, styrene, 1,3-butadiene, and vinyl chloride. Paints and nitrites may also be causal agents. Chemotherapeutic agents and immunosuppression have been linked to increased risk of leukemia, and genetic factors may play a role as well.

Epidemiologic Studies

Occupational Studies

Production Workers The NIOSH cohort study (Fingerhut et al., 1991) found no increase in leukemia deaths ($N = 6$), SMR = 0.7 (CI 0.2-1.5).

Workers with a 20 year latency period did not exhibit a significantly elevated SMR for leukemia whether they had less than one year of exposure (SMR = 1.3, CI 0.2-4.6) or more than one year of exposure (SMR = 0.8, CI 0.1-2.8).

Studies have been undertaken at several facilities that produced 2,4-D and 2,4,5-T. Zober and colleagues (1990) examined German workers exposed to TCDD in a chemical accident in 1953. A single death due to leukemia was reported among the three subcohorts studied, with 0.2 expected. A Dow study (Bond et al., 1988) of 878 workers who manufactured 2,4-D between 1945 and 1983, identified two cases of leukemia/aleukemia with at least 15 years latency (SMR = 3.6, CI 0.4-13.0).

Lynge (1985) examined 3,390 male and 1,069 female Danish workers who were employed in the manufacture of phenoxy herbicides. The main herbicide produced was not 2,4,5-T, although some 2,4,5-T production did exist. Nonsignificantly elevated relative risks of 2.1 were found for all female employees with no latency (two cases) and 4.0 for females involved in the manufacturing or packaging of phenoxy herbicides. Results for 10 year latency in this latter comparison were the same, based on one observed case. For male workers, the RR values were 1.1 with no latency (5 cases) and 1.4 for men involved in the manufacturing or packaging of phenoxy herbicides. Results for 10 year latency in this latter comparison were the same, based on one case. Bueno de Mesquita and colleagues (1993) examined a cohort of workers exposed to phenoxy herbicides and observed a nonsignificant elevation of leukemia in two factories, with an SMR = 2.2 (CI 0.3-7.9) in the exposed group.

The IARC study, which included the Lynge and Bueno de Mesquita studies, found no significant elevation of the SMR for leukemia in any of four exposure categories assigned based on questionnaires and job classifications (Saracci et al., 1991). The period studied was 10-19 years after the first exposure in the cohort. The four groups and their associated SMRs for leukemia were exposed, 1.2 (CI 0.7-1.9, $N = 18$); probably exposed, no cases; unknown exposure, no cases; and among those not exposed, the SMR = 0.9 (CI 0.2-2.6, $N = 3$).

Another of the cohorts contributing to the larger cohort of Saracci consisted of 5,784 chemical workers in the United Kingdom involved in the production of MCPA (Coggon et al., 1986). Fourteen deaths from leukemia were observed (SMR = 1.8, CI 1.0-3.0). A suggestion of a dose-response relationship to phenoxy herbicides was reported for three exposed groups: background exposure (SMR = 1.8, $N = 5$), low potential exposure (SMR = 1.3, $N = 3$), and high exposure (SMR = 2.1, $N = 6$). No relationship was identified with duration of exposure for three categories: < 1 month (SMR = 5.9, $N = 3$), 1-6 months (SMR = 1.1, $N = 2$), and > 6 months (SMR = 1.4, $N = 4$). Risk was not elevated with increasing latency period to 10 years from

first exposure. The leukemia results of the production worker studies are summarized in Table 8-33.

TABLE 8-33 Selected Epidemiologic Studies of Production Workers—Leukemia

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Bond et al., 1988	Dow workers with chloracne	2	3.6 (0.4-13.0)
Fingerhut et al., 1991	U.S. chemical workers	6	0.7 (0.2-1.5)
Saracci et al., 1991	Chemical workers		
	Exposed	18	1.2 (0.7-1.9)
	Probably exposed	0	—
	Nonexposed	3	0.9 (0.2-2.6)
	Unknown exposure	0	—
Zober et al., 1990	BASF production workers		
	Second additional cohort	1	5.2 (0.4-63.1)
Bueno de Mesquita et al., 1993	Netherlands production workers Workers exposed to phenoxy herbicides	2	2.2 (0.3-7.9)

[a] Given when available.

Agricultural/Forestry Workers Agricultural studies have focused primarily on farmers. Many of these studies have not attempted to quantify the intensity or duration of individual herbicide exposure (Burmeister, 1981; Blair et al., 1983; Wiklund, 1983; Blair and White, 1985; Zahm et al., 1990). Some studies of agricultural workers have attempted to categorize workers' exposure either by self-reported herbicide use or by job description. No association with elevated risk of leukemia was found among farmers licensed to apply pesticides in Italy (Corrao et al., 1989). Although many of these studies found a statistically significant elevation of mortality due to leukemia, interpretation of the association must be qualified, since other exposures, such as to pesticides or animals as vectors of viruses (Brownson et al., 1989; Brown et al., 1990), could have been the causal agent.

Brown and colleagues (1990) conducted a case-control study of leukemia for association with herbicide use. They identified an OR = 1.4 (CI 1.1-1.9) for chronic lymphocytic leukemia, based on 156 cases who were farmers. The remaining classifications of leukemia had odds ratios not statistically different from 1. They further classified exposure based on type of herbicide used. For those who used phenoxy herbicides, the odds ratio for all types of leukemia combined was 1.2 (CI 0.9-1.6); for those who used 2,4-D the odds ratio for all leukemias was 1.2 (CI 0.9-1.6); and for those who used 2,4,5-T the odds ratio for all leukemias was 1.3 (CI 0.7-2.2).

An excess of AML among those who used 2,4,5-T was reported, based on eight cases (OR = 2.1, CI 0.9-4.9). An excess of CLL was found among those who used 2,4,5-T (OR = 1.6, CI 0.7-3.4 for 10 cases). For those handling 2,4,5-T at least 20 years before interview, this excess for CLL was significant (OR = 3.3, CI 1.2-8.9). No dose-response relationship between number of years farmed and leukemia risk was identified.

Alavanja and colleagues (1988) studied cases of leukemia for association with being an agricultural extension agent and calculated an OR = 1.9 (CI 1.0-3.5) for all types of leukemia. The risk for myeloid leukemia was elevated (OR = 2.8, CI 1.1-7.2). However, they reported that "the association between mortality from cancers of lymphatic and hematopoietic systems, especially leukemia, suggests an occupational origin other than exposure to 2,4-D," since the OR for extension workers was higher for extension agents without prior farm experience than for agents with prior employment as farmers, and was also higher for those associated with chicken farming rather than with growing wheat or corn, yet the latter was more likely to involve herbicides.

In a case-control study using death certificates, Burmeister and colleagues (1982) found an elevated odds ratio (OR = 1.9, CI 1.2-3.1) for chronic lymphatic leukemia in farmers residing in Iowa counties that had higher use of herbicides.

Hansen and colleagues (1992) examined the prevalence of leukemia in a cohort of Danish gardeners (*N* = 4,015). They assumed that males work outdoors and therefore are exposed to herbicides, whereas females work indoors and are not exposed. The standardized morbidity ratio for chronic lymphatic leukemia was elevated among the 3,156 male gardeners (SMbR = 2.8, CI 1.0-6.0). However, pesticide and insecticide exposures were also reported by the outdoor workers, confounding the interpretation between herbicide exposure and elevated chronic lymphatic leukemia morbidity.

Ronco and colleagues (1992) calculated the standardized incidence ratio for male and female owners and employees at farms in Denmark and mortality odds ratios in Italy. Typically, the farmers in Italy applied their own herbicides, whereas professionals applied herbicides in Denmark rather than farmers. Statistically elevated SIRs were identified only for females who operated their own farms in Denmark, who were more likely to be raising animals and less likely to be exposed to herbicides. Wigle and colleagues (1990) stratified Canadian male farmers by the number of acres of herbicides sprayed in a region and observed no positive association with leukemia (OR = 0.9, CI 0.7-1.0).

Herbicides have been used by outdoor workers to clear heavy vegetative undergrowth. Bender and colleagues (1989) studied mortality among 4,849 male highway workers who used herbicides to clear roads, but did not stratify the workers by type or level of exposure. Leukemia was identified

in 17 workers; the overall SMR for leukemia was 1.1 (CI 0.6-1.7). However, when duration of employment was examined, the SMR for seven workers with 30-39 years experience was 4.3 (CI 1.7-8.8), whereas no deaths were noted for workers with less than 5 years experience, with 3.3 expected. Green (1991) compared death from leukemia as reported on death certificates with the number of years employed as a forestry worker. These workers were presumably exposed to phenoxy herbicides for more than six months. No positive association between years employed and leukemia was observed. Only a single death, out of a total of 80, was attributed to leukemia among the 1,222 men in the study. The author indicated that 96 percent of the workers were younger than 55 years; thus the cohort should be followed longer to determine disease incidence. The maximum latency period reported in the study was 30 years from the onset of exposure.

Results of the agricultural worker studies for leukemia are summarized in Table 8-34.

Pulp/Paper Workers To study the health effects of dioxins produced during the bleaching of pulp and paper, Solet and colleagues (1989) collected data on 201 deceased white male members of the United Paperworkers Union and identified a nonsignificant elevated PMR of 2.3 (CI 0.6-6.0) for leukemia and aleukemia combined. However, the job category of the workers was not indicated; therefore, exposure could not be assessed. Other risk factors were not controlled for in the analysis. Robinson and colleagues (1986) also did not determine individual exposure in their study of a cohort of 3,572 pulp and paper workers. An SMR of 0.5 (CI 0.2-1.5) was calculated for leukemia, based on four observed deaths. Henneberger and colleagues (1989) segregated the 883 employees within a pulp and paper mill by location, with those working in the chlorination section presumably having the highest exposure to dioxins. A nonsignificant elevated SMR was identified for leukemia in some subgroups, with a positive increase in SMR with consideration of a more than 20 year latency period. The total number of cases in each subgroup was small; for example, among 376 men who worked for at least a year in the paper mill, the SMR was 2.4 (CI 0.5-7.1).

Environmental Studies

Three papers have examined mortality from leukemia following exposure to TCDD that occurred during the 1976 accident at Seveso (Bertazzi et al., 1989a, 1992; Pesatori et al., 1992). However, since the longest latency period reported in any of these studies was 10 years, the length of follow-up may be insufficient to reach a final conclusion. An elevated RR = 2.5 (CI 0.9-7.3) for mortality from myeloid leukemia was observed in males from zones A, B, and R (Bertazzi et al., 1989a). The RR was also observed to increase with an increase in time since exposure; the RR for cases dying

between 1976 and 1981 was 1.6 (CI 0.3-7.5), whereas for 1982-1986 it was 4.2 (CI 0.9-19.0) (Bertazzi et al., 1989a). No excess for other types of leukemia among males or for any leukemia among females was found.

TABLE 8-34 Selected Epidemiologic Studies of Agricultural Workers—Leukemia

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Cohort studies			
Hansen et al., 1992	Danish gardeners		
	All gardeners—CLL	6	2.5 (0.9-5.5)
	All gardeners—all other types of leukemia	3	1.2 (0.3-3.6)
	Male gardeners—CLL	6	2.8 (1.0-6.0)
	Male gardeners—all other types of leukemia	3	1.4 (0.3-4.2)
Ronco et al., 1992	Danish and Italian farm workers		
	Danish self-employed farmers		0.9
	Danish male farmers		1.0
	Italian self-employed farmers		0.7
	Italian male farmers		0.9
Wigle et al., 1990	Saskatchewan farmers	138	0.9 (0.7-1.0)
Case-control studies			
Brown et al., 1990	Residents of Iowa and Minnesota		
	All types of leukemia, ever farmed		1.2 (1.0-1.5)
	CLL, ever farmed		1.4 (1.1-1.9)
	All types of leukemia, any herbicide use		1.2 (0.9-1.6)
	CLL, any herbicide use		1.4 (1.0-2.0)
	Herbicide users, phenoxy acid use		1.2 (0.9-1.6)
	All types of leukemia, 2,4-D use		1.2 (0.9-1.6)
	All types of leukemia, 2,4,5-T use		1.3 (0.7-2.2)
Alavanja et al., 1988	USDA agricultural extension agents		1.9 (1.0-3.5)
Blair and White, 1985	Residents of Nebraska		
	All cases, all leukemia—farming		1.3
Burmeister et al., 1982	Residents of Iowa		
	CLL in white, male farmers		1.9 (1.2-3.1)

[a]Given when available.

A separate study (Bertazzi et al., 1992) examined mortality among individuals who were between 1 and 19 years old at the time of the accident. Again, a nonsignificant but elevated relative risk was calculated for lymphatic leukemia for males (RR = 9.6, CI 0.9-106.0). The relative risk for overall leukemia among males was 2.1 (CI 0.7-6.9), and for overall leukemia

for females 2.5 (CI 0.2-27.0), but these estimates were based on four male cases and a single female case.

In a cancer morbidity study of men and women found in zones A and B, there were two leukemia cases for males (RR = 1.4, CI 0.3-5.5), and two cases for females (RR = 1.5, CI 0.4-6.2) (Pesatori et al., 1992). One of the male cases and both of the female cases were myeloid leukemia, yielding relative risks of 1.6 (CI 0.2-11.9) and 3.3 (CI 0.8-13.9), respectively.

Vietnam Veterans Studies

Epidemiologic studies of Vietnam veterans have been conducted by several states, the DVA, the CDC, the Australian government, the Air Force, and others. None of these studies found an elevated risk for leukemia among Vietnam veterans (Anderson et al., 1986a,b; Boyle et al., 1987; Fett et al., 1987b; Breslin et al., 1988). In contrast to NHL and HD, the DVA studies (e.g., Breslin et al., 1988; Watanabe et al., 1991) did not report any unexpected deficit of leukemia cases among the Vietnam era comparison group. However, since none of these studies specifically defined an individual's exposure to herbicides, any potential association between herbicide exposure and leukemia incidence would have been greatly diluted.

Thomas and Kang (1990) studied mortality among members of the U.S. Army Chemical Corps, which served in Vietnam between 1966 and 1971. This group was responsible for the storage, preparation, and application of herbicides and other chemicals. Two deaths from leukemia were found with only 0.5 expected; this increase was not statistically significant. The authors note that since this group was in contact with chemicals other than herbicides in Vietnam, and since postwar exposures were not documented, the increase in leukemia deaths could not be attributed to military herbicide use alone. No cases of leukemia were reported for the Ranch Hand cohort (Michalek et al., 1990), the group responsible for the U.S. military aerial spraying of herbicides in Vietnam and having documented exposures to herbicides.

Summary

The epidemiologic evidence for an association between exposure to herbicides and leukemia comes primarily from studies of farmers and residents of Seveso, Italy. A number of studies of farmers show a consistently elevated risk of leukemia. These results are not necessarily due to herbicide use, however, since other confounding exposures exist and were not adequately controlled for in the analyses of these studies. Furthermore, when farmers are stratified by suspected use of herbicide, the incidence of leukemia is generally not elevated. Some studies of chemical workers found an

increased risk of leukemia, but the number of cases was small in all of these studies.

In the accident that occurred at Seveso, the observed overall relative risk for leukemia mortality and incidence were elevated, but not significantly. The increase was significant, however, for cases who died five to ten years after the accident and were in the most highly exposed zone. Since only 10 years of follow-up are currently available, the follow-up studies through 1996 should provide further evidence of whether the suggested association is real.

The available data on Vietnam veterans are generally not conclusive because the exposure data are inadequate for the cohort being studied. For example, exposure estimates based on region of service are inadequate for differentiating levels of exposure, and possible alternative exposures exist within the Army Chemical Corps. Small sample sizes weaken the studies of the Ranch Hands or Chemical Corps, where excesses are not likely to be detected.

Since no study has adequately differentiated between exposure solely to either herbicides or TCDD, or demonstrated a dose-response for any subtype of leukemia, it is not possible to attribute any symptom or subtype of leukemia as a result of exposure.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and leukemia.

Biologic Plausibility

TCDD has been shown to have a wide range of effects in laboratory animals on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. In addition, TCDD has been shown to cause cancer in laboratory animals at a variety of sites. If TCDD has similar effects on cell regulation in humans, it is plausible that it could have an effect on human cancer incidence. In contrast to TCDD, there is no convincing evidence of, or mechanistic basis for, the carcinogenicity in animals of any of the herbicides.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential

risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

SUMMARY

Based on the occupational, environmental, and veterans studies that it has reviewed, the committee has reached one of four standard conclusions about the strength of the epidemiological evidence regarding association between exposure to herbicides and/or TCDD and each of the cancers under study. As explained in Chapter 5, these distinctions—leading to four categories—reflect the committee's judgment that if an association between exposure and an outcome were "real," it would be found in a large, well-designed epidemiologic study in which exposure to herbicides or dioxin was sufficiently high, well-characterized, and appropriately measured on an individual basis. Consistent with the charge to the Secretary of Veterans Affairs in Public Law 102-4, the distinctions between these standard conclusions are based on statistical association, not on causality as is common in scientific reviews. To summarize the committee's conclusions, the data are reviewed here by category, with emphasis on the factors that led the committee to assign the cancer to this category and not some other.

Cancers with Sufficient Evidence of an Association

The committee found sufficient evidence of an association with herbicides and/or TCDD for three cancers: soft tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease. For cancers in this category, a positive association between herbicides and the outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. The committee regards evidence from several small studies that are free from bias and confounding, and show an association that is consistent in magnitude and direction, as sufficient evidence for an association.

Soft tissue sarcomas are a rare but diverse group of tumors that share a common International Classification of Diseases code but have a wide variety of forms and causes. The strongest evidence for an association between STS and exposure to phenoxy herbicides comes from a series of case-control studies involving a total of 506 cases conducted by Hardell and colleagues in Sweden (Hardell and Sandstrom, 1979; Eriksson et al., 1981;

Hardell and Eriksson, 1988; Eriksson et al., 1990; Wingren et al., 1990) that show an association between STS and exposure to phenoxy herbicides, chlorophenols, or both. Although these studies have been criticized, the committee feels that there is insufficient justification to discount the consistent pattern of elevated risks, and the clearly described and sound methods employed. These findings are supported by a significantly increased risk in the NIOSH study (SMR = 9.2, CI 1.9-27.0) for the production workers most highly exposed to TCDD (Fingerhut et al., 1991), and a similar increased risk in the IARC cohort (SMR = 6.1, CI 1.7-15.5) for deaths that occurred between 10 and 19 years after the first exposure (Saracci et al., 1991). These are the two largest, as well as the most highly exposed occupational cohorts. Some studies in other occupational, environmental, and veterans groups showed an increased risk for STS, but the results were commonly nonsignificant possibly because of small sample sizes related to the relative rarity of STS in the population. Because of difficulties in diagnosing this group of tumors, the epidemiologic studies reviewed by the committee were inconsistent with regard to the specific types of tumors included in the analyses. The available data did not permit the committee to determine whether specific forms of STS were or were not associated with TCDD and/or herbicides. Therefore, the committee's findings relate to the class as a whole.

Non-Hodgkin's lymphoma includes a group of malignant lymphomas, that is, neoplasms derived from lymphoreticular cells in lymph nodes, bone marrow, spleen, liver, or other sites in the body. One large, well-conducted case-control study in Sweden by Hardell and colleagues (1981) examined NHL and Hodgkin's disease together and found an odds ratio of 6.0 (CI 3.7-9.7) based on 105 cases for exposure to phenoxy acids or chlorophenols, and these results held up under further investigation of the validity of exposure assessment and other potential biases (Hardell, 1981). A more recent case-control study by Persson and colleagues (1989) showed increased risk for NHL in those exposed to phenoxy acids (OR = 4.9, CI 1.0-27.0), based on a logistic regression analysis of 106 cases. Other studies of farmers and agricultural workers (Tables 8-22, 8-23, 8-24) are generally positive for an association between NHL and herbicides/TCDD; however, only some are significant. All of the studies of U.S. agricultural workers reviewed showed elevated relative risks (although none were significant), and two NCI studies of farmers in Kansas and Nebraska (Hoar et al., 1986; Zahm et al., 1990) show patterns of increased risk linked to use of 2,4-D. The CDC Selected Cancers Study found an increased risk of NHL in association with service in Vietnam; other studies of veterans, generally with small sample sizes, are consistent with an association (Tables 8-26 and 8-27). In contrast, studies of production workers, including the largest, most heavily exposed cohorts (Fingerhut et al., 1991; Saracci et al., 1991; Zober et al., 1990;

Manz et al., 1991) indicate no increased risk. Thus, unlike most of the other cancers studied by the committee for which the data do not distinguish between the effects of herbicides and TCDD, the available epidemiologic data suggest that the phenoxy herbicides, including 2,4-D, rather than TCDD may be associated with non-Hodgkin's lymphomas.

Hodgkin's disease, also a malignant lymphoma, is a neoplastic disease characterized by progressive anemia and enlargement of lymph nodes, spleen, and liver. Fewer studies have been conducted of HD in relation to exposure to herbicides or TCDD than have been conducted of STS or NHL, but the pattern of results is strikingly consistent. The 60 HD cases in the study by Hardell and colleagues (1981) were later examined by Hardell and Bengtsson (1983), who found odds ratios of 2.4 (CI 0.9-6.5) for low-grade exposure to chlorophenols and 6.5 (CI 2.7-19.0) for high-grade exposures. Persson and colleagues' study (1989) of 54 HD cases showed a large, but not statistically significant, OR = 3.8 (CI 0.5-35.2) for exposure to phenoxy acids. Furthermore, nearly all of the 13 case-control and occupational cohort studies summarized in Tables 8-28, 8-29, and 8-30 show increased risk for HD, although only a few of these results are statistically significant. As with NHL, even the largest studies of production workers exposed to TCDD do not indicate an increased risk. The few studies of HD in Vietnam veterans tend to show elevated risks, but all but one are not statistically significant (Table 8-31).

When these three cancers (STS, NHL, and HD) are considered as a whole, it is noteworthy that the strongest evidence for an association with exposure to phenoxy herbicides is the series of case-control studies conducted by Hardell and colleagues and the cohort studies of herbicide applicators and agricultural workers. Studies in other countries are sometimes positive, but not as consistently. Whether this reflects higher typical exposure levels in workers in the countries studied, genetic differences in susceptibility to these diseases, the fact that more intensive studies have taken place, or other risk factors is not known. With regard to STS, the study of Woods and colleagues (1987) suggests that both exposure levels and genetic differences are at play. However, although there may be differences from population to population in the increased risk associated with exposure to herbicides and TCDD, the committee regards the available evidence as sufficient to indicate that there is a statistical association between the herbicides used in Vietnam and STS, NHL, and HD.

Cancers with Limited/Suggestive Evidence of An Association

The committee found limited/suggestive evidence of an association for three other cancers: respiratory cancers, prostate cancer, and multiple myeloma. For outcomes in this category, the evidence must be suggestive of an association

between herbicides and the outcome, but may be limited because chance, bias, or confounding could not be ruled out with confidence. Typically, at least one high-quality study indicates a positive association, but the results of other studies may be inconsistent.

Among the many epidemiologic studies of respiratory cancers (specifically cancers of the lung, larynx, and trachea), positive associations were found consistently only in those studies in which TCDD or herbicide exposures were probably high and prolonged, especially the largest, most heavily exposed cohorts of chemical production workers exposed to TCDD (Zober et al., 1990; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991) (see Table 8-8) and herbicide applicators (Axelson and Sundell, 1974; Riihimaki et al., 1982; Blair et al., 1983; Green, 1991). Studies of farmers tended to show a decreased risk of respiratory cancers (perhaps due to lower smoking rates), and studies of Vietnam veterans are inconclusive. The committee felt that the evidence for this association was limited/suggestive rather than sufficient because of the inconsistent pattern of positive findings across populations with various degrees of exposure and because the most important risk factor for respiratory cancers—cigarette smoking—was not fully controlled for or evaluated in all studies.

Several studies have shown elevated risk for prostate cancer in agricultural or forestry workers. In a large cohort study of Canadian farmers (Morrison et al., 1993), an increased risk of prostate cancer was associated with herbicide spraying, and increasing risk was shown with increasing number of acres sprayed. For the entire cohort, the relative risk for prostate cancer and spraying at least 250 acres was 1.2 (CI 1.0-1.5). When the analysis was restricted to the farmers most likely to be exposed to phenoxy herbicides or other herbicides, and those with no employees, no custom workers to do the spraying for them, age between 45-69 years, and sprayed $\geq$ 250 acres RR = 2.2 (CI 1.3-3.8); the test for trend over increasing number of acres sprayed was significant. The risk was elevated in a study of USDA forest conservationists (PMR = 1.6, CI 0.9-3.0) (Alavanja et al., 1989), and a case-control study of white male Iowans who died of prostate cancer (Burmeister et al., 1983) found a significant association (OR = 1.2) that was not associated with any particular agricultural practice. These results are strengthened by a consistent pattern of nonsignificant elevated risks in studies of chemical production workers in the United States and other countries, agricultural workers, pesticide applicators, paper and pulp workers, and the Seveso population (see Table 8-18). Studies of prostate cancer among Vietnam veterans or following environmental exposures have not consistently shown an association. However, prostate cancer is generally a disease of older men, and the risk among Vietnam veterans would not be detectable in published epidemiologic studies. Because there was a strong indication of a dose-response relationship in one study and a consistent positive association

in a number of others, the committee felt that the evidence for association with herbicide exposure was limited/suggestive for prostate cancer.

Multiple myeloma, a cancer of specific bone marrow cells, has been less extensively studied than other lymphomas, but a consistent pattern of elevated risks appears in the studies that have been conducted, as can be seen in Table 8-32. Ten studies of agricultural and forestry workers provide information on MM risk in relation to herbicide or pesticide exposure. All demonstrated an odds ratio or SMR greater than 1.0; seven did so at a statistically significant level. This finding is made more specific for herbicide exposure by subanalyses in four of these studies (Burmeister et al., 1983; Cantor and Blair, 1984; Alavanja et al., 1989; Boffetta et al., 1989) that suggest higher risks for those exposed to herbicides, and higher risks for the studies of herbicide applicators summarized in Table 8-32 (Riikimaki et al., 1983; Swaen et al., 1992). The committee determined that the evidence for this association was limited/suggestive because the individuals in the existing studies—mostly farmers—have, by the nature of their occupation, probably been exposed to a range of potentially carcinogenic agents other than herbicides and TCDD. Multiple myeloma, like non-Hodgkin's lymphoma and Hodgkin's disease for which there is stronger epidemiologic evidence of an association, is derived from lymphoreticular cells, which adds to the biologic plausibility of an association.

Cancers with Limited/Suggestive Evidence of No Association

For a small group of cancers the committee found a sufficient number and variety of well-designed studies to conclude that there is limited/suggestive evidence of no association between these cancers and TCDD or the herbicides under study. This group includes gastrointestinal tumors (colon, rectal, stomach, and pancreatic), skin cancer, brain tumors, and bladder cancer. For outcomes in this category, several adequate studies covering the full range of levels of exposure that human beings are known to encounter are mutually consistent in not showing a positive association between exposure to herbicides and the outcome at any level of exposure and which have relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, level of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.

The data on colon cancer exemplify the situation that led the committee to say that there was evidence of no association between a cancer and exposure to herbicides and/or TCDD. Colon cancer is relatively common, so an increase in the risk of these cancers would be relatively easy to detect in occupational studies. Table 8-4 summarizes the epidemiologic studies

reviewed by the committee that address colon cancer; they include a mixture of occupational studies of various types, environmental studies, and studies of Vietnam veterans. Some of the studies such as the NIOSH (Fingerhut et al., 1991) and IARC (Saracci et al., 1991) cohorts are large and have relatively high exposures. The number of studies with estimated relative risks above and below 1.0 are roughly evenly distributed, and a number of studies have tight confidence intervals that include 1.0. The NIOSH study, for instance, based on 25 exposed cases, finds an odds ratio of 1.2 with a 95 percent confidence interval of 0.8 to 1.8. The IARC study finds an odds ratio of 1.1 (CI 0.8-1.5) based on 41 cases. Thus, this pattern suggests that there is no association between herbicides/TCDD and colon cancer, at least in the situations represented in the available studies.

Cancers with Inadequate/Insufficient Evidence to Determine Whether an Association Exists

The scientific data for the remainder of the cancers reviewed by the committee were inadequate or insufficient to determine whether an association exists. For cancers in this category, the available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association. For example, studies fail to control for confounding or have inadequate exposure assessment. This group of cancers includes hepatobiliary cancers, nasal/nasopharyngeal cancer, bone cancer, female reproductive cancers (breast, cervical, uterine, ovarian), renal cancer, testicular cancer, and leukemia.

For example, there are relatively few occupational, environmental, or veterans studies of liver cancer, and most of these are small in size and have not controlled for life-style-related risk factors. One of the largest studies (Hardell et al., 1984) indicates an increased risk for liver cancer and exposure to herbicides, but another study of Swedish agricultural workers (Wiklund, 1983) estimates a relative risk that is significantly less than 1.0. The estimated relative risks from other studies are both positive and negative. As a whole, when bearing in mind the methodological difficulties associated with most of the few existing studies, the evidence regarding liver cancer is not convincing about either an association with herbicides/TCDD or the lack of an association.

The epidemiologic evidence for an association between exposure to herbicides and leukemia comes primarily from studies of farmers and residents of Seveso, Italy. The observed overall relative risk for leukemia mortality and incidence in Seveso was elevated, but not significantly. A number of studies of farmers that the committee found convincing for NHL, HD, or MM also show a consistently elevated risk of leukemia (see Table 8-34), but these results are not necessarily due to herbicide use because confounding

exposures were not controlled for adequately in the analyses of these studies and because when farmers are stratified by suspected use of herbicide, the incidence of leukemia is generally not elevated. Some studies of chemical workers found an increased risk of leukemia, but the number of cases was small in all of these studies. The available data on Vietnam veterans are generally not conclusive because the exposure data are inadequate for the cohort being studies. Small sample sizes weaken the studies of the Ranch Hands or Chemical Corps, where excesses are not likely to be detected.

Increased Risk in Vietnam Veterans

Although there have been numerous health studies of Vietnam veterans, most have been hampered by relatively poor or non-existent measures of exposure to herbicides or TCDD, in addition to other methodologic problems. Most of the evidence on which the conclusions in this chapter are based comes from studies of people exposed to dioxin or herbicides in occupational and environmental settings, rather than from studies of Vietnam veterans. The committee found this body of evidence adequate for reaching the conclusions about statistical associations between herbicides and health outcomes in this chapter. However, the lack of adequate data on Vietnam veterans per se complicates the determination of the increased risk of disease among individuals exposed to herbicides during service in Vietnam. To estimate the magnitude of risk for a particular health outcome among herbicide-exposed Vietnam veterans, quantitative information about the dose-time-response relationship for each health outcome in humans, information on the extent of herbicide exposure among Vietnam veterans, and estimates of individual exposure are needed. Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the studies that have been reviewed, the inadequate control for important confounders, and the uncertainty about the nature and magnitude of exposure to herbicides in Vietnam (as discussed in Chapter 6), none of the ingredients necessary for a quantitative risk assessment are available. Thus, it is not possible for the committee to quantify the degree of risk likely to be experienced by veterans because of their exposure to herbicides in Vietnam.

NOTE

[*] The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

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9

Reproductive Effects

This chapter reviews the evidence for an association between herbicide exposure and reproductive and developmental endpoints including spontaneous abortion, birth defects, perinatal and infant mortality, low birthweight, childhood cancer, and sperm abnormalities and infertility. The evidence for an increased risk among veterans exposed to herbicides in Vietnam and the biologic plausibility of herbicides causing the outcomes of interest are also reviewed. As a reminder, most of the studies of occupational groups and veterans involve exposure of men to the herbicides in question, the exception being a small number of environmental studies that included maternal (and probably paternal) exposure. This introduction reviews two other topics pertinent to this chapter: some specific methodologic issues of relevance when evaluating the reproductive epidemiology studies discussed, and general comments regarding the plausibility of male-mediated developmental effects.

METHODOLOGIC ISSUES

Chapter 5 describes the general methodologic issues pertaining to the evaluation and interpretation of epidemiologic studies. This section will expand on some of these issues as they relate specifically to reproductive epidemiology. Many of these issues are important in the interpretation of the findings of a number of studies of herbicides and reproductive outcomes in which the relative risk estimate is "weak" or "moderate," that is, between 1 and 2. It is often stated that the closer the relative risk gets to 1, the

greater is the likelihood that confounding or recall bias may explain the association (Wynder, 1987; Khoury et al., 1992a).

Confounding can result in the estimate of the relative risk being biased either toward or away from the null value of 1.0 (no association). By definition, confounding factors have to be risk factors for the disease under consideration. Confounding with respect to reproductive outcomes is a difficult issue. For some outcomes, such as birth defects, there are few suspected, let alone established, risk factors (e.g., maternal age and Down's syndrome). The possibility of unmeasured confounding could therefore play a role in explaining some of the associations reported. Conversely, it is possible that in some situations, confounding could mask a stronger association. The extent of confounding in the studies examined for this chapter is uncertain.

Biased recall of exposure or outcome is another potential problem in reproductive epidemiology studies. Misclassification of exposure to herbicides has been discussed in Chapter 5. Misclassification of outcome can be a problem leading to either an under- or an overestimate of the true relative risk. For certain reproductive outcomes and childhood conditions (e.g., specific birth defects), accurate recall of the outcome may be difficult, especially if the pregnancy or event occurred in the distant past. If both the study and the comparison groups (e.g., veterans versus nonveterans) have similarly flawed recall, the relative estimate will be reduced toward 1.0. If, however, recall differs between the groups, a biased estimate may be obtained. Medical record verification of many reproductive and childhood health conditions is needed to minimize this potential bias.

The statistical power of reproductive epidemiology studies to detect an elevated relative risk, if one exists, should also be borne in mind when interpreting the evidence (see Chapter 5). In a cohort study evaluating herbicide exposure (e.g., among occupational groups or Vietnam veterans) and spontaneous abortion, approximately 266 total pregnancies would have to be studied to detect a doubling of risk (relative risk = 2, alpha = .05, beta = .80). For other outcomes, the sample sizes (exposed and unexposed groups combined) required to detect a doubling of risk would be 656 live births for low birthweight; 2,478 live births for all major birth defects; 16,932 live births for the most common major birth defect; 17,902 live births for chromosomal abnormalities; and 1,856 live births for infant death. Some of the studies reviewed had adequate statistical power for assessment of some of the more common reproductive outcomes such as spontaneous abortion, but power may have been lacking for rarer outcomes such as specific birth defects. When evaluating a given study finding, examination of the confidence interval around the point estimate of the relative risk will provide guidance as to the degree of precision and study size.

PLAUSIBILITY

In this evaluation of herbicide exposure, adverse reproductive outcomes, and potentially increased risk among Vietnam veterans, the primary emphasis is on exposure to the male. As noted in the research recommendations (Chapter 12), further study of female veterans is called for; nonetheless, the majority of potentially exposed workers and veterans are men. This situation requires further general discussion, given the fact that the vast majority of animal and human data on adverse reproductive outcomes pertain to maternal preconceptional or in utero exposure not paternal exposure. Further, as noted in Chapter 4, herbicides have not been fully evaluated in male animal test systems for many developmental endpoints. Therefore, a general summary of the animal and human evidence on paternal exposure to chemicals or radiation and adverse reproductive or developmental outcomes can help put the review of herbicides in context.

The role of paternal exposures in the etiology of many reproductive and developmental outcomes has not been investigated extensively (Olshan and Faustman, 1993). The effects of chemicals and radiation on sperm parameters and, possibly, infertility have been demonstrated (Wyrobek et al., 1983). On the other hand, the prevailing view is that exposure of the human male to chemicals and radiation is largely unrelated to the occurrence of developmental endpoints such as miscarriage, birth defects, growth retardation, and cancer (Brown, 1985). This view has been held despite the fact that some animal studies have indicated that male exposure can lead to a variety of developmental outcomes. The paucity of human data and definitive mechanistic models has hindered progress in this area. Several potential mechanisms have been proposed to explain possible male-related effects on offspring. A direct effect of an agent on male germ cell DNA is the traditional explanation for the induction of some developmental abnormalities. The majority of the available animal test data involve this mechanism. More indirect mechanisms involving transfer to toxic agents in seminal fluid and maternal exposure to agents brought home by the father have been suggested, although data supporting these routes are lacking at present.

As noted above, experimental animal evidence for direct effects of exposure on male germ cell DNA (germ cell mutagenicity assays) has been available for a number of years. In fact, an important animal test for germ cell mutagenicity, the specific locus test, was developed in 1951 with tests involving ionizing radiation (Russell, 1951). The majority of the animal test data include the evaluation of radiation and chemicals in relation to expression of defined visible phenotypes due to mutations at recessive loci (specific locus test), fetal loss (dominant lethal test), inherited chromosomal aberrations (heritable translocation test), and defined congenital anomalies (dominant skeletal and dominant cataract tests). Ionizing radiation and a

small number (compared to animal carcinogenicity assays) of chemicals have yielded positive results in these test systems. One pattern that has emerged from the specific locus test data is that for many chemicals the shorter-lived, postmeiotic spermatogenic cells are more sensitive to the induction of mutations (Russell et al., 1990). There are also animal data showing that male exposure to radiation and some chemicals can produce other outcomes in offspring such as congenital anomalies, tumors, growth retardation, and neurobehavioral effects. Animal test data for these endpoints are suggestive but are still limited at the present time (Selby, 1990).

Epidemiologic associations between paternal exposures and developmental abnormalities in offspring have been reported, although further replication of the suggestive findings remains to be done. Two general types of exposure have been studied, namely, occupational and life-style exposures such as tobacco and alcohol consumption. Unfortunately, few other categories of exposure have been examined. A summary of the findings from epidemiologic studies is provided here. Paternal occupational exposure to vinyl chloride, anesthetic gases, dibromochloropropane, mercury, lead, other metals, and various solvents has been linked to an increased risk of fetal loss (spontaneous abortion) (Olshan and Faustman, 1993). Use of tobacco and alcohol has not been associated with an elevated relative risk of spontaneous abortion, although there have been few studies directly focusing on paternal exposure to these substances. Paternal employment in the textile, mining, rubber, plastics, and synthetics industries was associated with prematurity and low birthweight in offspring in one study (Savitz et al., 1989). Another study found an association between paternal alcohol consumption and reduced birthweight of offspring, after adjustment for maternal factors (Little and Sing, 1987). A number of epidemiologic studies have examined the relationship between paternal occupation and birth defects. A variety of occupations have yielded positive associations including painters, welders, auto mechanics, firemen, forestry and logging workers, motor vehicle operators, wood workers, farm workers, metal workers, and plywood mill workers (Olshan and Faustman, 1993). Some associations between birth defects and fathers' smoking and alcohol use have been noted (Savitz et al., 1991).

Several recent large case-control studies have suggested some paternal occupations and exposures that may be associated with childhood cancer in the offspring (Savitz and Chen, 1990; O'Leary et al., 1991). These associations include painters, mechanics, machinists, and motor vehicle drivers and leukemia; painters, metal workers, electronics-related industries, and motor vehicle-related jobs and childhood brain tumors; auto mechanics and machinists, welders, and painters and Wilms' tumor (childhood kidney tumor); electronics-related occupations and neuroblastoma. Additional study of these occupational exposures is needed to establish the potential importance of

previous findings. Paternal cigarette consumption has been related to an increased risk of childhood cancer in some studies, but not in others (Olshan and Faustman, 1993). A recent study of an excess of leukemia and lymphoma among the children of nuclear plant workers in Great Britain has been the subject of much discussion and controversy (Gardner et al., 1990; MacMahon, 1992). The pregnancies and offspring of two groups of uniquely exposed individuals, atomic bomb survivors and survivors of childhood cancer, have been the subject of epidemiologic study. There does not appear to be an increased risk of adverse reproductive and developmental outcomes in these groups, although some concerns regarding statistical power and methodology persist.

The animal and human data indicate that the exposure of the male to various toxic agents may increase the risk of the full spectrum of adverse developmental endpoints from fetal loss to cancer. However, the evidence is not firm and requires much more study in both laboratory and epidemiologic settings.

SPONTANEOUS ABORTION

Introduction

Definition

Spontaneous abortion (or miscarriage), according to the World Health Organization (1977), is a "nondeliberate fetal death of an intrauterine pregnancy before 22 completed weeks of gestation, corresponding to a fetal weight of approximately 500 grams or more." Pregnancy losses prior to implantation (preimplantation) are not clinically detectable with currently available diagnostic procedures. In contrast to preimplantation losses, all postimplantation losses are, at least in theory, clinically detectable, since measurable human chorionic gonadotropin production begins at implantation (Kline et al., 1989). However, early postimplantation losses occurring prior to the first missed menstrual period (e.g., 25 to 28 days after the last menstrual period) will also tend to go undetected, since they generally occur prior to pregnancy recognition. The rate of these early detectable (but often unrecognized) pregnancy losses has been estimated to be approximately 30 percent (Wilcox et al., 1988). Because preimplantation and early postimplantation losses are difficult to ascertain for epidemiologic studies of pregnancy loss, the appropriate epidemiologic end point for these studies is not all spontaneous abortions but rather all clinically recognized spontaneous abortions—those that come to the attention of a woman or her physician. All subsequent discussions of pregnancy loss, miscarriage, or spontaneous abortion refer to clinically recognized outcomes unless otherwise specified.

Descriptive Epidemiology

Approximately 10 to 15 percent of all clinically recognized pregnancies end in a clinically recognized loss. Of these clinically recognized pregnancy losses, 35 to 40 percent are losses of chromosomally abnormal embryos and fetuses (Kline et al., 1989).

A wide range of maternal characteristics and exposures has been linked to miscarriage; however two major risk factors have been established—maternal age and history of previous miscarriage (Kline et al., 1989). The risk of pregnancy loss is known to increase with increasing maternal age, especially after age 30 or 35. A woman's risk of having a second loss once she has had a first is elevated about 60 percent over that of women with no history of miscarriage. The risk of losing a pregnancy may also be increased among women with a history of multiple induced abortions. Pregnancy losses also occur more frequently among nonwhite than among white women. Women of lower socioeconomic status (SES) appear to have a higher proportion of chromosomally normal spontaneous abortions relative to their higher-SES counterparts. Other maternal medical conditions and exposures that have been associated, at least in some studies, with an increased risk of miscarriage include diabetes and epilepsy, a history of maternal fever during pregnancy, uterine and hormonal abnormalities, immunologic (e.g., Rh factor) incompatibilities, maternal exposure to ionizing radiation, and maternal contraceptive use (Kallen, 1988; Kline et al., 1989). An increased risk of miscarriage has also been associated with maternal smoking and consumption of alcohol (Kline et al., 1989). Maternal intake of caffeine during pregnancy has been suggested as a possible risk factor for miscarriage; to date, however, study findings on this topic are inconclusive (Dlugosz and Bracken, 1992).

A variety of maternal occupational exposures may also be related to the risk of miscarriage, including exposure to ethylene oxide, antineoplastic agents, and possibly anesthetic gases (Hemminki et al., 1982; Selevan et al., 1985; Kline et al., 1989). As noted in the introduction, paternal occupational exposures (including vinyl chloride, lead dibromochloropropane, and anesthetic gases) may be related to increased risk of miscarriage (Olshan and Faustman, 1993).

Epidemiologic Studies of Spontaneous Abortion

Occupational Studies

There have been six studies of miscarriage among wives of workers occupationally exposed to herbicides. These include the studies of May (1982), Suskind and Hertzberg (1984), Moses and colleagues (1984), Smith

and colleagues (1982), Carmelli and colleagues (1981), and Townsend and colleagues (1982). At a production facility manufacturing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), May (1982) conducted a follow-up of 41 workers who developed chloracne after accidental exposure to 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD), 31 workers with no exposure, and 54 workers with potential TCDD exposure. No evidence was provided in the workers' histories to suggest an association between workers' exposure and their wives' history of miscarriage. The major limitation of this study was the small number of exposed and unexposed workers, which resulted in inadequate statistical power to detect anything other than a very large relative risk (RR).

Suskind and Hertzberg (1984) and Moses et al. (1984) studied workers exposed to dioxin in an explosion that occurred at a 2,4,5-T manufacturing plant in Nitro, West Virginia. No significant difference in the incidence of miscarriage was found between exposed (105/1,000 pregnancies) and unexposed (119/1,000 pregnancies) worker cohorts [relative risk (RR) = 0.9, CI 0.6-1.2] (Suskind and Hertzberg, 1984). Some concerns about this study include limited power (204 exposed and 163 unexposed workers), low response rates (61 percent of eligible exposed workers and 46 percent of eligible unexposed men), and the fact that the events being asked about in the study questionnaire occurred in the distant past (the mean age at time of interview was 57 years for the exposed workers and 46 years for the unexposed workers). Moses and colleagues (1984) investigated self-reported symptoms among 226 workers. A history of chloracne was used as a surrogate measure of dioxin exposure; 117 of the 226 participants reported current or past chloracne. No consistent differences in spontaneous abortion were found between the groups with and without chloracne. For example, among the 717 pregnancies occurring after 1948 (when 2,4,5-T production began), 6 percent ended in miscarriages in the chloracne group compared to 7 percent in the group without chloracne [odds ratio (OR) = 0.9, CI 0.4-1.8]. The potential misclassification from using chloracne as a measure of exposure, the small number of reported pregnancies, and the low study participation (55 percent of workers contacted participated in the survey) limit the interpretation of these results.

Carmelli and colleagues (1981) examined husband's occupational exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) in a case-control study of spontaneous abortion among women in Oregon and Washington. In the entire sample, the crude odds ratio for fathers who reported work exposure to herbicides compared to those without work exposure was 0.8 (CI 0.5-1.2). When stratified by industry, the odds ratio estimate was 0.7 (CI 0.3-1.8) for farm exposure and 0.9 (CI 0.5-1.6) for forest/commercial exposure. When exposure only during the period around conception was considered, the odds ratio for forest/commercial workers was 1.6 (CI 0.7-3.3) and 1.0

(CI 0.4-2.1) for farm workers. Further stratification by father's age revealed some suggestive associations. Among forest/commercial exposed workers in the age group 18-25 years, the odds ratio for spontaneous abortion was 3.1 (CI 0.9-9.6). Within the 31-35 year age group, farm workers with exposure around conception had an elevated relative risk (OR = 2.9, CI 0.8-10.9). The findings for the forest/commercial workers are suggestive, although the increased odds ratio in another age group for farm workers adds uncertainty to the interpretation of the study findings.

One of the larger occupational studies evaluated reproductive outcomes among wives of 370 workers involved in the processing of chlorophenol at the Dow Chemical Midland plant (Townsend et al., 1982). The rates of miscarriage were similar in the group exposed to any dioxin (133 per 1,000 live births) and the unexposed workers (119 per 1,000) (adjusted OR = 1.03, CI 0.8-1.4). The study had good statistical power for the evaluation of spontaneous abortion, and various confounding factors were considered. Potential study limitations include the low proportion of eligible subjects that participated in the interview and the possibility of poor recall given that conception may have occurred up to 40 years prior to the interview. Nonetheless, this relatively well-designed study did not indicate an association with spontaneous abortion.

The study by Smith and colleagues (1982) of 548 herbicide applicators in New Zealand did not find an increased risk of spontaneous abortion, with an odds ratio of 0.9 (CI 0.6-1.5) for the comparison of 2,4,5-T sprayers to men who were employed as agricultural workers without herbicide exposure. The study included men with a high likelihood of exposure to 2,4,5-T, confounding factors were considered, and the study's statistical power was adequate.

Environmental Studies

The available studies assessing the effect of environmental exposure to herbicides or dioxin on the risk of miscarriage have been reviewed. The Alsea, Oregon study by the U.S. Environmental Protection Agency (U.S. EPA, 1979) found a significantly higher rate of spontaneous abortion in an area that had been sprayed with 2,4,5-T than in a comparison area. In addition, a correlation between spontaneous abortion rate and spray pattern (pounds applied by month) was reported. Although suggestive, the study was of ecologic design and has been criticized for a number of reasons (Sharp et al., 1986).

Spontaneous abortion rates among residents of Seveso, Italy, were not associated with "zone" of exposure (Bisanti et al., 1980). Although actual exposures were unknown, residence in high versus low exposure areas (based on TCDD contamination levels in the soil) was used to differentiate levels

of exposure. A cytogenetic analysis of maternal, placental, and aborted (induced) fetal tissue from Seveso (Tenchini et al., 1983) noted a higher frequency of chromosomal aberration in fetal tissue from TCDD-exposed women than from the unexposed comparison group. Potential problems with variability in the cell culture process were noted by the authors. In the context of no increase in the spontaneous abortion rate among exposed women, and without cytogenetic analysis of fetal tissue from exposed spontaneous abortions, the relevance of these findings is uncertain.

Vietnamese Studies

Several studies of spontaneous abortion have been conducted by Vietnamese researchers. These included both studies of potential exposure to herbicides among residents, male and female, residing in South Vietnam (sprayed versus unsprayed areas) and studies in North Vietnam involving men who were potentially exposed during service in the South. Only a few of the studies have been published in scientific journals, but most of the results were reported at a 1983 conference in Vietnam (Vietnam Courier, 1983). The conference presentations have been summarized and reviewed in several reports (Westing, 1984; Constable and Hatch, 1985; Sterling and Arundel, 1986). The Constable and Hatch report (1985) serves as the primary source for this review.

Three studies conducted in the South reported an increase in spontaneous abortion among people living in sprayed areas. Khoa (1983) reported a 10.1 percent rate of miscarriage among the Montagnard people, compared with 6.1 percent for individuals living in an unsprayed area. Trung and Chien (1983) found an increase in miscarriage in an area after spraying and no similar increase in an unsprayed comparison area. Spontaneous abortion increased in the sprayed area from 5.6 percent before the spraying to 13.9 percent after the time of spraying, and in the unsprayed area from 7.3 to 7.4 percent. An analysis of reproductive outcomes from 1952 to 1981 at a referral hospital in Ho Chi Minh City showed an increase in spontaneous abortion starting in 1967 (14.6 percent versus 4.1 percent in 1966) that reached a peak in 1978 (18.1 percent), possibly consistent with times of heaviest spraying (Huong and Phuong, 1983). Another study in the South (Phuong and Huong, 1983) examined spontaneous abortion in a sprayed village (7,327 pregnancies) compared to a group of women in Ho Chi Minh City who were considered unexposed (6,690 pregnancies). Among the exposed group, 8 percent of pregnancies ended in miscarriage compared to 3.6 percent among the unexposed group.

Tung (1980) compared obstetrical statistics from two villages—one with veterans who had served in the South and were considered exposed to herbicides, and another village with unexposed veterans who remained in the

North. Among the 1,748 pregnancies involving exposed veterans, 14.4 percent ended in spontaneous abortion as opposed to 9 percent of 1,581 unexposed pregnancies. A follow-up to this study (Lang et al., 1983) attempted to classify veterans with potential herbicide exposure into low-, moderate-, and high-exposure subgroups based on the areas in which they lived while in the South. An association between spontaneous abortion and extent of potential herbicide exposure was reported, but only among older mothers. A large survey (40,064 interviews) of reproductive health was conducted in three districts in the North (Can et al., 1983). Pregnancy outcomes were evaluated with respect to the exposure status of the father. The proportion of miscarriages was higher in the exposed group (2,274 of 32,069; 7.1 percent) compared to the unexposed group (7,148 of 121,933; 5.9 percent).

Studies from South Vietnam and of veterans returning to the North suggest an increased risk of spontaneous abortion. A major problem with these studies is that, at present, they have not been published extensively in a form that provides all the relevant information needed to evaluate study design and findings. The studies summarized in several papers appear to have problems with respect to the selection of subjects and collection of exposure data. Ascertainment of the reproductive outcomes, usually based on self-report, seems to be incomplete, given the lower than expected rates of miscarriage among the unexposed pregnancies. Further, control of potentially confounding factors also appears inadequate. At present, from available reports, the studies from Vietnam, although suggesting an increased risk of spontaneous abortion, are of insufficient quality to weigh heavily in the final evaluation of an association with herbicides.

Several studies conducted in Vietnam have also examined the risk for hydatidiform mole (Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989a). This disorder, associated with the death of a fetus, produces a mass of degenerated placental tissue in the uterus. It is benign, but has been considered a precursor of choriocarcinoma, a malignant tumor of embryonic tissue. It is more common in Asian populations with an incidence of 1 in 120 deliveries in Taiwan compared to 1 in 1,000-2,000 deliveries in the United States (Bracken et al., 1984; Hayashi and Bracken, 1984).

Most of the studies have been conducted in the south of Vietnam, and one study (Can et al., 1983) examined the risk of hydatidiform moles in wives of veterans who had returned to the north. The studies in the South have reported an association between exposure to herbicides and the occurrence of moles. For example, Phuong and colleagues (1989a) conducted a case-control study using patients seen at the Ho Chi Minh City Ob-Gyn Hospital during 1982. Exposure was defined as the patient's residence in villages that had been sprayed, mostly in 1965 to 1970. The odds ratio (calculated from the data presented) for hydatidiform mole was 13.1 (CI 5.6-30.9). The study in the North of Vietnam did not report an increased

incidence in moles in villages with potentially exposed veterans. As described previously, interpretation of the Vietnamese studies is difficult because of the inadequate reporting of the studies and concerns about potential biases. Separating maternal from paternal exposure is a problem in the Vietnamese studies conducted in the south because both parents were likely to have been exposed in their village of residence. Further, hydatidiform moles are less common in the United States and have not been an endpoint in occupational, environmental, or U.S. veteran studies. Thus, the direct relevance to this review is limited.

Vietnam Veterans Studies

The latest report on reproductive outcomes from the Air Force Health Study (AFHS, 1992) stated that there was no significant association with miscarriage and paternal serum dioxin levels based on comparisons between 791 Ranch Hand veterans and 942 comparison veterans. Examination of the results showed some analyses that indicated a possible increased risk; however, given the problems with data analysis and presentation of results (see Appendix C), it is difficult to evaluate the Ranch Hand findings.

The Centers for Disease Control (CDC) Vietnam Experience Study (VES) of 7,924 Vietnam veterans and 7,364 non-Vietnam veterans detected a significant, slightly increased relative risk estimate for spontaneous abortion (adjusted OR = 1.3, CI 1.2-1.4; CDC, 1989). Veterans' self-reported herbicide exposure showed an apparent dose-response gradient, with an odds ratio of 1.2 (CI 1.0-1.4) for "low" exposure, 1.4 (CI 1.2-1.6) for "mid" exposure, and 1.7 (CI 1.3-2.1) for the "high" exposure level. A pattern of excess risk for Vietnam veterans was found for many conditions examined in this study. One possible explanation for this pattern is recall bias, that is, the differential recall of past events or exposures among the exposed (Vietnam veterans) compared to unexposed (non-Vietnam veterans). This bias could artificially increase the relative risk estimate. An additional problem with data quality is that the fathers, rather than the mothers, were interviewed, and they are generally a less reliable source of information on reproductive events. Some potentially confounding factors were accounted for, but an important factor—history of previous miscarriage—was not included. The most important limitation of this study is the characterization of Agent Orange exposure primarily by service in Vietnam. The validity of self-reported herbicide exposure, although related to an increased risk of miscarriage, is highly uncertain and, as noted above, may be affected by recall bias.

The American Legion study (Stellman et al., 1988) involved the interview of 6,810 veterans (2,858 Vietnam veterans, 3,933 non-Vietnam veterans, and 19 veterans with an undetermined service location) and the use of

the HERBS tapes to estimate herbicide exposure (see Chapter 6). Among pregnancies of wives of Vietnam veterans, 7.6 percent ended in miscarriage compared with 5.8 percent for veterans who did not serve there (OR = 1.4, CI 1.1-1.6). When stratified by estimated herbicide exposure level, the proportions of pregnancies ending in miscarriage increased from 7.3 (low exposure) to 8.5 (medium exposure) to 9.6 percent (high level of exposure). The corresponding odds ratios were 1.3 (CI 1.0-1.7) for low exposure, 1.5 (CI 1.1-2.1) for medium exposure, and 1.7 (CI 1.2-2.4) for high exposure, relative to the risk for men who did not serve in Vietnam. Relative to men with low exposure, the odds ratios were 1.2 for men with medium exposure and 1.4 for those with high exposure. The authors also examined the risk for men who stated that they had handled herbicides. Compared with men who did not serve in Vietnam, they had an odds ratio of 1.6 (CI 0.7-3.3). The investigators further evaluated the association between Agent Orange exposure and risk of miscarriage by performing an analysis of variance. They found that mean levels of Agent Orange exposure were higher in pregnancies that ended in miscarriage, after adjusting for mother's age and smoking during pregnancy. Multivariate logistic regression analysis was also conducted to examine the risk of miscarriage for Agent Orange while adjusting for potentially confounding factors such as mother's smoking during pregnancy, mother's age, father's age, combat, and father's year of birth. The regression coefficients for Agent Orange exposure and mother's smoking were statistically significant. The authors did not present the number of outcomes classified by Agent Orange exposure and maternal smoking or the corresponding odds ratio estimates from the logistic regression model.

A strength of the American Legion study was the use of a more refined measurement of potential Agent Orange exposure based on the HERBS tapes. However, there are several areas of concern. First, the use of a self-administered mail questionnaire does not yield the best response rate and quality of information. There is some evidence of underreporting, given that the reported prevalence of miscarriage is lower than the level found in most studies (5.8 versus 15 percent). The majority of odds ratio estimates were not statistically significant and were under 2.0. Despite these concerns, their results are suggestive of an increased risk for miscarriage with increased estimated levels of Agent Orange exposure.

A case-control study of spontaneous abortion in Massachusetts (Aschengrau and Monson, 1989) did not indicate an overall increased risk among women married to Vietnam veterans (adjusted OR = 0.9, CI 0.4-1.9). When the data were subdivided by trimester there was a slightly increased, nonsignificant relative risk estimate for first trimester abortions among Vietnam veterans' wives (OR = 1.2, CI 0.6-2.8). The analysis included adjustment for potentially confounding factors; however, the possibility of selection bias resulting from the procedure for approaching patients for interview (interviews

were attempted for 32 percent of women admitted to the hospital with a spontaneous abortion) should be noted. In addition, there were only 10 Vietnam veterans among the cases and 60 among the controls, leading to rather imprecise relative risk estimates as evidenced by the wide confidence intervals. Most importantly, the use of service in Vietnam as a surrogate for herbicide exposure is problematic, as described in Chapter 6.

State surveys of the health of Vietnam veterans, including reproductive history, have been conducted for veterans living in Iowa, Maine, and Hawaii (Rellahan, 1985; Wendt, 1985; Deprez et al., 1991). These studies have not generally indicated an increased risk of spontaneous abortion, although Deprez and colleagues (1991) did report a higher percentage of miscarriages among wives of Maine Vietnam veterans (15 percent) than was reported among the entire Maine population (1.6 percent) in 1988. Limitations such as low response rate, small sample size, use of mail questionnaires, and lack of comparison groups apply to varying degrees to this group of studies.

The study of a cohort of 357 Tasmanian veterans who had served in Vietnam from 1965 to 1972 reported a significant excess of a variety of adverse reproductive outcomes when compared with a group of matched friend/neighborhood families (Field and Kerr, 1988). Fetal loss was reported to be significantly higher in the veteran group compared to nonveterans, 24 percent and 15 percent, respectively (crude RR = 1.6, CI 1.3-2.0). The report did not provide enough details about the selection of veteran and comparison subjects, and the fact that the final sample was only 31 percent of the original Vietnam veteran cohort may reflect potential selection bias. As with U.S. veteran studies, the simple use of veteran status as a measure of exposure is a problem. The authors reported an association with jungle duty, a better indicator of potential herbicide exposure, but specific results were not reported.

Summary

The studies involving occupational and environmental herbicide exposure generally reported no association with spontaneous abortion; however, these studies were inadequate with respect to sample size, elimination of potential bias, and assessment of exposure. A list of the studies considered in this review appears in Table 9-1.

The available epidemiologic studies of veterans are generally limited by inadequate sample sizes, potential bias, and other methodologic problems. There are some suggestive findings indicating an increased risk for Vietnam veterans, including a possible dose-response gradient of increasing risk with increasing estimated (self-reported or inferred) Agent Orange exposure. Nonetheless, the inconsistency with environmental and occupational

TABLE 9-1 Epidemiologic Studies—Spontaneous Abortion

Reference	Description	*N*	OR/RR (95% CI)
Occupational			
Townsend et al., 1982	Wives of men employed at Dow involved in chlorophenol processing	85	1.0 (0.8-1.4)
Smith et al., 1982	Follow-up of 2,4,5-T sprayers		
	Sprayers compared to non-sprayers	43	0.9 (0.6-1.5)
Carmelli et al., 1981	Spontaneous abortion among wives of men occupationally exposed to 2,4-D		
	All reported work exposure to herbicides (high and medium)	63	0.8 (0.5-1.2)
	Farm exposure	32	0.7 (0.3-1.8)
	Forest/commercial exposure	31	0.9 (0.5-1.6)
	Exposure during conception period:		
	Farm exposure	15	1.0 (0.4-2.1)
	Forest/commercial exposure	16	1.6 (0.7-3.3)
	All exposures, father aged 18-25 years: Forest/commercial exposure	8	3.1 (0.9-9.6)
	Exposure during conception period:		
	Father aged 31-35 years, farm	10	2.9 (0.8-10.9)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers	69	0.9 (0.6-1.2)
Moses et al., 1984	Follow-up of 2,4,5-T production workers	14	0.9 (0.4-1.8)
Vietnam veterans			
CDC, 1989	Vietnam Experience Study	1,566	1.3 (1.2-1.4)
	Self-reported low exposure	489	1.2 (1.0-1.4)
	Self-reported medium exposure	406	1.4 (1.2-1.6)
	Self-reported high exposure	113	1.7 (1.3-2.1)
Stellman et al., 1988	Assessment of reproductive effects among American Legionnaires who served in Southeast Asia (1961-1975)		
	Vietnam veterans compared to:		
	Vietnam-era veterans	231	1.4 (1.1-1.6)
	Low exposure	72	1.3 (1.0-1.7)
	Medium exposure	53	1.5 (1.1-2.1)
	High exposure	58	1.7 (1.2-2.4)
	Herbicide handlers	9	1.6 (0.7-3.3)
	Compared to low exposure:		
	Medium exposure	53	1.2 (0.8-1.7)
	High exposure	58	1.4 (0.9-1.9)
Aschengrau and Monson, 1989	Spontaneous abortion and husband's Vietnam service		
	Spontaneous abortions	10	0.9 (0.4-1.9)
	First-trimester abortions	NA	1.2 (0.6-2.8)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	195	1.6 (1.3-2.0)

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval; NA = not available.

studies, uncertainty of the methods of exposure determination, marginal magnitude of the increased risk, and failure to exclude chance are of enough concern that the evidence can be considered insufficient. Future analyses of the Ranch Hand data may contribute important evidence regarding an increased risk for spontaneous abortion among exposed Vietnam veterans.

Conclusions

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and spontaneous abortion.

Biologic Plausibility

TCDD has been reported to reduce fertility in male laboratory animals at doses high enough to produce other toxic effects. Studies of the potential reproductive toxicity of the herbicides are too limited to permit conclusions.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, the effects of selection, information, and confounding bias in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

BIRTH DEFECTS

Introduction

Definition

The March of Dimes defines a birth defect as "an abnormality of structure, function or metabolism, whether genetically determined or as the result of an environmental influence during embryonic or fetal life" (Bloom, 1981). Other terms often used interchangeably with birth defects are congenital anomalies and congenital malformations. Major birth defects are

usually defined as those abnormalities that are present at birth and severe enough to interfere with viability or physical well-being. Birth defects are usually recorded and classified according to the organ or anatomic system affected (Bloom, 1981). Ascertainment and correct diagnosis of birth defects can be difficult, and require careful patient evaluation and registration procedures. The proper grouping of birth defects for epidemiologic study is an area of considerable interest and debate (Khoury et al., 1992b).

Descriptive Epidemiology

Major birth defects are seen in approximately 2 to 3 percent of live births (Kalter and Warkany, 1983a). An additional 5 percent of birth defects can be detected with follow-up through the first year of life. Given the general frequency of major birth defects of 2 to 3 percent and the number of men who served in Vietnam (2.6 million), and by assuming that they had at least one child, it has been estimated that 52,000 to 78,000 babies with birth defects have been fathered by Vietnam veterans, even in the absence of an increase due to exposure to herbicides or other toxic substances (Erickson et al., 1984a). Among U.S. whites, the highest rates of major congenital malformations are for hypospadias (32.7/10,000 births), hip dislocation (32.3), clubfoot (27.5), and patent ductus arteriosus (26.5) (Chavez et al., 1988). As infant mortality has declined in the United States, the proportion of infant deaths attributable to birth defects has increased. Birth defects are currently the single greatest underlying cause of infant deaths in the United States, representing more than 20 percent of such deaths in 1990 (CDC, 1993).

Nonetheless, the etiology of most birth defects is difficult to ascertain; approximately 60 percent of all birth defects in humans have no known cause (Kalter and Warkany, 1983a). Another 20 percent may be due to the effects of many genes or to the interaction of genes and the environment (multifactorial). Among birth defects with a known or suspected genetic cause, those due to a single gene or chromosomal abnormality are responsible for approximately 10 percent of all birth defects. Another 10 percent are known to be caused by environmental (or nongenetic) factors such as maternal disease or infection, radiation, or chemical exposure.

Although approximately 800 drugs and chemicals have been identified as teratogenic in animals, only a handful of them are known to produce birth defects in humans as well (Schardein, 1985; Shepard, 1992). Thalidomide, one of the earliest identified human teratogens, is associated with limb, ear, and other defects. Examples of other known or suspected teratogens include methylmercury, diethylstilbestrol, alcohol, cocaine, tetracycline, warfarin, valproic acid, forms of vitamin A (retinoic acid), androgenic hormones, chlorambucil, cyclophosphamide, fluorouracil, methotrexate,

norethindrone, para- and trimethadione, phenytoin, and streptomycin. A variety of maternal infections are known to result in birth defects including rubella, cytomegalovirus, herpes simplex, syphilis, toxoplasmosis, and Venezuelan equine encephalitis. Maternal diabetes and phenylketonuria are also known to produce defects and other disorders in offspring. Older maternal age is associated with several birth defects, most notably Down's syndrome. Exposures such as anesthetic gases, smoking, and certain maternal occupations have also been examined as risk factors for birth defects, with inconsistent results (Kalter and Warkany, 1983b). Some paternal occupations have been suggested as being associated with an increased risk of birth defects in offspring (see discussion of plausibility).

Epidemiologic Studies of Birth Defects

Occupational Studies

Four occupational epidemiology studies have examined the potential association between herbicide exposure of male workers and birth defects. The Townsend study (Townsend et al., 1982) of workers with potential dioxin exposure at a Dow Chemical plant did not find an increased risk of birth defects among dioxin-exposed workers (30 births with anomalies; 47/1,000 births) compared to unexposed workers (87 births with anomalies; 49/1,000 births; OR = 0.9, CI 0.5-1.4). A major limitation of this study is its limited statistical power to detect an elevated odds ratio for specific defects. The authors noted that the study had 26 percent power to detect a doubling of risk due to exposure for a group of indicator malformations (anomalies thought to be easily recognized and reported by the mother, such as an oral cleft, spina bifida, Down's syndrome). An additional problem is that despite the use of these "indicator malformations," without medical records, validation of the accuracy of maternal self-report of birth defects is questionable for many conditions.

Two studies of workers from a Nitro, West Virginia, 2,4,5-T plant did not report an association with birth defects among offspring (Moses et al., 19841; Suskind and Hertzberg, 1984). The relative risk estimates for any birth defect were 1.3 (CI 0.5-3.4) for Moses et al. and 1.1 (CI 0.5-2.2) from the Suskind and Hertzberg study. Both studies had limited statistical power, given the small number of subjects (204 exposed workers in the Suskind and Hertzberg study; 117 exposed workers in the Moses study). This is especially problematic for the evaluation of most specific birth defects. Both studies also relied on self-reports for the ascertainment of birth defects.

A study of 2,4,5-T sprayers found only a slightly elevated odds ratio for congenital anomalies (OR = 1.2, CI 0.5-3.0) associated with the spraying

group (Smith et al., 1982). The study used self-administered questionnaires to determine outcomes. As with the other studies, it had limited power for the analysis of individual birth defects.

Environmental Studies

A variety of environmental studies have examined the relationship between herbicide exposure and prevalence of birth defects (Nelson et al., 1979; Gordon and Shy, 1981; Hanify et al., 1981; Mastroiacovo et al., 1988; Stockbauer et al., 1988; White et al., 1988; Fitzgerald et al., 1989; Jansson and Voog, 1989). Some studies reported a statistical association with specific birth defects (clubfoot, Fitzgerald et al., 1989; cleft lip with or without cleft palate, Gordon and Shy, 1981; heart, hypospadias, clubfoot, Hanify et al., 1981; oral clefts, Nelson et al., 1979), although others have not reported an association (Stockbauer et al., 1988; Fitzgerald et al., 1989; Jansson and Voog, 1989) including the Seveso study (Mastroiacovo et al., 1988). Interpretation of the results of these environmental studies is difficult because most of the studies were inconsistent, were based on ecologic correlations, had inadequate statistical power, did not validate birth defects recorded from vital statistics or self-reports, and included both male and female exposures.

A recently published study from Vietnam evaluated the risk of birth defects among the offspring of mothers who resided in a village in the South that had been sprayed during the Vietnam war (Phuong et al., 1989b); 81 cases of birth defects (diagnosis not specified) were identified. There were no differences reported between cases and controls for the potentially confounding factors investigated. Strong associations were found for birth defects (calculated from data presented; OR = 3.8, CI 1.1-13.1). The paper is difficult to evaluate given the sparse details presented. Study design factors such as how birth defects were diagnosed and what types were detected, the size of the original case and control groups from which the final groups were sampled, the pattern of patient accrual for this hospital, the method of data collection, and how the potential herbicide spraying histories were determined were not specified. Finally, to put the study in the context of this review, the potential exposure 17-22 years earlier pertains to both the mother and the father.

Results from a number of other studies from Vietnam, both of sprayed villages in the South and of veterans returning to the unsprayed North, have been reported, mostly in a review by Constable and Hatch (1985). These studies indicate an increased risk of birth defects including anencephaly, oral clefts, and a variety of other anomalies. Nonetheless, these studies generally suffer from poor reporting and a variety of methodologic problems such as limited control of confounding factors, use of a referral hospital,

lack of comparison group, uncertainty of exposure classification, and no validation of reported birth defects. Although the findings are suggestive of an association between herbicide spraying and birth defects, the available studies are insufficient to draw firm conclusions.

Vietnam Veterans Studies

As part of the CDC (1989) Vietnam Experience Study, the reproductive outcomes and the health of children of male veterans were examined. The VES assessment of reproductive outcomes and child health included three components: (1) a telephone interview; (2) hospital record review of birth defects for a subsample of veterans who underwent a medical examination; and (3) medical records review of selected birth defects for all study subjects.

Results from analysis of the interview data found that Vietnam veterans reported more birth defects (64.6 per 1,000 total births) among offspring than did non-Vietnam veterans (49.5 per 1,000 total births). The adjusted odds ratio estimate for congenital anomalies as a group was 1.3 (CI 1.2-1.4). When examined by specific defect category, elevated adjusted odds ratios were found for defects of the nervous system (OR = 2.3, CI 1.2-4.5); ear, face, neck (OR = 1.6, CI 0.9-2.8); and integument (OR = 2.2, CI 1.2-4.0). A small but statistically significant odds ratio of 1.2 (CI 1.1-1.5) was found for musculoskeletal defects. An analysis of specific defects considered by the investigators to be relatively common and reliably diagnosed was also conducted. Elevated (crude) odds ratios were reported for hydrocephalus (OR = 5.1, CI 1.1-23.1), spina bifida (OR = 1.7, CI 0.6-5.0), and hypospadias (OR = 3.1, CI 0.9-11.3). Vietnam veterans also reported having more children with multiple defects (OR = 1.6, CI 1.1-2.5) than non-Vietnam veterans. An analysis of Vietnam veterans' self-reported herbicide exposure found a dose-response gradient, with an adjusted odds ratio for birth defects of 1.7 (CI 1.2-2.4) at the highest level of exposure.

The VES also examined serious health problems in the veterans' children; that is, the veterans were asked to report physician-diagnosed major health problems or impairments during the first five years of life. It was found that about half of the health conditions reported by veterans were respiratory disease (mostly asthma and pneumonia) and otitis media. For most of the conditions, Vietnam veterans reported more health conditions than non-Vietnam veterans (all conditions, OR = 1.3, CI 1.2-1.4). After excluding children with a serious health condition and either a birth defect or cancer, the overall crude OR was 1.2 (CI 1.1-1.3). Elevated crude odds ratios were found for anemias (OR = 2.0, CI 1.2-3.3), diseases of the skin (OR = 1.5, CI 1.1-1.9), rash (OR = 2.3, CI 1.1-4.9), and allergies (OR = 1.6, CI 1.2-2.1). Without medical records validation for many of these types of

common conditions and health problems, recall bias may be an explanation for many of these findings.

The CDC (1989) did conduct two substudies using hospital records to identify birth defects among the veterans offspring. The first, the General Birth Defects Study (GBDS), compared the occurrence of birth defects recorded on hospital records for the children of Vietnam and of non-Vietnam veterans (130 cases and 112 cases, respectively) that participated in the medical examination component of the VES. There were no apparent differences between the group of men who participated in the medical examination and the total interview group for a variety of characteristics. The GBDS analysis did not find any difference in the prevalence of birth defects between the two groups of children (crude OR = 1.0, CI 0.8-1.3). There was a slight, nonsignificant excess for major birth defects (OR = 1.2, CI 0.8-1.9). When analyzed by organ system, only digestive system defects appeared to be elevated (OR = 2.0, CI 0.9-4.6), although the small number of defects precluded the analysis of several broad categories. The number of defects was also too small for the analysis of specific individual defects. An analysis by race did indicate an elevated odds ratio (3.4, CI 1.5-7.6) for black Vietnam veterans. An examination of the specific defects listed on hospital records for children of black veterans did not reveal any particular pattern. A comparison of interview and hospital records was also conducted to evaluate the extent of potential misclassification of veteran responses. In general, interview responses were not predictive for the presence of a defect for either veteran group. The agreement between interview and hospital records was slightly poorer for Vietnam veterans. For example, the positive predictive value of the interview response for the presence of a defect in the hospital record was 24.8% among Vietnam veterans and 32.9% among non-Vietnam veterans. Sensitivity was 27.1% among Vietnam veterans and 30.3% among non-Vietnam veterans. The kappa measure of agreement was also lower (20.9% versus 27.6%) among Vietnam veterans.

The second substudy, the Cerebrospinal Malformation (CSM) Study, involved the acquisition of medical records for all cases of cerebrospinal malformations (spina bifida, anencephalus, hydrocephalus) and stillbirths reported by veterans in the interview study. The CSM record review substudy found 26 cerebrospinal malformations (live and stillbirths) among children of Vietnam veterans and 12 among children of non-Vietnam veterans. No formal analysis of the difference in CSM between the veteran groups was conducted due to the fact that negative responses (i.e., children without a reported CSM) were not verified and the participation rates differed between groups (7.8 percent of Vietnam veterans in contrast to 22.1 percent of non-Vietnam veterans refused to participate).

The VES did find suggestive associations for birth defects. It is interesting to note that some potential associations were found for birth defects

considered by the investigators to be "relatively common, easily diagnosed, and observable at birth" (CDC, 1989). These include hydrocephalus (OR = 5.1, CI 1.1-23.1) and hypospadias (OR = 3.1, CI 0.9-11.3). The GBDS did not replicate these findings, but this sample had limited power for the analysis of specific defects. Although associations were not found for all conditions, there was clearly a general pattern of a greater prevalence of birth defects in the offspring of Vietnam veterans, according to self-reports. The authors properly note the potential for recall bias as an explanation for the pattern of excess risk. As an attempt to evaluate recall bias, two record validation studies of birth defects were conducted. Overall, the GBDS did not find any association with an increased risk of birth defects among offspring of Vietnam veterans. However, this validation study had limited power to detect an increased risk for specific birth defects. The second validation substudy, the CSM review, was flawed by the differentially poor response rate among the non-Vietnam veteran group. This result and the fact that negative responses were not pursued discouraged the investigators from estimating the relative risk for CSMs.

Another important study of Vietnam veterans was the CDC Birth Defects Study (Erickson et al., 1984a,b). In this study, children with birth defects among 428 fathers who were reported to have been Vietnam veterans were compared to children with birth defects among 268 control fathers who were non-Vietnam veterans. The odds ratio for Vietnam veteran status in relation to any major birth defect among offspring was 1.0 (CI 0.8-1.1). Analysis of the Agent Orange exposure opportunity index (EOI; see Chapter 6 for details) based on both military records and self-reports did not indicate a statistically significant trend of increasing risk of all types of birth defects (combined) with increasing levels of Agent Orange exposure. No association was noted between Vietnam veteran status or self-reported Agent Orange exposure and risk of fathering a child with multiple birth defects (OR = 1.1, CI 0.7-1.7). The odds ratios for Vietnam veteran status, self-reported Agent Orange exposure, and logistic regression coefficients for EOI based on self-report and military records for most of the 95 birth defect groups were not significantly elevated. Although the odds ratio for spina bifida with Vietnam veteran status was not elevated (OR = 1.1), the EOI indices showed a pattern of increasing risk. For example, the odds ratios for the EOI based upon information obtained during the interview for low to high levels of exposure (levels 1 to 5) were 1.2 (CI 1.0-1.4), 1.5 (CI 1.1-2.1), 1.8 (CI 1.1-3.0), 2.2 (CI 1.2-4.3), 2.7 (CI 1.2-6.2). A similar pattern was found for cleft lip with/without cleft palate, namely, EOI-1 (OR = 1.2, CI 1.0-1.4), EOI-2 (OR = 1.4, CI 1.0-1.9), EOI-3 (OR = 1.6, CI 1.0-2.6), EOI-4 (OR = 1.9, CI 1.0-3.6), and EOI-5 (OR = 2.2, CI 1.0-4.9). The category "specified anomalies of nails" had an increased odds ratio for Vietnam veteran status and elevated coefficients (not statistically significant)

for the two exposure indices. The category "other neoplasms" was related to the EOI based on the father's self-reported Agent Orange exposure. This group included a variety of congenital neoplasms such as cysts, teratomas, and benign tumors. In an attempt to search for a Vietnam veteran birth defect "syndrome," pairs and triplets of defects were examined for combinations that yielded significant differences in the distribution among Vietnam veterans and controls. According to the authors, these analyses did not produce any important associations or patterns among defect combinations.

The results of this study were generally negative; that is, there was not a general pattern of increased risk for birth defects among the offspring of Vietnam veterans. However, the analysis of the Agent Orange EOIs based on military records found a significant trend for increased risk for spina bifida with increased exposure. As the authors note, this must be viewed with caution because a related defect, anencephalus, was not found to be associated with a significant EOI trend. Another positive association was noted for cleft lip, without cleft palate, where a significant regression coefficient was found for the EOI index based on the father's interview. No association was found for the EOI from military records.

The CDC Birth Defects Study has many strengths including the use of a population-based registry system with careful classification of birth defects for analysis. The statistical power of the study was excellent for many major birth defects. Use of the Agent Orange EOIs is an attempt to refine exposure assessment procedures compared to measures used in most other studies. The study did have several important limitations. First, the response rates among cases and controls were problematic, with approximately 56 percent of eligible case and control fathers interviewed. Examination of the nonparticipation group revealed lower participation among persons classified as "nonwhite." The analyses by race did not find important differences, but the potential for bias should not be overlooked. Another problem relates to the fact that case births occurred from 1968 through 1980, but interviews took place during 1982 and 1983, up to 14 years after the birth. To minimize the potential recall bias induced by this long lag period, controls were matched on year of birth.

The latest Air Force report on the analysis of the Ranch Hand study updates the previous analysis of baseline data (AFHS, 1992). This new evaluation incorporates the verification of self-reported birth defects and analysis by measured serum dioxin levels. The report stated that there were few significant associations between serum dioxin level and the risk of congenital anomalies. Some analyses of all defects combined and musculoskeletal defects found significant associations, but the authors felt that no consistent patterns among defects and dioxin levels were discernible. They concluded that the apparent statistical associations were due to chance and

there is no "underlying association" in their data between birth defects and dioxin.

As the authors noted, the total prevalence of birth defects in the Ranch Hand group is greater than in the unexposed comparison group (veterans involved in air cargo missions, presumably with "background" levels of herbicide or dioxin exposure). This finding was also noted in the baseline evaluation of both groups. The odds ratio comparing the Ranch Hand group with the "comparisons" for total congenital anomalies was 1.3 (CI 1.1-1.6). Although the numbers were rather small, elevated odds ratios (for births after service in Southeast Asia) were found for nervous system (OR = 1.9, CI 0.5-7.2), respiratory system (OR = 2.6, CI 0.6-10.7), circulatory and heart (OR = 1.4, CI 0.7-2.6), urinary (OR = 2.5, CI 1.3-5.0), chromosomal (OR = 1.8, CI 0.6-6.1), and other (OR = 2.6, CI 0.6-10.7) defects. These results are only suggestive but do indicate some difference in the risk of birth defects between these two groups. Based on their analyses incorporating serum dioxin levels, the investigators concluded that dioxin levels do not explain the difference. However, the committee is concerned (see Appendix C) about the methods and presentation employed in the analysis of dioxin levels, and it is unclear what the findings from this important study mean, other than some indication that the Ranch Hand subjects seem to be at a higher risk of fathering a child with a birth defect than the comparison group. Attribution of this increase to herbicides or dioxin must await further analysis.

Aschengrau and Monson (1990) studied late adverse pregnancy outcomes among 14,130 obstetric patients who delivered at Boston Hospital for Women from August 1977 to March 1980. History of the fathers' military service in Vietnam was determined from Massachusetts and national military records by using the husbands' names and Social Security numbers. The likelihood of combat experience, based on branch of service and military occupation, was used to estimate potential herbicide exposure. The analyses compared the risk of malformations among children of 107 Vietnam veterans to that for children of 1,432 men without known military service; the risk in 313 non-Vietnam veterans compared to the 1,432 men without military service; and the 107 Vietnam veterans with the 313 non-Vietnam veterans. There was a slight, nonsignificant increase in the odds ratio for all congenital anomalies for Vietnam veterans compared to men without known military service, (OR = 1.3, CI 0.9-1.9) and for Vietnam veterans compared with non-Vietnam veterans (OR = 1.2, CI 0.8-1.9). The odds ratio was elevated for major malformations for the Vietnam veteran—men without known military service comparison (OR = 1.8, CI 1.0-3.1), but decreased for the Vietnam veteran—non-Vietnam veteran comparison (OR = 1.3, CI 0.7-2.4). Only slight increases were found for the analysis of minor malformations and "only normal variants." Although based on small numbers,

the analyses of 12 malformation groups found that children of Vietnam veterans relative to children of men with no known military service had an increased (nonsignificant) risk of malformations of the nervous system, cardiovascular system, genital organs, urinary tract, and musculoskeletal system. Confidence intervals were not presented with the odds ratio estimates, but it was noted that they included 1.0, so elevated risks were not significantly increased. Further examination of specific anomaly diagnoses for the 18 infants of Vietnam veterans with major malformations did not reveal any pattern of association with potential herbicide exposure.

The study did find a positive association between paternal military service in Vietnam and the risk of major malformations in offspring. The authors suggest cautious interpretation of their findings, given the small number of subjects in many of the comparisons involving specific groups of birth defects. Additionally, it was noted that some of the malformations observed can also be due to maternal and delivery factors (endocrine condition and fetal presentation). An important problem relates to misclassification of herbicide exposure due to equating exposure to service in Vietnam.

Two state health surveys of veterans (Iowa and Hawaii) did not indicate an increased prevalence of birth defects (Rellahan, 1985; Wendt, 1985). A survey in Maine reported an increased risk of birth defects among veterans (Deprez et al., 1991). The limitations of these general survey studies affect their usefulness in this evaluation.

As part of the National Vietnam Veterans Birth Defects/Learning Disabilities Registry and database, a joint project of the Association of Birth Defect Children and the New Jersey Agent Orange Commission, a self-administered questionnaire was sent to Vietnam veterans to inquire about birth defects and a variety of conditions and disabilities in the children of Vietnam veterans and non-Vietnam veterans (Lewis and Mekdeci, 1993). A preliminary analysis indicated no differences in birth defects between the two groups; however, for a variety of conditions including allergies, frequent infections, benign tumors, cysts, and chronic skin disorders, the veterans showed a higher frequency. The possibility of recall bias and the self-selected nature of the registry are of concern. Nonetheless, a carefully designed and comprehensive epidemiologic study with medical record review could address the possibility of an association with some of these childhood health conditions.

A study of birth defects among offspring of Australian Vietnam veterans was conducted by using a total of 8,517 matched case-control pairs, with 127 infant cases and 123 infant controls having a father who served in Vietnam (Donovan et al., 1984). There were 202 cases and 205 controls whose fathers were in the Army but did not serve in Vietnam. The adjusted odds ratio for birth defects among children of Vietnam veterans versus all other men was 1.02 (CI 0.8-1.3). Analysis of subgroups based on type of

Army veteran (Australian Regular Army enlistees, National Service draftees) did not detect any increased odds ratios for these comparisons. There was a slight, statistically nonsignificant increase in the odds ratio for National Service Vietnam veterans versus National Service veterans who did not serve in Vietnam (OR - 1.3, CI 0.9-2.0). It was found that the risk was independent of the length of Vietnam service and the time between service and conception. Analyses by diagnostic group (central nervous system, cardiovascular, oral clefts, hypospadias, musculoskeletal, dislocation of hip, chromosomal anomalies) did not show an excess risk for Vietnam veterans. However, two defects had odds ratios above 1.5 (statistically nonsignificant), namely, ventricular septal defects (OR = 1.8) and Down's syndrome (OR = 1.7).

Overall, this study was negative; that is, there was no evidence of an increased risk of fathering a child with a congenital anomaly for Australian Army veterans who served in Vietnam. As indicated by the upper confidence limit (1.3) this study had adequate power to rule out an odds ratio greater than 1.3 for congenital anomalies. Assessment of potential Agent Orange exposure in this study is limited because of the use of history of service in Vietnam as the primary "exposure" variable. Further, this uncertainty is compounded by potential differences in the location and nature of service of Australian veterans in Vietnam and their resultant herbicide exposure.

The Australian study of veterans living in Tasmania reported more congenital anomalies among the 357 Vietnam veterans than among the comparison families (Field and Kerr, 1988). The authors suggested that the results indicated a pattern of association with congenital heart disease and anomalies of the central nervous system. As described earlier in the section on spontaneous abortion, there are several notable problems with this study including inadequate presentation of results, potential selection bias, self-reported health outcomes, and using service in Vietnam as a surrogate for herbicide exposure.

Summary

There is little evidence of a statistical association between father's occupational exposure to herbicides or dioxin and birth defects among offspring. The available epidemiologic studies have been limited by a number of problems, especially inadequate statistical power for the evaluation of specific birth defects. Studies involving environmental exposure have yielded inconsistent results; furthermore, given the ecologic nature of the studies, the limited sample sizes (for specific defects), and the failure to verify reported birth defects, the evidence can be considered inadequate for an association. A list of the studies considered in this review appears in Table 9-2.

TABLE 9-2 Epidemiologic Studies—Birth Defects

Reference	Description	*N*	OR/RR (95% CI)
Occupational			
Townsend et al., 1982	Follow-up of Dow chemical plant workers	30	0.9 (0.5-1.4)
Smith et al., 1982	Follow-up of 2,4,5-T sprayers		
	Sprayers compared to nonsprayers	13	1.2 (0.5-3.0)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers	18	1.1 (0.5-2.2)
Moses et al., 1984	Follow-up of 2,4,5-T production workers	11	1.3 (0.5-3.4)
Environmental			
Fitzgerald et al., 1989	Follow-up of an electrical transformer fire		
	Total birth defects	1	SIR = 212 (5.4-1,185.1)
Hanify et al., 1981	All malformations	164	1.7 (1.4-2.2)
	All heart malformations	20	3.9 (1.7-8.9)
	Hypospadias, epispadias	18	5.6 (2.1-15.1)
	Talipes	52	1.7 (1.1-2.4)
	Anencephaly	10	1.4 (0.6-3.3)
	Spina bifida	13	1.1 (0.6-2.3)
	Cleft lip	6	0.6 (0.2-1.5)
	Isolated cleft palate	7	1.4 (0.5-3.8)
Mastroiacovo et al., 1988	Reproductive outcomes of Seveso, Italy, residents		
	Zones A and B total defects	27	1.2 (0.8-1.8)
	Zones A, B, R total defects	137	1.0 (0.8-1.2)
	Zones A and B mild defects	14	1.4 (0.9-2.6)
Stockbauer et al., 1988	TCDD soil contamination in Missouri		
	Total birth defects	17	0.8 (0.4-1.5)
	Major defects	15	0.8 (0.4-1.7)
	Midline defects	4	0.6 (0.2-2.3)
	Central nervous system defects	3	3.0 (0.3-35.9)
Vietnam veterans			
Erikson et al., 1984a	Birth Defects Study		
	Any major birth defects	428	1.0 (0.8-1.1)
	Multiple birth defects with reported exposure	25	1.1 (0.7-1.7)
	EOI-5: spina bifida	1	2.7 (1.2-6.2)
	EOI-5: cleft lip with/without cleft palate	5	2.2 (1.0-4.9)
CDC, 1989	Vietnam Experience Study		
	Interview study		
	Any congenital anomaly	826	1.3 (1.2-1.4)
	Nervous system defects	33	2.3 (1.2-4.5)

Reference	Description	*N*	OR/RR (95% CI)
	Ear, face, neck defects	37	1.6 (0.9-2.8)
	Integument	41	2.2 (1.2-4.0)
	Musculoskeletal defects	426	1.2 (1.1-1.5)
	Hydrocephalus	11	5.1 (1.1-23.1)
	Spina bifida	9	1.7 (0.6-5.0)
	Hypospadias	10	3.1 (0.9-11.3)
	Multiple defects	71	1.6 (1.1-2.5)
	Defects with high exposure	46	1.7 (1.2-2.4)
CDC, 1989	GBDS study		
	Birth defects	130	1.0 (0.8-1.3)
	Major birth defects	51	1.2 (0.8-1.9)
	Black Vietnam veterans with children with birth defects	21	3.4 (1.5-7.6)
	Digestive system defects	18	2.0 (0.9-4.6)
Aschengrau and Monson, 1990	Birth defects and father's Vietnam service		
	Vietnam veterans compared to men without known military service	55	1.3 (0.9-1.9)
	Vietnam veterans compared to non-Vietnam veterans	55	1.2 (0.8-1.9)
	Major malformations		
	Vietnam veterans compared to men without known military service	18	1.8 (1.0-3.1)
	Vietnam veterans compared to non-Vietnam veterans	18	1.3 (0.7-2.4)
Donovan et al., 1984	Birth defects and father's Vietnam service (Australia)		
	Vietnam veterans vs. all other men	127	1.02 (0.8-1.3)
	National Service veterans Vietnam service vs. no Vietnam service	69	1.3 (0.9-2.0)
AFHS, 1992	Follow-up of Air Force Ranch Hands		
	Birth defects in conceptions following service in Southeast Asia:		
	Congenital anomalies	229	1.3 (1.1-1.6)
	Nervous system	5	1.9 (0.5-7.2)
	Respiratory system	5	2.6 (0.6-10.7)
	Circulatory system or heart	19	1.4 (0.7-2.6)
	Urinary system	21	2.5 (1.3-5.0)
	Chromosomal	6	1.8 (0.6-6.1)
	Other	5	2.6 (0.6-10.7)

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval; SIR = standardized incidence ratio.

The results of available epidemiologic studies of Vietnam veterans are also inconsistent; some studies suggest a potential association with defects of the central nervous system and other systems, although others find no increased risk. As noted for other end points, the veteran studies were generally limited by inadequate sample size, the marginal magnitude of the increased risk, and the failure to exclude bias and chance. Moreover, uncertainty regarding the assessment of exposure among Vietnam veterans and the limited evidence from other cohorts do not permit one to draw a conclusion about an increased risk of birth defects among the offspring of men exposed to herbicides in Vietnam. Future analyses of the Ranch Hand data may contribute important evidence regarding an increased risk of birth defects among offspring of exposed Vietnam veterans.

Conclusions

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and birth defects among offspring.

Biologic Plausibility

Laboratory studies of the potential developmental toxicity of TCDD and herbicides using male animals are too limited to permit conclusions.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, the effects of selection and information bias and low statistical power in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

STILLBIRTH, NEONATAL DEATH, AND INFANT DEATH

Introduction

Definitions

The use of the terms stillbirth and neonatal death can be confusing and

has differed in epidemiologic studies. Varying definitional criteria and vital statistics registration problems have lead to difficulties in interpreting national and international rates (Golding, 1991). Stillbirth (or late fetal death) is typically defined as the delivery of a fetus occurring at or after 28 weeks of gestation and showing no signs of life at birth, although a more recent definition includes deaths among all fetuses weighing more than 500 g at birth, regardless of gestational age at delivery (Kline et al., 1989). Neonatal deaths are usually defined as the death of a live-born infant within the first 28 days of life. Early neonatal deaths occur within the first week of life. Because there are no clear biological differences between late fetal deaths (stillbirths) and deaths in the early neonatal period, these are commonly referred to together as perinatal deaths (Kallen, 1988). The perinatal mortality rate of a population is often calculated as the sum of the number of stillbirths plus the number of deaths in the first week of life occurring per 1,000 live and stillbirths. Late neonatal death is usually defined as death of the infant after the first week but within the first 28 days of life, and infant death as death within the first year.

Descriptive Epidemiology

Stillbirths occur in approximately 1 to 2 percent of all births (Kline et al., 1989). Among low birthweight live- and stillborn infants (weighing 500-2,500 grams), placental and delivery complications such as abruptio placentae, placenta previa, malpresentation, and umbilical cord complications are the most common causes of perinatal mortality (Kallen, 1988). Among infants weighing more than 2,500 grams at birth, the most common causes of perinatal death are lethal congenital malformations and placental complications (Kallen, 1988).

Both maternal age and parity appear to be associated with perinatal mortality, although neither relationship is straightforward. In particular, antepartum (before labor or childbirth) stillbirths increase with increasing maternal age, primarily for women aged 35 years or older (Kline et al., 1989). There is disagreement as to whether this maternal age association varies with birth order (Bakketeig et al., 1984). Perinatal mortality risk appears to be higher among nulliparous women and among those with at least three or four previous births. The parity association is especially strong for intrapartum (during labor and delivery or childbirth) stillbirths (Kline et al., 1989).

Maternal smoking during pregnancy also appears to be a risk factor for perinatal death, although not all studies of this topic have reported positive findings (Bakketeig et al., 1984). The association between maternal smoking and perinatal mortality is probably attributable at least in part to an increase in obstetrical complications (especially abruptio placentae) among

smokers. Socioeconomic status (SES) and race are two additional factors that have been linked to perinatal death. The rate of perinatal mortality is known to increase with decreasing SES (Bakketeig et al., 1984). Furthermore, in the United States, black infants have a higher rate of perinatal mortality than white infants; this difference in risk is associated with SES differences, although other factors may also be involved (Wilcox and Russell, 1986).

It has been noted earlier in this section that stillbirths and early neonatal deaths are often combined under the rubric "perinatal deaths." Unfortunately, the precise definitions of neonatal death used in the epidemiologic studies being considered has not been provided in the majority of studies. Thus, for this review of the evidence, the studies have been categorized into stillbirths, neonatal deaths (early and late), and infant deaths, using the terms as applied by the studies' authors.

Epidemiologic Studies of Stillbirth

Occupational and Environmental Studies

The five occupational studies that have directly evaluated stillbirth have not reported any association with potential herbicide or dioxin exposure of male workers (May, 1982; Smith et al., 1982; Townsend et al., 1982; Moses et al., 1984; Suskind and Hertzberg, 1984). The largest study investigated the reproductive histories of male workers at the Dow Midland plant and reported 15 stillbirths to workers exposed to any dioxin (OR = 1.1, CI 0.5-2.1) (Townsend et al., 1982). The limitations of most of the occupational studies with respect to exposure assessment and statistical power have been described previously for other outcomes.

Two environmental studies that have analyzed stillbirth have not reported an overall increased risk related to potential herbicide exposure (Stockbauer et al., 1988; White et al., 1988). White and colleagues (1988) in an ecologic study of agricultural chemical use in New Brunswick, Canada, did find, however, a statistically significant risk ratio for second trimester stillbirths, although not for all stillbirths (results not reported in the article). They also noted that a dose-response relationship was present. The potential for exposure misclassification, as well as the ecologic design of this and other environmental studies, limits the interpretation of these data.

Vietnam Veterans Studies

Three studies of stillbirths to veterans have yielded somewhat mixed results. The Massachusetts study of adverse pregnancy outcomes (Aschengrau and Monson, 1990) found two elevated odds ratios for stillbirth, including 1.5 (CI 0.4-3.9) for the comparison of Vietnam veterans with nonveterans

and 3.2 (CI 0.7-14.5) for the comparison with non-Vietnam veterans. Although suggestive, these relative risk estimates are unstable, with rather wide confidence intervals. The Field and Kerr (1988) study of Australian Vietnam veterans did not find an association with stillbirth, (crude RR = 1.4, CI 0.5-3.5). Results from the interview portion of the VES (CDC, 1989) did not show an increased odds ratio (OR = 0.9, CI 0.7-1.1); there was also no difference found based upon the GBDS analysis of hospital records (OR = 1.0, CI 0.4-2.4). Self-reported herbicide exposure in the interview data was not related to stillbirths in a dose-response manner ("low" OR = 1.1; "mid" OR = 1.2; "high" OR = 0.5). Problems with inadequate determination of herbicide exposure affect all these studies.

Epidemiologic Studies of Neonatal Death

Occupational and Environmental Studies

Neonatal death was not associated with herbicide exposure in three occupational and environmental studies (May, 1982; Suskind and Hertzberg, 1984; Fitzgerald et al., 1989). The study by Suskind and Hertzberg (1984) of workers at the Nitro, West Virginia, 2,4,5-T plant found a relative risk estimate of 1.8 (CI 0.7-4.5). A Missouri study of exposure to TCDD-contaminated soil found a nonsignificant odds ratio of 1.3 (CI 0.4-4.2) for perinatal death (Stockbauer et al., 1988).

Vietnam Veterans Studies

The Massachusetts veterans study did not report an increased risk of neonatal death for Vietnam veterans (OR = 1.1, CI 0.2-4.5) versus non-Vietnam veterans or men without military service (OR = 1.2, CI 0.2-4.2) (Aschengrau and Monson, 1990). The Australian veterans study by Field and Kerr (1988), reported that a statistically significant association was found based on 12 neonatal deaths among Vietnam veterans and 1 death among nonveterans (RR = 18.1, CI 2.4-134.4). The VES (CDC, 1989) did not evaluate overall neonatal death, but the GBDS analysis (CDC, 1989) showed an elevated odds ratio of 2.0 (CI 0.8-4.9) for early neonatal death for all veterans and an odds ratio of 4.0 (CI 1.2-14.0) among whites. The study of Ranch Hand veterans (AFHS, 1992) was interpreted by the authors as having found no association with the small number of neonatal deaths (nine cases) identified. There seemed to be a pattern of increasing rates of neonatal deaths (postservice) with increasing estimated dioxin levels among the Ranch Hand cohort relative to the comparison cohort. However, the rates also showed a similar pattern for the time period prior to service in Southeast Asia, which potentially minimizes the importance of these results.

Epidemiologic Studies of Infant Death

Occupational and Environmental Studies

The results for infant death are inconsistent with some studies reporting an elevated risk among male workers or veterans. The Dow study (Townsend et al., 1982) found a decreased odds ratio (OR = 0.6, CI 0.3-1.4). The Missouri study of TCDD-contaminated soil (Stockbauer et al., 1988) found a twofold increased odds ratio, but with a wide confidence interval (CI 0.5-8.7).

The Vietnamese studies conducted in the South have generally reported an increased risk of infant death among individuals in villages with presumed exposure to herbicides (Constable and Hatch, 1989; Dai et al., 1990). The problems with interpreting the results of many of these studies have been described by Constable and Hatch (1985) and in an earlier section of this chapter.

Vietnam Veterans Studies

Overall, the studies of Massachusetts veterans (Aschengrau and Monson, 1990), the VES (CDC, 1989), and the study of Ranch Hand veterans (AFHS, 1992) did not report an association with infant death. Self-reports of infant death from the interview study of the CDC's VES did not indicate an increased risk of deaths among children of Vietnam veterans relative to non-Vietnam veterans (OR = 1.0, CI 0.8-1.3), although there was a dose-response gradient with self-reported herbicide exposure. The adjusted odds ratio for the highest level of reported herbicide exposure was 2.7 (CI 1.4-5.4). The Ranch Hand study did not report an association with infant death, although there were a small number of deaths. Similarly, the Field and Kerr study of Australian veterans (1988) did not find an association with infant deaths occurring between the ages of 1 month and 1 year (RR = 0.9, CI 0.2-3.5).

Summary

Stillbirth and Neonatal Death

A statistical association with stillbirth has not been reported in the available occupational and environmental epidemiologic studies. The majority of studies did not have adequate statistical power, and the assessment of exposure was incomplete. Some studies of veterans have reported an increased risk, whereas others have indicated no statistical association. Interpretation of these veteran studies is constrained by limited statistical power, and most importantly, uncertainty of correctly assigning herbicide exposure to study groups. A list of the studies considered in this review appears in Table 9-3.

TABLE 9-3 Epidemiologic Studies—Stillbirth and Neonatal Death

Reference	Description	*N*	OR/RR (95% CI)
Stillbirth			
Occupational			
Townsend et al., 1982	Follow-up of Dow chemical plant employees	15	1.1 (0.5-2.1)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers	11	1.4 (0.5-4.1)
Smith et al., 1982	Follow-up of 2,4,5-T sprayers	3	—
Environmental			
White et al., 1988	Follow-up of agricultural activity in New Brunswick; highest level of potential exposure in second trimester	NA	1.5 (1.0-2.3)
Stockbauer et al., 1988	TCDD soil contamination in Missouri	4	1.6 (0.3-7.4)
Vietnam veterans			
CDC, 1989	Vietnam Experience Study		
	Interview study	126	0.9 (0.7-1.1)
	Low exposure	41	1.1 (0.7-1.7)
	Medium exposure	32	1.2 (0.7-1.9)
	High exposure	3	0.5 (0.2-1.6)
	Validation study	10	1.0 (0.4-2.4)
Aschengrau and Monson, 1990	Stillbirth and paternal Vietnam service		
	Vietnam veterans compared to men with no military service	5	1.5 (0.4-3.9)
	Vietnam veterans compared to non-Vietnam veterans	5	3.2 (0.7-14.5)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	11	1.4 (0.5-3.5)
Neonatal death			
Occupational			
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers	17	1.8 (0.7-4.5)
May, 1982	Follow-up of 2,4,5-T production workers perinatal deaths	1	—
Environmental			
Stockbauer et al., 1988	TCDD soil contamination in Missouri perinatal deaths (includes stillbirths)	6	1.3 (0.4-4.2)
Vietnam veterans			
Aschengrau and Monson, 1990	Neonatal death and paternal Vietnam service		
	Vietnam veterans compared to men with no known military service	3	1.2 (0.2-4.2)
	Vietnam veterans compared to non-Vietnam veterans	3	1.1 (0.2-4.5)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	12	18.1 (2.4-134.4)
CDC, 1989	Vietnam Experience Study		
	GBDS study—early neonatal death	16	2.0 (0.8-4.9)

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval; NA = not available.

TABLE 9-4 Epidemiologic Studies—Infant Death

Reference	Description	*N*	OR/RR (95% CI)
Occupational			
Townsend et al., 1982	Follow-up of Dow chemical plant workers	9	0.6 (0.3-1.4)
Environmental			
Stockbauer et al., 1988	TCDD soil contamination in Missouri	5	2.0 (0.5-8.7)
Vietnam veterans			
CDC, 1989	Vietnam Experience Study		
	Interview study	152	1.0 (0.8-1.3)
	Low exposure	58	1.9 (1.2-2.9)
	Medium exposure	38	2.0 (1.2-3.1)
	High exposure	11	2.7 (1.4-5.4)
Field and Kerr, 1988	Follow-up of Australian Vietnam Veterans deaths between ages 1 month and 1 year	4	0.9 (0.2-3.5)

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval.

Infant Death

The available occupational and environmental epidemiologic studies (see Table 9-4) were limited by low statistical power, potential selection bias, and inadequate exposure classification. One study of veterans reported an association of infant death with self-reported herbicide exposure. Nonetheless, other studies did not report an increased risk, but as noted for other outcomes, problems with exposure determination, statistical power, and potential bias limit interpretation.

Conclusions

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and stillbirth, neonatal, and infant death among offspring.

Biologic Plausibility

TCDD has been reported to reduce fertility in male laboratory animals at doses high enough to produce other toxic effects. Studies of the potential reproductive toxicity of the herbicides are too limited to permit conclusions.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, the effects of information bias and low statistical power in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

LOW BIRTHWEIGHT

Introduction

Definition

Reduced infant weight at birth is one of the most important causes of neonatal mortality and morbidity in the United States. The World Health Organization recommends a 2,500-gram cutpoint for the determination of low birthweight (Alberman, 1984). Although often treated as a single entity, the concept of low birthweight actually encompasses two different causal pathways: (1) low birthweight secondary to intrauterine growth retardation (IUGR), which is more related to neonatal morbidity; and (2) low birthweight secondary to preterm delivery, which is more strongly associated with neonatal mortality (Alberman, 1984; Kallen, 1988). The concept of IUGR represents birthweight adjusted for gestational age. The currently used definition of preterm delivery (PTD) is delivery at less than 259 days, or 37 completed weeks of gestation, calculated on the basis of the date of the last menstrual period (Bryce, 1991).

Descriptive Epidemiology

Approximately 7 percent of live births have low birthweight. The incidence of IUGR is much more difficult to quantify since there are no universally applied standards for dividing the distribution of birthweight for gestational age. In 1982, the incidence of prematurity in the United States was 8 percent in whites and more than twice that in blacks (Bryce, 1991).

When no distinction is made between the causes of low birthweight (i.e., IUGR versus PTD), the factors most strongly associated with reduced birthweight are maternal smoking during pregnancy, multiple births, and race/ethnicity. Other potential risk factors for low birthweight include SES, maternal size, birth order, maternal complications during pregnancy (e.g.,

severe preeclampsia) and obstetric history, job stress, and cocaine or caffeine use during pregnancy (Kallen, 1988).

Epidemiologic Studies of Low Birthweight

Occupational and Environmental Studies

In a study of individuals potentially exposed to TCDD in an electrical transformer fire in Binghamton, New York, Fitzgerald and colleagues (1989) found 3 cases of low birthweight infants (3.7 expected) resulting in a standardized incidence ratio (SIR) of 81 (CI 16.7-236.4). One environmental study examined birthweight as an outcome (Stockbauer et al., 1988). This study of TCDD exposure via soil contamination in Missouri reported an odds ratio of 1.5 (CI 0.9-2.6) for low birthweight (8 2,500 g). The odds ratio for IUGR was 1.1 (CI 0.5-2.3). There was no association between birthweight and level of potential TCDD exposure, as estimated by comparison of births to high-risk versus low-risk exposures, or by time period of birth.

Vietnam Veterans Studies

The Field and Kerr (1988) study of Australian veterans showed an increased risk ratio (RR = 1.6, CI 1.0-2.5) for low birthweight. Interpretation of results from this study is difficult because the authors failed to present their findings in a manner that allows a full epidemiologic and statistical assessment. The GBDS validation component of the VES (CDC, 1989) found similar rates of low birthweight among the offspring of Vietnam veterans and non-Vietnam veterans (OR = 1.1, CI 0.8-1.4). Analysis by self-reported herbicide exposure did not indicate a pattern of increase with mid to high levels of exposure (CDC, 1989). The American Legion study did not find an association between low birthweight and service in Vietnam or estimated herbicide exposure (Stellman et al., 1988). The Ranch Hand report (AFHS, 1992) did note some associations with low birthweight. Among births after service in Southeast Asia, the unadjusted odds ratio comparing the high dioxin level to the control group was 2.3 (CI 1.3-4.0). Other analyses yielded inconsistent results. This inconsistency and the general problems with the Ranch Hand report limit the interpretation of these results.

Summary

Given the lack of available occupational and environmental studies, the evidence on low infant birthweight is considered inadequate. Its inadequacy

is due to the paucity of occupational studies, lack of consistent findings, and concerns about the interpretation of the Ranch Hand analyses. One available epidemiologic study of dioxin exposure (soil contamination) reported a weak association (statistically nonsignificant) with low birthweight. Studies of veterans were inconsistent; some indicated no increased risk, whereas others suggested an increased risk among certain subgroups. Future analyses of the Ranch Hand data may contribute important evidence regarding an increased risk for low birthweight among offspring of exposed Vietnam veterans. A list of the studies considered in this review appears in Table 9-5.

TABLE 9-5 Epidemiologic Studies—Low Birthweight

Reference	Description	*N*	OR/RR (95% CI)
Occupational			
Fitzgerald et al., 1989	Follow-up of an electrical transformer fire	3	SIR = 81 (16.7-236.4)
Environmental			
Stockbauer et al., 1988	TCDD soil contamination in Missouri		
	Intrauterine growth retardation	14	1.1 (0.5-2.3)
	Low birthweight	27	1.5 (0.9-2.6)
Vietnam veterans			
CDC, 1989	Vietnam Experience Study (GBDS)	99	1.1 (0.8-1.4)
Filed and Kerr, 1988	Follow-up of Australian Vietnam veterans	48	1.6 (1.0-2.5)
AFHS, 1992	Follow-up of Air Force Ranch Hands; conceptions during or after Southeast Asian service with high current dioxin levels	20	2.3 (1.3-4.0)

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval; SIR = standardized incidence ratio.

Conclusions

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and low birthweight in offspring.

Biologic Plausibility

Laboratory studies of the potential developmental toxicity of TCDD and herbicides using male animals are too limited to permit conclusions.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, the effects of selection and information bias in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

CHILDHOOD CANCER

Introduction

Definition and Descriptive Epidemiology

In most epidemiologic studies, childhood cancer usually refers to cancer diagnosed from birth through age 15 years. Childhood cancers are usually classified by primary anatomic site or tumor cell type.

The distribution of childhood cancers by type includes leukemia (23 percent), lymphoma (13 percent), central nervous system (19 percent), neuroblastoma (8 percent), soft tissue sarcoma (7 percent), kidney (6 percent), bone (5 percent), retinoblastoma (3 percent), liver (1 percent), and other (8 percent). There are approximately 6,500 new cases of cancer diagnosed each year in the United States in persons under age 15 (Young et al., 1986). About 2,200 deaths each year result from childhood cancer. The age-adjusted annual incidence of childhood cancers is estimated to be 136 per million in white children and 108 per million in black children. The cumulative incidence is approximately 1 in 600 before age 15. International rates of some childhood cancers have been found to vary geographically (and ethnically) (Parkin et al., 1988).

Compared with adult cancers, relatively little is known about the etiology of most childhood cancers, especially potential environmental risk factors. Important advances have been made in the study of genetic and molecular factors, and certain cancers in children. Acute leukemia, the most common type in children, has been the subject of most epidemiologic studies, although recent analytic epidemiology studies have been completed

on many other cancer types. Recently published reviews can be consulted for further details (Greenberg and Shuster, 1986; Robison et al., 1991). Some of the potential risk factors for childhood cancer that have been suggested (sometimes with inconsistent results) include advanced maternal age, prior fetal loss, birthweight of more than 4,000 grams, prenatal diagnostic radiation, postnatal irradiation, electromagnetic fields, parental occupation, pesticides, prenatal viral exposure, maternal use of marijuana during pregnancy, and parental cigarette smoking. In addition, some childhood cancers involve a familial aggregation of cancer (familial cancer syndromes) or defined chromosomal abnormalities; in some cases, their molecular origins have been delineated (Murphee and Benedict, 1984; Malkin et al., 1990).

Epidemiologic Studies of Childhood Cancer

Occupational and Environmental Studies

There were no studies that directly examined occupational or environmental exposure to herbicides or dioxin in relation to childhood cancer in offspring.

Vietnam Veterans Studies

The Field and Kerr (1988) study of Australian veterans found four cases of childhood cancer among the offspring of Vietnam veterans and none among the offspring of the comparison group. A variety of conditions appeared to be involved including a thalamic tumor, cerebral teratoma, vascular hamartoma, and lipoblastoma.

There was a slight excess of childhood cancers among children of Vietnam veterans in the VES (CDC, 1989). Twenty-five cases of childhood cancer were reported among the children of Vietnam veterans and 17 cases among non-Vietnam veterans (adjusted OR = 1.5, CI 0.7-2.8). Leukemia was the predominant diagnostic category, accounting for 12 cases among Vietnam veterans and 7 among non-Vietnam veterans (OR = 1.6, CI 0.6-4.0). The CDC's Birth Defects Study (Erickson et al., 1984b) reported an odds ratio of 1.8 (CI 1.0-3.3) for "other neoplasms." These included dermoid and epidermoid cysts (26 cases), and teratospermia (14 cases), lipomas (9 cases), neuroblastomas (3 cases), hepatoblastomas (1 case), rhabdomyosarcomas (1 case), and miscellaneous benign tumors (24 cases). This diverse group of congenital neoplasms was also associated with the Agent Orange exposure opportunity index based on interview data for service location (EOI level 5: OR = 2.0, CI 0.6-7.0).

TABLE 9-6 Epidemiologic Studies—Childhood Cancer

Reference	Description	N	OR/RR (95% CI)
Vietnam veterans			
CDC, 1989	Vietnam Experience Study		
	Childhood cancer	25	1.5 (0.7-2.8)
	Leukemia	12	1.6 (0.6-4.0)
Erikson et al., 1984a	CDC Birth Defects Study, "other" neoplasms	87	1.8 (1.0-3.3)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	4	—

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval.

Summary

There are no available occupational and environmental epidemiologic studies of herbicide exposure that address childhood cancer as an outcome.

Two studies of Vietnam veterans found some suggestion of an increased risk of cancer among offspring (see Table 9-6). The evidence is, however, inadequate, given the lack of other studies, failure to exclude chance and bias, and problems with herbicide exposure assessment.

Conclusions

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and childhood cancer in offspring.

Biologic Plausibility

Laboratory studies of the potential developmental toxicity of TCDD and herbicides using male animals are too limited to permit conclusions.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, the effects of information bias and low statistical power in these studies, and the lack of information

needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

SPERM PARAMETERS AND INFERTILITY

Introduction

Definition

Sperm Parameters There are several biologic markers of physiologic damage to human male reproduction using seminal sperm (Overstreet and Katz, 1987; NRC, 1989). The most common parameters used to evaluate effects due to toxic agents have been sperm number, sperm motility, and sperm structure or morphology. Sperm number is the number of sperm in the ejaculate (total number or per milliliter of semen). Men with sperm counts of less than 20 million/ml appear to be at an increased risk of infertility, although there is some uncertainty about the specific nature of the relationship between sperm number (or concentration) and fertility. Sperm motility refers to the swimming or "movement" ability of the sperm. Motility scoring has been shown to be subjective and sensitive to time and temperature, although automated approaches have been developed (Boyers et al., 1989). A strong correlation between sperm motility and fertility has been demonstrated. Sperm structure (morphology) refers to the evaluation of sperm size and shape. Classification systems based on sperm head, midpiece, and tail characteristics have been used. A general association has been found between a greater proportion of abnormal sperm and an increased likelihood of infertility. More recent work has found that a morphometric parameter, mean sperm width-to-length ratio, was associated with measures of infertility (Boyle et al., 1992).

Infertility Reduced reproductive capacity usually incorporates two concepts: (1) fecundity, the ability to conceive; and (2) fertility, the ability to produce live children. Typically, in epidemiologic studies, "infertility" is evaluated in two ways: (1) the number of children fathered, and (2) whether the couple had tried for a year or longer to conceive a child without success. Other measures of impaired fertility such as time to pregnancy have also been proposed (Baird et al., 1986). The definitions of male infertility are limited because they do not consider the female's role in the ability of the couple to conceive and produce live children. In addition, these measures usually do not take into account the desire for children, contraceptive practices, and other factors.

Descriptive Epidemiology

Most studies have reported that 10-15 percent of couples have impaired fertility (NRC, 1989). Accurate population statistics for sperm parameters are difficult to obtain, given the fact that most studies involve a group of selected men who enter infertility clinics for evaluation. A comprehensive literature review of environmental, occupational, and therapeutic chemical exposures and sperm tests found that, of 87 chemicals tested, exposure to 51 significantly reduced sperm count (Wyrobek et al., 1983). Of the 59 chemicals tested with respect to sperm motility, 22 were related to significant decreases in the numbers of motile sperm. Among the 44 chemicals tested for effects on sperm structure, 17 showed significant evidence for a decrease in the number of morphologically normal sperm.

Epidemiologic Studies of Sperm Parameters

Occupational and Environmental Studies

One study of Italian farmers evaluated sperm parameters in relation to 2,4-D exposure (Lerda and Rizzi, 1991). The study involved 32 farmers exposed to herbicides and 25 control farmers not working with herbicides. Urine measurements of 2,4-D confirmed exposure status, at least for that chemical. An association was found with one type of abnormal sperm morphology and reduced sperm motility. No association with decreased sperm count was noted.

Vietnam Veterans Studies

The Air Force study of 417 Ranch Hand veterans did not report decreased sperm count or increased percentage of abnormal sperm compared to the controls (AFHS, 1992). In fact, the reported relative risks were lower in the groups of exposed men. The VES (CDC, 1989) evaluation of 324 Vietnam veterans found associations between Vietnam service and lower sperm concentration (<20 million cells/ml; OR = 2.3, CI 1.2-4.3), Lower proportion of normal sperm (<40 percent normal; OR = 1.6, CI 0.9-2.8), and to a lesser extent, reduced sperm motility (<40 percent motile cells; OR = 1.2, CI 0.8-1.8). Further analysis did not show any pattern of association between these sperm parameters or the ability to father children, and history of combat experience or self-reported herbicide exposure.

Epidemiologic Studies of Infertility

Occupational and Environmental Studies

No studies of occupational or environmental exposure to herbicides and infertility were available.

Vietnam Veterans Studies

Several studies of veterans have examined infertility. Some studies have used other measures including "difficulty conceiving or having children" and number of conceptions or number of children fathered. The Australian veteran's study did not find an association either with difficulty in conceiving or with number of children (Field and Kerr, 1988). The study of American Legionnaires found a slightly increased odds ratio for difficulty in having children (OR = 1.3), but no association with the number of children fathered (Stellman et al., 1988). The Ranch Hand study reported no difference in the total number of conceptions (AFHS, 1992). The VES (CDC, 1988) did not find an association with the number of children fathered.

Summary

Only one occupational epidemiology study is available for assessing the association between herbicide exposure and altered sperm parameters (sperm count, motility, morphology). This study of sperm parameters and 2,4-D exposure did indicate an association with abnormal sperm morphology; however, given the small sample size and lack of additional studies, the evidence for determination of an association is considered inadequate. No studies were identified that examined occupational or environmental exposure and impaired fertility. One study of veterans reported an association with altered sperm measures (reduced sperm concentration and increased percentage of abnormal sperm), although there was no relationship to the number of children fathered, self-reported herbicide exposure, or the extent of combat experience (see Table 9-7). The paucity of occupational studies, lack of

TABLE 9-7 Epidemiologic Studies—Sperm Parameters and Infertility

Reference	Description	*N*	OR/RR (95% CI)
Vietnam veterans			
Stellman et al., 1988	Assessment of reproductive effects among American Legionnaires who served in Southeast Asia		
	Difficulty having children	349	1.3, $p < .01$
CDC, 1989	Vietnam Experience Study		
	Lower sperm concentration	42	2.3 (1.2-4.3)
	Proportion of abnormal sperm	51	1.6 (0.9-2.8)
	Reduced sperm motility	83	1.2 (0.8-1.8)

NOTE: OR/RR = Odds ratio/relative risk; CI = confidence interval.

consistent findings in veteran studies, and methodologic problems in the studies reviewed do not permit a valid assessment of an increased infertility risk.

Conclusions

Review of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and altered sperm parameters or infertility.

Biologic Plausibility

TCDD has been reported to reduce fertility in male laboratory animals at doses high enough to produce other toxic effects. Studies of the potential reproductive toxicity of the herbicides are too limited to permit conclusions.

Increased Risk of Disease in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, effects of information bias in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

NOTE

* The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

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10

Neurobehavioral Disorders

Neurologic problems in clinical medicine cover a wide variety of disorders. The nervous system is anatomically and functionally divided into central and peripheral subsystems. The central nervous system (CNS) includes the brain and spinal cord, and CNS dysfunction can be subdivided into two general categories, neurobehavioral and motor/sensory. Neurobehavioral difficulties involve two primary categories: cognitive decline, including memory problems and dementia; and neuropsychiatric disorders, including neurasthenia (a collection of symptoms including difficulty concentrating, headache, insomnia, and fatigue), depression, posttraumatic stress disorder (PTSD), and suicide. Other CNS problems can be associated with motor difficulties, characterized by problems such as weakness, tremors, involuntary movements, incoordination, and gait/walking abnormalities. These are usually associated with subcortical or cerebellar system dysfunction. The anatomic elements of the peripheral nervous system (PNS) include the spinal rootlets that exit the spinal cord, the brachial and lumbar plexus, and the peripheral nerves that innervate the muscles of the body. PNS dysfunctions, involving either the somatic nerves or the autonomic system, are known as neuropathies.

Neurologic dysfunction can be further classified as either global or focal. For example, in neurobehavioral disorders, global dysfunction can involve altered levels of consciousness or agitated behavior, whereas focal changes give rise to isolated signs of cortical dysfunction such as aphasia or apraxia. Likewise, global neuropathies could affect all peripheral nerves of the body, whereas a focal lesion would damage only a single nerve.

The usual evaluation of neurologic function involves the clinical neurologic examination in conjunction with several testing procedures. The neurologic examination is a five-part battery of tests performed by a physician that systematically evaluates cerebral (mental status), cranial nerve, motor, sensory, and cerebellar/gait functions. Special ancillary tests that can be performed by additional professionals (physicians, neuropsychologists, or technicians) may include detailed neuropsychological evaluations with standardized and validated test protocols, electromyography (EMG) and nerve conduction studies for PNS function, neuroimaging for identifying CNS anatomic lesions, and neurophysiology tests such as electroencephalography (EEG) for the assessment of epilepsy and metabolic disorders.

The neuropsychologic battery of tests chosen depends on the age of the patient and the type of behavioral alterations being evaluated. Although there are literally hundreds of standardized tests available for neuropsychological assessment, a few of the most commonly used are the Wechsler Adult Intelligence Scales (WAIS) and its revised version (WAIS-R), the Minnesota Multiphasic Personality Inventory (MMPI), and the Self-Report Symptom Inventory (known as SCL-90). The WAIS and WAIS-R assess general intelligence as well as verbal and nonverbal cognitive abilities using 11 different subtests. The MMPI, a standardized 566-item questionnaire, provides objective assessment of personality characteristics and psychopathology (Green, 1980). The MMPI consists of three validity scales and ten clinical scales; test norms are based on scores of a sample of Minnesota men who took the test before World War II. The SCL-90 is a 90-question, self-administered checklist that examines various personality characteristics, psychiatric disorders, health-related concerns, anxiety, and depression.

Although the neurologic examination and the specialty tests described above are widely available, they are not all uniformly standardized and their results can be affected by a number of factors. They are often able to detect neurologic dysfunction but cannot always distinguish it from the effects of abnormal emotional states or diseases outside the nervous system that can alter a patient's function. For example, body temperature can modify EMG data, examiner style and native intelligence can affect patient performance on neuropsychologic tests, and fatigue or medications can profoundly affect EEG patterns. For these reasons, rigorous methodology and maximally matched control or comparison populations are especially important for the scientific study of the causes of neurologic and behavioral alterations.

Case identification in neurology is also often difficult. Despite the advances in neuroimaging, many types of neurologic alternations are biochemical and show no abnormalities on scanning tests. The nervous system is not usually accessible for biopsy, so pathologic confirmation is not feasible

for many neurologic disorders. Behavioral and neurophysiologic changes can be partly or largely subjective and, even when objectively documented, may often be reversible. Timing is important in assessing the effect of chemical exposures on neurologic function. Some symptoms of neurologic importance will appear acutely but be short-lived, whereas others will appear slowly and be detectable for extended periods. These caveats must be considered in the design and critique of epidemiologic studies evaluating an association between exposure to any chemical agent and neurologic or neurobehavioral dysfunction.

Many reports have addressed the possible contribution of herbicides and pesticides to nervous system dysfunction, and reported abnormalities have ranged from mild and reversible to severe and long-standing. These assessments have been conducted in three general settings, related to occupational, environmental, and Vietnam veteran exposures (see Table 10-1). Several case reports of patients ingesting 2,4-dichlorophenoxyacetic acid (2,4-D) are mentioned under environmental exposures. This chapter reviews reports of neurologic alterations associated with exposure to herbicides, TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), or other compounds used in herbicides in Vietnam and focuses on chronic effects of neurotoxicity. It emphasizes the small number of cross-sectional studies with comparison samples in which both exposed and unexposed were neurologically assessed by systematic physical examinations and/or ancillary tests such as neuropsychological evaluations or EMG measurements.

In approaching this study of the health effects of herbicide exposure, the committee discussed meta-analysis for certain health outcomes with consideration of sample size, measurement of exposure as well as outcome, selection of controls, period of observation, and other methodologic factors. For neurobehavioral disorders, however, a sufficient number of studies of neurological disorders with similar exposure classifications and disease outcome diagnostic groups were not available for this type of analysis.

The potential neurotoxicity of TCDD and herbicides in animal studies has not been thoroughly investigated. A large number of acute and subchronic toxicity studies have been conducted with TCDD but the majority of these studies was not designed specifically to investigate neurotoxicity. Available data imply that CNS alterations or changes in the responsiveness of neurochemical processes in the CNS may be associated with lethal or near lethal dose levels of TCDD in some animal species, however, the changes observed may also be regulatory responses occurring secondary to changes induced in other organ systems (see Chapter 4). TCDD concentrations in the brain after systemic exposure are low, and quite similar among rodent species. Relatively little work has been done to quantify the concentration of Ah receptors in the central or peripheral nervous systems.

TABLE 10-1 Neurobehavioral Studies of Herbicide Exposure

Reference	Study Group, *N*	Tests of Neurological Dysfunction	Exposure Measures	Comparison Group, *N*
Occupational				
Alavanja et al., 1989	1,411 forest/soil conservationists	No: mortality data only	No	None
Alderfer et al., 1992	281 TCP or 2,4,5-T production workers	Psychological evaluation, Becks, SCL-90-R	Serum TCDD	260 community matched referents
Bashirov, 1969	292 2,4-D production workers	No: interviews	No	None
Bond et al., 1987	322 Dow chemical workers (with chloracne)	No: mortality data only	Chloracne	2,026 workers without chloracne
Bond et al., 1989	2,072 Dow chemical workers (with chloracne)	No: mortality data only	Job classification	Internal comparison
Coggon et al., 1986	5,754 MCPA production and spraying workers	No: mortality data only	Occupational records	British national population
Green, 1991	1,222 forestry workers	No; mortality data only	Job classification	Ontario male population
Klawans, 1987	45 railroad workers	Neuropsychological evaluation	No	None
Moses et al., 1984	117 2,4,5-T production workers	Neurologic examination,	Self-reports, chloracne	109 workers without chloracne

Reference	Study Group, *N*	Tests of Neurological Dysfunction	Exposure Measures	Comparison Group, *N*
Manz et al., 1991	1,184 production workers	No: mortality data only	Job classification	a) 3,120 workers at gas supply company b) German population
Pazderova-Vejlupkova et al., 1981	55 2,4,5-T production workers	Neurologic examination, EMG	No	None
Poland et al., 1971	73 2,4-D and 2,4,5-T production workers	Neurologic examination, MMPI	Job classification	Internal comparison
Singer et al., 1982	56 2,4-D and 2,4,5-T production workers	EMG, nerve conduction	No	25 nonexposed referents
Suskind and Hertzberg, 1984	204 2,4,5-T production workers	Neurologic examination, EMG	Job classification	163 nonexposed workers
Sweeney et al., in press	281 TCP and 2,4,5-T production workers	EMG	Serum TCDD	260 community-matched referents
Thomas, 1987	1,412 fragrance and flavor plant workers	No: mortality data only	Job classification	U.S. male population
Environmental				
Assennato et al., 1989	Seveso residents, 152—1st follow-up (FU); 142—2nd FU; 141—3rd FU	EMG	Chloracne	Unexposed subjects from nearby towns 123—1st FU 196—2nd FU 167—3rd FU

Barbieri et al., 1988	152 Seveso residents of TCDD contaminated area	Neurologic examination, EMG	Chloracne	123 residents from nearby towns
Bertazzi et al., 1989	Seveso residents, 556 zone A; 3,920 zone B; 26,227 zone R	No: mortality data only	Residence in contaminated area	167,391 nonexposed residents of surrounding area
Boeri et al., 1978	470 zone A Seveso residents	Interview, neurologic exam, EMG	Residence in zone A	152 zone R residents
Filippini et al., 1981	308 Seveso residents	EMG, neurologic examination	Chloracne, abnormal hepatic enzymes	305 nearby nonexposed residents
Gilioli et al., 1979	35 lab technicians in Seveso	EMG	No	35 subjects from more distant areas
Pocchiari et al., 1979	446 Seveso residents	Neurologic examination	Residence in zone A	255 inhabitants from low exposure area (zones B and R)
Stehr et al., 1986	68 Missouri residents of TCDD contaminated area	Neurologic examination	Self-reports	36 individuals with little or no TCDD exposure
Hoffman et al., 1986 Stehr-Green et al., 1987	154 Missouri residents, Quail Run	Neurologic examination, neuropsychologic battery	Residence in contaminated area 6+ months	155 unexposed residents

Reference	Study Group, *N*	Tests of Neurological Dysfunction	Exposure Measures	Comparison Group, *N*
Webb et al., 1987	68 Missouri residents of TCDD contaminated area	Neurologic examination	Soil measurements	36 individuals with little or no TCDD exposure
Vietnam Veterans				
AHFS, 1984	1,208 Air Force Ranch Hands	Neurologic examination	Exposure index	1,238 Air Force veterans who did not participate in Ranch Hand
AFHS, 1987	1,016 Air Force Ranch Hands	Neurologic examination	Exposure index	1,293 Air Force veterans who did not participate in Ranch Hand
AFHS, 1990	995 Air Force Ranch Hands	Neurologic examination	Exposure index	939 Air Force veterans who did not participate in Ranch Hand
AFHS, 1991	888 Air Force Ranch Hands	Neurologic examination, questionnaire	Serum TCDD	856 Air Force veterans who did not participate in Ranch Hand
Boyle et al., 1987	9,324 Vietnam veterans	No: mortality data only	Vietnam service	8,989 Vietnam era veterans
Breslin et al., 1988	24,235 Army and Marine Vietnam veterans	No: mortality data only	Vietnam service	26,685 Vietnam era veterans

Bullman et al., 1991	374 Vietnam veterans with PTSD	No	Vietnam service	373 Vietnam veterans without PTSD
CDC, 1987	9,324 veterans, Vietnam Experience Study	No: mortality data only	Vietnam service	8,989 Vietnam era veterans
CDC, 1988	2,490 veterans, Vietnam Experience Study	Neuropsychiatric test, EMG, hearing test	Vietnam service	1,972 Vietnam era veterans
Decoufle et al., 1992	7,924 Vietnam veterans	Questionnaire data	Self-reported exposure	7,364 Vietnam era veterans
Eisen et al., 1991	2,260 Vietnam veteran twin pairs	Self-reported symptoms	Service in SEA	Twin siblings who did not serve in SEA
Fett et al., 1987	19,205 Australian Vietnam veterans	No: mortality data only	No	25,677 Vietnam era veterans
Fiedler and Gochfeld, 1992	10 New Jersey Vietnam veterans	Neuropsychiatric test, WAIS-R	Serum TCDD	10 nonexposed Vietnam veterans; 7 Vietnam era veterans
Goldberg et al., 1990	2,092 Vietnam veteran twin pairs	Questionnaire data	No	Twin siblings who did not serve in Vietnam
Kogan and Clapp, 1985	840 Massachusetts Vietnam veterans	No: mortality data only	Vietnam service	Massachusetts population and 2,515 Vietnam era veterans
Lawrence et al., 1985	555 New York State Vietnam veterans	No: mortality data only	Vietnam service	941 Vietnam era veterans

Reference	Study Group, *N*	Tests of Neurological Dysfunction	Exposure Measures	Comparison Group, *N*
Levy, 1988	6 Massachusetts Vietnam veterans with chloracne	Standardized interview for PTSD, WAIS-R	Chloracne	25 Vietnam veterans without chloracne
Rellahan, 1985	232 Vietnam veterans, Hawaii residents	Self-reported symptoms	Vietnam service, combat	186 Vietnam era veterans
Snow et al., 1988	2,858 Vietnam veterans	Self-reported symptoms	Vietnam service, combat	Internal comparison
True et al., 1988	775 Vietnam veterans	Self-reported symptoms	Vietnam service, combat	1,012 Vietnam era veterans
Wendt, 1985	10,846 Iowa Vietnam veterans	Questionnaire data	Service in SEA	None

COGNITIVE AND NEUROPSYCHIATRIC EFFECTS

Epidemiologic Studies of Cognitive and Neuropsychiatric Effects

Occupational Studies

Numerous occupational studies of neurobehavioral effects of herbicides have been conducted, but most are limited by methodologic problems. In 1971, Poland and colleagues (1971) reported on personality changes in 73 male workers involved in manufacturing of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Physical neurologic examination detected no abnormalities in these workers. Mean MMPI scores for 28 production workers (with potentially higher exposures) and 20 administrative staff (with potentially lower exposures) each differed significantly from published norms. Production workers differed on 9 of 13, and administrative staff on 6 of 13, clinical scales. Chloracne was found in a total of 13 workers, but there was no significant correlation between job location within the plant and chloracne. The subgroup of workers with chloracne had significantly higher mean mania scores when compared to workers with less severe acne ($p < .05$). The authors note, however, that the workers are not comparable to the population on which the test norms are based. The overall results of this study did not show a strong association between exposure to 2,4-D and 2,4,5-T and significant personality change.

A 10 year follow-up study examined 55 men in Czechoslovakia who were exposed to TCDD during the production of 2,4,5-T and who developed signs of illness during the study period (Pazderova-Vejlupkova et al., 1981). During the initial evaluation at onset of illness, 7 percent of the workers demonstrated encephalopathy, and 75 percent neurasthenia. The severity of symptoms was unrelated to length of exposure, job classification, or age. Over time (i.e., mean of 9.3 years), the number of patients with neurasthenia decreased, and the accompanying anxiety and depression disappeared completely. The authors proposed two possible mechanisms for these results. First, psychiatric symptoms could have been caused directly by the neurotoxicant and gradually resolved. Alternatively, these symptoms could be related to a severe somatic condition that developed as a result of fears and changes in daily activities, both at home and at work. In this case, adaptation and new coping mechanisms resulted in a diminution of symptoms over time. Methodologic problems in this study included the use of self-reported symptoms, no objective measure of exposure (all workers were considered exposed), and selection bias (i.e., only sick workers were studied).

The results of a study of 45 railroad workers exposed to TCDD in early

1979 were reported by Klawans (1987). The workers were exposed to TCDD during cleanup of a chemical spill from damage to a tank car that contained polychlorinated phenols. The concentration of TCDD found in the tank car was approximately 45-46 parts per billion (ppb). Initial complaints of the workers were fatigue (91 percent), muscle ache (51 percent), and distal paresthesia (93 percent). The results of a neuropsychological evaluation given two years later revealed cognitive impairment in 49 percent of the workers (i.e., decreased attention/concentration as assessed by the Mental Control and Digit Span subtest of the Wechsler Memory Scales and Reaction Time task). Some degree of depression as measured by Beck's Depression Inventory was found in 69 percent of the workers. The same percentage of workers was found to be depressed at the six year follow-up examination. Although this study performed detailed psychometric evaluations of the CNS, only exposed workers were examined. No control group of workers was included for comparison, and no direct or indirect measures of TCDD exposure were employed.

Self-reports of sleep difficulties (Bashirov, 1969), headaches (Bashirov, 1969), fatigue (Kimmig and Schulz, 1957; Goldman, 1973), and other subjective neurologic complaints have appeared frequently in case studies and some cohort reports, but studies with comparison populations have not regularly suggested CNS problems. In a cohort mortality study of 1,222 male Canadian forestry workers at an electrical utility presumed to have been exposed to 2,4-D and 2,4,5-T (Green, 1991), a significant excess in the number of suicides was observed for the cohort as a whole compared to the total male population of Ontario (SMR = 2.1, CI 1.1-3.8). However, the overall mortality rate was lower in the forestry workers than in the general population, and fewer deaths from diseases of the nervous system occurred than were expected. Although the author of this study speculated that CNS toxicity from phenoxy herbicides might be the cause of the increased rate of suicides, the rationale for this is not stated. Because of the nature of the workers' routine job duties at the utility, length of employment was used as a surrogate measure of exposure. The study was limited by the young age of the cohort (the mean age at the end of the last follow-up was 43 years), no mention of whether protective equipment was used, and no control for potential confounders (age, education, general health). The limitations imposed by the "healthy worker effect" and the use of death certificates to ascertain the cause of death are described in Chapter 5.

Of all studies assessing neurobehavioral outcomes after occupational exposure to dioxin, the National Institute for Occupational Safety and Health (NIOSH) study stands out as particularly rigorous (Alderfer et al., 1992). A group of 281 workers who manufactured trichlorophenol (TCP) or 2,4,5-T was compared with an unexposed referent group (N = 260) recruited from the same communities as the workers and matched on age, race, and sex. A

comprehensive medical and psychological evaluation was administered over a three day period in 1987 or 1988. Depressed mood was assessed using the Beck Depression Inventory and the depression subscale of the Self-Report Symptom Inventory-Revised-90 (SCL-90-R). These depression scales are well standardized and validated. The authors noted that the study had statistical power of 85 percent to detect a twofold increase in the prevalence of depressive symptoms. Separate multiple regression models were used to evaluate the relationships between two measures of TCDD exposure, current serum TCDD level and status as a worker or referent, and each of the two outcome measures of depression. Considered in the analysis were the following covariates: age, race, income, education, family history of psychiatric disorders, current treatment for psychiatric disorders, current treatment with neurotoxic drugs or drugs that can produce depression as a side effect, history of head injury, history of low vertebral disk herniation, history of chronic medical disorders, self-reported occupational exposure to neurotoxicants, self-reported exposure to Agent Orange during military service in Vietnam, smoking history, and lifetime alcohol use.

Workers had a mean serum TCDD level of 220 parts per trillion (ppt) and the referents had a mean TCDD level of 6 ppt ($p = .0001$). Neither serum level nor group membership was significantly associated with scores on the Beck Depression Inventory or the SCL-90 in either the unadjusted or the adjusted analyses. These results suggested that current TCDD level was not associated with current depression in this group of highly exposed workers. The authors warned that since this study was conducted many years after TCDD exposure, depression may have been present following exposure but resolved prior to data collection. Additional analyses showed self-reported Agent Orange exposure during the Vietnam war to be a significant covariate in the model for the Beck depression score. However, only seven referents and one worker reported Agent Orange exposure; the mean serum TCDD level in these eight participants was 43 ppt compared to a mean level of 116 ppt in participants reporting no Agent Orange exposure ($p < .10$).

Environmental Studies

The largest environmental accident, in which TCDD contaminated about 2,000 hectares, occurred in 1976 at Seveso, Italy. Pocchiari and colleagues (1979) reported preliminary clinical findings on 446 inhabitants living in the most highly contaminated area (zone A), closest to the plant where the explosion occurred, and 255 inhabitants who lived a distance from the plant (zones B and R) who were thought to be less exposed. TCDD was found in environmental soil samples from areas next to the factory at levels as high as 15,000 ppb. An initial neurologic evaluation was done in 1977; a second evaluation was completed in 1978 on about one-half of the individuals from

zone A who were screened in 1977. Idiopathic clinical neurologic damage (not specifically defined) was found in 6.7 percent of zone A residents, compared to 1.2 percent of zone B and R residents; subclinical damage was found in 3.1 percent and 1.2 percent of residents of the respective zones (no statistical analysis provided). Two years later, 11.7 percent of 205 exposed residents had evidence of neurologic dysfunction (no data for comparison group).

Bertazzi and colleagues (1989) examined the mortality of adults (aged 20-74 years) residing in zones A, B, and R for the period from July 1976 to December 1986. A referent cohort of subjects who lived in the immediate surrounding area was also examined. Men who ever lived in a TCDD contaminated zone demonstrated excess mortality from chronic ischemic heart disease in the first five years following the accident compared to the male reference population. The authors proposed that both the psychosocial stress caused by the accident (e.g., relocation, loss of jobs, loss of property) and the TCDD toxicity may have contributed independently or interactively to increased cardiovascular morbidity. No long-term effects on the CNS were reported.

In 1971, sludge waste containing TCDD was sprayed for dust control purposes on residential, recreational, and commercial areas of eastern Missouri (Stehr-Green et al., 1987). TCDD levels were measured in composite soil samples from this area at levels between 39 and 1,100 ppb. In 1984, comprehensive examinations of 154 exposed residents (defined as residing in the contaminated area for six months or more) and 155 unexposed persons (defined as residents for six months or longer of sites with no potential exposure to TCDD) were performed to assess possible acute and subacute health effects. Results of the neurobehavioral tests showed no significant differences on tests of cognitive functioning, with the exception of the Vocabulary subtest from the WAIS. This subtest is a good estimate of premorbid level of verbal intelligence, which significantly influences performance on neurobehavioral tests (Bolla-Wilson and Bleeker, 1986). Group differences on the Vocabulary subtest indicate that the control and exposed groups were not comparable on this confounding variable, and no statistical adjustments were used to control for the possible confounding effects. Higher scores were found for the exposed group relative to the unexposed group for a number of scales on the Profile of Mood States (tension/anxiety, anger/hostility, depression/dejection, and fatigue/inertia). An implicit confounder in all neurobehavioral research related to environmental disasters is the inability to separate confidently symptoms related to stress from the disaster itself from those related to the putative neurotoxicant. Because the differences cited above could reasonably be attributed to the stress associated with environmental disasters (exaggerated media coverage, relocation, or inability to relocate due to limited financial resources), no specific effect could be identified for exposure to TCDD.

Neurobehavioral effects were described in two case reports of ingestion of 2,4-D. Severe CNS depression was reported in a 61 year old woman who intentionally consumed dandelion killer (Friesen et al., 1990). Initial serum 2,4-D concentration after hospitalization was 392 mg/liter. Clinically, the woman was comatose, and showed depressed respiration, pinpoint pupils, and no deep tendon reflexes. As her 2,4-D concentrations decreased, her mental status improved. At discharge, she showed no neurologic or other systemic effects from the 2,4-D overdose. In a case of fatal 2,4-D ingestion described by Dudley and Thapar (1972), a 76 year old man with senile dementia ingested a large amount of 2,4-D. The patient was comatose when admitted to the hospital, with minimal response to deep pain and hyperactive deep tendon reflexes. Three days after hospitalization the patient died from a trial fibrillation. Autopsy revealed tissue concentrations of 2,4-D in the following organs: liver, 408 parts per million (ppm); brain, 93 ppm; and blood, 58 ppm. In the brain, plaques of perivascular demyelination similar to those found in multiple sclerosis or after arsenic and carbon monoxide poisoning were found. These two case studies demonstrate a positive association between ingestion of extremely high doses of an herbicide and CNS depression. However, it is not possible to determine whether the CNS depression may have been related to chemical components in the herbicides other than 2,4-D. In the second case, where dementia was already present, the relationship between histologic changes and 2,4-D exposure remains unclear.

Vietnam Veterans Studies

As with environmental disasters, neurotoxicant studies conducted on war victims are confounded by the stress of the war experience. For many centuries it has been well recognized that catastrophes, personal tragedies, and armed conflict lead to a variety of somatic and psychologic symptoms. The American Civil War resulted in descriptions of palpitations, chest pains, and other cardiac disturbances, which were called "soldier's heart." Many World War I soldiers developed a syndrome called "shell shock" or war gas syndrome. During and after World War II and the Korean War, there was further experience with these symptoms, particularly because large numbers of civilians were exposed to physical catastrophe. Thus, the concentration camp syndrome/prisoner syndrome and the traumatic neurosis of war were recognized as ramifications of this pattern of response.

In the 1950s, psychiatrists developed a diagnosis called posttraumatic distress disorder (PTSD) and established specific criteria for this diagnostic category. PTSD is described in the *Diagnostic and Statistical Manual of Mental Disorders*—third edition, revised (American Psychiatric Association, 1987) as a disabling complex of memories, behaviors, and affective

states occurring in the aftermath of an extraordinarily stressful experience. The salient features of the disorder are recurrent reexperiencing of the traumatic event (e.g., repetitive dreams or intrusive memories), hyperarousal (e.g., exaggerated startle response or insomnia), avoidance of stimuli reminiscent of the traumatic event, and diminished emotional responsiveness. Symptoms of anxiety and depression are common and may be severe. There may also be symptoms of organic mental disorder (i.e., failing memory, difficulty concentrating, emotional lability, headache, and vertigo).

It has been estimated that 10 to 20 percent of veterans who served in Vietnam reported that they had at one time suffered from psychological disturbances (neurasthenia) or PTSD (Hall and Macphee, 1985; CDC, 1988). The prevalence of similar psychiatric disorders in the general population is between 10 and 20 percent, with severe disturbances found in 3 percent (Andrews et al., 1981; Hall and Macphee, 1985). Therefore, although Vietnam veterans show higher rates of psychiatric disturbance than veterans who did not serve in Vietnam, they appear to be no more likely than the general population to develop these difficulties. However, it should be noted that Vietnam veterans are predominantly young and male, and the prevalence of these psychological conditions may be different than for the general population. Some investigators have found that service in Vietnam was associated with increased mortality due to trauma, such as suicide, motor vehicle accidents, and "accidental poisonings" (Lawrence et al., 1985; Fett et al., 1987; Breslin et al., 1988). In some cases, psychiatric difficulties are reported as direct manifestations of the neurotoxicity of Agent Orange (Barr, 1982; Levy, 1988). A suggestion that Agent Orange causes neuropsychiatric disorders by producing metabolic disturbance or temporal lobe seizures (Barr, 1982) has not been tested with empirical research.

Other studies employing different methodologies failed to find these same associations. A body of evidence has shown that the development of PTSD is likely to be related to being wounded (Bullman et al., 1991), high intensity of combat experience (Blum et al., 1984; Hall and Macphee, 1985; Hall, 1986; Snow et al., 1988; True et al., 1988; Goldberg et al., 1990; Decoufle et al., 1992) young age, low level of education, and race (True et al., 1988).

Levy (1988) compared 6 Vietnam veterans with chloracne to a matched control group of 25 Vietnam veterans without chloracne on a battery of neuropsychological tests and interviewed them for PTSD. Active cases of chloracne were used to estimate Agent Orange exposure. The groups were matched on age, education, and period of Vietnam service. In order to control for the effects of combat stress, control subjects had to report a similar number of contacts with the enemy. The exposed group scored significantly lower on six of the nine neuropsychological tests. One of the largest group differences was on the WAIS-R Vocabulary subtest, which is a good estimate of verbal intelligence. In general, the lower the score on this

test, the poorer is the performance on other neurobehavioral tests. These data suggest that the exposed group was not comparable to the referent group on intellectual level, and therefore, the evidence for organic psychological deficits (reported group differences on the neuropsychological tests) is questionable. Exposed Vietnam veterans reported a significantly higher rate of PTSD-related symptoms, although the study involved only a small number of Vietnam veterans, was conducted nearly 20 years after potential exposure in Vietnam, and did not control for confounding variables.

The National Vietnam Veterans Readjustment Study (Kulka et al., 1988) examined current and lifetime prevalence of PTSD and other psychological disorders (e.g., depression, anxiety reaction, obsessive compulsive disorder, alcohol abuse or dependence) in both men and women Vietnam theater veterans. The *lifetime* prevalence of PTSD (disorder occurred at sometime during their lives) was 30.6 percent in the male veterans and 26.9 percent among female veterans. Exposure to war zone stress in Vietnam was positively associated with higher rates of all the psychiatric disorders. These disorders all tended to be chronic rather than acute. It was hypothesized that the development of PTSD in an individual was related to a variety of risk factors, which included individual vulnerability (biological, psychological, and sociodemographic factors) and war zone stressors that were independent of other risk factors. Exposure to herbicides in Vietnam was not examined as a potential risk factor in this large-scale study.

Boyle and colleagues (1987) examined the postservice mortality of a cohort of 9,324 Army Vietnam veterans compared to 8,989 Vietnam era Army veterans who did not serve in Vietnam. In the first five years after discharge, Vietnam veterans had a 17 percent higher mortality rate than Vietnam era veterans and most deaths were from external causes (e.g., motor vehicle accidents, drug and alcohol use). The authors suggested that the excess of traumatic deaths among Vietnam veterans was probably related to unusual stresses the veterans endured while stationed in a war zone. On the other hand, drug-related deaths were felt to be linked to intensity of combat experience, rather than the "Vietnam experience" per se. Unfortunately, it is extremely difficult to separate effects of herbicide exposure and combat since it is possible that those who were the most heavily exposed to herbicides were also most likely to be in combat zones. The results of the Wisconsin Vietnam Veteran Mortality Study (Anderson et al., 1986) revealed that alcohol consumption was related to such demographic characteristics as region of residence, age, income, and marital status rather than to veteran status. This suggests that differences in alcohol consumption between veterans and nonveterans were related to demographic differences between the two groups rather than veteran status.

Farberow and colleagues (1990) examined potential risk factors for suicide among 38 Vietnam veterans compared to 46 Vietnam veterans who

died from motor vehicle accidents. Veterans combat exposure was assessed using information from military personnel records on military occupational specialty code (MOSC) and from psychological autopsy. Suicide was not associated with specific combat experiences or military occupation, however symptoms related to PTSD were observed more frequently among suicide cases than accident cases.

Several other studies of Vietnam veterans have evaluated suicide as an outcome (Lawrence et al., 1985; CDC, 1987; Fett et al., 1987). However, because of methodologic problems with these studies, including variability and lack of information on herbicide exposure, and inadequate consideration of potential confounding variables in the veterans' studies, such as combat experience, it was not possible to determine whether there is an increased suicide risk associated with herbicide or TCDD exposure.

There are few studies that directly assess the cognitive effects of herbicide exposure by using standardized neuropsychological tests. In two studies that have been completed—the Vietnam Experience Study conducted by the CDC (1988) and the New Jersey Pointman study (Fiedler and Gochfeld, 1992)—neither found consistent associations between exposure and decrements in performance. One explanation for this could be the length of time that elapsed between exposure and evaluation. If herbicide exposure was associated with cognitive effects at the time of exposure, it is likely that these effects would have disappeared or been compensated for in the time since exposure ceased. A poor measure of herbicide exposure could also lead to a lack of apparent differences; problems with self-reported exposures and the use of current serum TCDD levels to estimate prior dioxin exposure are discussed in Chapter 6.

Serum TCDD concentrations from 888 participants in Operation Ranch Hand were compared to those of 856 Air Force veterans who did not participate in Ranch Hand (AFHS, 1991). The highest levels were found among the ground crew, but the variation in individual TCDD scores was considerable. In the Ranch Hands, no associations were found between body burden of TCDD (current serum levels) and reports of sleep disturbances or SCL-90 variables (e.g., anxiety, depression, somatization). Although significant results were found between serum TCDD and certain elements of the self-administered Millon Chemical Multiaxial Inventory test, which assesses basic personality patterns, pathological personality disorders, and clinical symptom syndromes, these findings were not consistent with similar variables on the SCL-90-R and other reported information.

Summary of Cognitive and Neuropsychiatric Effects

The existing literature on neurobehavioral effects of occupational, environmental, and Vietnam veterans' exposure to herbicides and related compounds

is insufficient to determine whether an association exists between herbicide exposure and chronic cognitive or neuropsychiatric disorders. As suggested by Sharp and colleagues (1986), the delayed effects of pesticides on human health are difficult to detect, and the health risks may be sufficiently small that they are below the power of present epidemiologic studies to detect.

Although there are no shortages of studies concerning this topic, methodologic problems make it difficult to reach definitive conclusions. Shortcomings in defining exposure include absent or poor exposure assessments; inconsistencies in identifying exposed individuals for study (i.e., some studies rely upon the presence of chloracne for inclusion, others assumed all subjects were exposed); and concomitant exposure to different chemicals, mixtures of chemicals, or concentrations. Studies of cognitive or neuropsychiatric disorders are also weakened by the small numbers of subjects; poor selection, or absence of, comparison groups; confounding of the possible effects of herbicides with the effects of stress; and inadequate statistical analyses. Self-reports of exposure and symptoms may not be verified independently. To maximally define the direct effects of herbicides and dioxin on cognitive and neuropsychiatric function, future studies should focus primarily on occupationally exposed groups for whom levels of exposure are better known and neurobehavioral testing can be conducted in relative proximity to the time of exposure.

Based on past findings, if herbicide or TCDD exposure is associated with neurobehavioral disorders, these are in all likelihood subtle. Since the central nervous system can compensate for minor damage, the chances of finding subtle effects 20 years after exposure are small given the assessment tools currently available. However, it is also possible that subtle CNS changes acquired in early adulthood could manifest themselves in later adult life when compounded by "normal age-related changes" of the CNS. That is, exposure to neurotoxins could produce "accelerated aging" of the brain due to premature neuronal loss, which could then result in neurobehavioral deficits. Therefore, a prospective study of individuals with documented significant exposure, after they reach the age of 60 years, would be of interest in investigating the interactive effects of exposure to herbicides and dioxins with age on neurobehavioral functioning.

Conclusions for Cognitive and Neuropsychiatric Effects

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and cognitive or neuropsychiatric disorders.

Biologic Plausibility

Studies in laboratory animals do not support an association between exposure to TCDD or herbicides and cognitive or neuropsychiatric disorders.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the studies that have been reviewed and inadequate control for important confounders, it is not possible for the committee to quantify the degree of risk likely to be experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

MOTOR/COORDINATION DYSFUNCTION

Epidemiologic Studies of Motor/Coordination Dysfunction

Occupational Studies

Tremor and dystonia were described in a series of exposed railroad workers (Klawans, 1987), but no comparison group was studied. The high prevalence of the findings (53 percent with dystonia and 78 percent with tremor), detected specifically by a movement disorder specialist, suggests that movement disorders may be subtle but present in many subjects if specifically sought. Other reports of occupational exposure have not confirmed these findings, and thus a strong association between herbicides and motor/sensory CNS dysfunction cannot be established.

Mortality studies that have included evaluations of strokes or vascular lesions of the CNS as a cause of death have not reported a significant increase in risk from occupational exposure to TCDD (Thomas, 1987; Alavanja et al., 1989; Bond et al., 1989). Using chloracne as a clinical measure of TCDD exposure, one study found that strokes occurred more frequently than expected in chloracne subjects compared to nonchloracne subjects (RR = 3.8, CI 1.8-12.1; Bond, 1987). This risk estimate, however, was based on only four cases, and multiple chemical exposures were likely.

Environmental Studies

Nonbehavioral CNS effects that included increased tendon jerks, ataxia, and extrapyramidal signs occurred in 37 of 470 patients in the high-exposure Seveso group compared to 8 of 152 in the comparison group, but no statistical analysis was presented (Boeri et al., 1978). Mortality studies

based on combined Seveso exposure zones (A, B and R) showed no increased risk for death from strokes (RR = 1.0, CI 0.8-1.3; Bertazzi et al., 1989). However, in the highest exposure group, five subjects died from cerebrovascular disease, resulting in increased relative risk values for zone A. The small number of strokes in the entire group limits the impact of the observation. Other environmental reports did not focus on CNS effects.

Effects seen in fatal intoxication cases have included depressed levels of consciousness, increased reflexes, altered pupillary signs, and other neurologic deficits, but it is not clear whether these signs were direct toxic manifestations of herbicide exposure or nonspecific effects of cardiopulmonary collapse (Nielsen et al., 1965; Dudley and Thapar, 1972). One patient received 2,4-D as therapy for a life-threatening fungal infection, disseminated coccidioidomycosis (Seabury, 1963). After 2,000 mg of 2,4-D delivered intravenously had no neurologic effects, he received 3,600 mg over two hours. The patient became semistuperous, developed fibrillary movements about his mouth and upper extremities, and showed hyporeflexia on neurologic examination. Within 48 hours after cessation of treatment, these signs reversed. Although the patient died 17 days after this treatment, autopsy examination showed no signs to suggest a specific lesion from the 2,4-D treatment.

Vietnam Veterans Studies

Several of the Vietnam veteran studies have examined other CNS functions besides behavior. In the 1982 baseline examination for the Ranch Hand study (AFHS, 1984), an increased frequency of abnormal Babinski signs was reported in Ranch Hands compared to the control group, but this finding did not reoccur in the 1985 examination (AFHS, 1987). In the 1987 follow-up examination, coordination difficulty and postural tremor reportedly occurred more frequently in Ranch Hands (AFHS, 1990). This clinical finding was reinforced in the serum TCDD analysis of the 1987 examination data; Ranch Hands with elevated serum TCDD levels experienced more coordination difficulties than the comparison group (AFHS, 1991). Serum TCDD was also shown to be positively and significantly associated with the CNS index, a composite score based on coordination, gait, and tremor. Although the Ranch Hand studies are difficult to interpret (see Appendix C), these findings recall the CNS observations of tremor and dystonic postures in the series of railroad cleanup workers reported by Klawans (1987).

A final neurologic effect reported with greater frequency for Vietnam veterans than Vietnam era veterans was hearing loss (CDC, 1988). This observation was interpreted as consistent with exposure to high levels of noise during military service in Vietnam and not specifically related to

herbicide exposure, because the study population was not selected to assess toxic exposure but rather for the Vietnam experience.

Questionnaires on perceived neurologic problems in Vietnam veterans showed more tiredness, headaches, dizziness, ringing in the ears, and complaints of hearing loss compared to a control group of Vietnam era veterans (Rellahan, 1985; Eisen et al., 1991; Decoufle et al., 1992). A survey of 10,846 Vietnam veterans in Iowa (Wendt, 1985) showed that chronic headaches and "nerve/brain" problems were more frequent after Vietnam service than before military experience, but no statistical analysis was performed. This assessment, like the other questionnaire studies discussed in this report, focused on Vietnam experience as opposed to herbicide exposure. None of these cited surveys included neurologic examinations.

In mortality studies, the only neurologic disorder commonly investigated is stroke or vascular disease of the CNS. In the mortality study of Massachusetts veterans (Kogan and Clapp, 1985), the mortality risk for cerebrovascular disease was significantly higher for Vietnam veterans than for non-Vietnam veterans. The 19 Vietnam veteran deaths observed between 1978 and 1983 resulted in a proportionate mortality ratio of 1.6, adjusted for age, (p = .02) when compared with the mortality experience of non-Vietnam veterans. The study was limited to those veterans who applied for a state military service bonus. Information on potential confounders, such as smoking and alcohol use, was not available for study, and herbicide or TCDD exposure was not specifically addressed.

A final area of focus has been the neurologic function of Vietnam veterans' offspring. One study found that children whose fathers reported exposure to Agent Orange in Vietnam scored significantly worse on a variety of sensory integrative function tests. Because of the small number of subjects and the lack of distinction between Vietnam service and specific TCDD exposure, it is not possible to determine whether herbicide exposure was associated with the test scores (Becker, 1982).

Summary of Motor/Coordination Dysfunction

There are no definitive studies to determine whether exposure to dioxin or related herbicides is associated with CNS motor/sensory/coordination problems. However, follow-up of veterans, and, to a lesser extent, environmental observations suggest that motor and coordination difficulties should be assessed further in exposed subjects. Additional longitudinal assessments of motor and coordination problems are warranted in exposed subjects, especially those with high exposure as in the NIOSH cohort studied by Fingerhut and colleagues (1991). Vietnam veterans represent the most systematically evaluated group with chronic TCDD exposure, and the findings in this group suggest that CNS disorders may focus on the subtle

clinical area of coordination and abnormal involuntary movement disorders. Since this area is a specific subspecialty of neurology, future evaluations should involve specialists in this field. Internationally respected scales for movement disorders have been developed and should be used in future studies of such problems, as well as assessments that capture the disability related to any objective findings.

In the past decade—and unrelated specifically to the question of TCDD and the CNS—an increasing concern has developed scientifically over the possible link between parkinsonism and chemicals used as herbicides and pesticides (Semchuk et al., 1992). The most dramatic and well-studied chemical is MPTP, which is a narcotic derivative with a chemical composition resembling paraquat. It has been reported that laboratory workers exposed to the compound have developed parkinsonism (Langston and Ballard, 1983). Parkinsonian syndromes have also been described in association with the fungicide manganous ethylene bisdithiocarbamate (Ferraz et al., 1988) and the grain fumigant consisting of 80 percent carbon tetrachloride and 20 percent carbon disulfide (Peters et al., 1988). Paraquat has been shown to be associated with pathologic damage to the substantia nigra (Grcevic et al., 1977). These data support the concept that some herbicides and pesticides could possibly be associated with parkinsonism. Furthermore, since the laboratory workers had insidious onset of parkinsonism, chronic exposure could be associated with the problem, without a massive accident or evidence of acute intoxication (Langston and Ballard, 1983).

As Vietnam veterans move into the decades when Parkinson's disease becomes more prevalent, attention to the frequency and character of new cases of parkinsonism in exposed versus nonexposed individuals may be highly useful in assessing whether dioxin or herbicide exposure is a risk factor for eventual Parkinson's disease.

Conclusions for Motor/Coordination Dysfunction

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and motor/coordination dysfunction.

Biologic Plausibility

Studies in laboratory animals do not support an association between exposure to TCDD and motor/coordination dysfunction. However, the herbicide MPTP, which does not share strong structural similarities to TCDD, has been associated with the onset of parkinsonism in animals and in humans.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the studies that have been reviewed and inadequate control for important confounders, it is not possible for the committee to quantify the degree of risk likely to be experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

PERIPHERAL NERVOUS SYSTEM DISORDERS

Epidemiologic Studies of Peripheral Nervous System Disorders

Occupational Studies

A large number of reports have suggested that acute or subacute peripheral neuropathies can be associated with occupational exposure to herbicides (Ashe and Suskind, 1950; Baader and Bauer, 1951; Kimmig and Schulz, 1957; Goldstein et al., 1959; Todd, 1962; Berkley and Magee, 1963; Poland et al., 1971; Jirasek et al., 1974; Oliver, 1975; Pazderova-Vejlupkova et al., 1981). Only a very limited number of studies on the PNS provide any control or comparison group data. Since peripheral neuropathies can be induced by common medical and environmental disorders such as diabetes and poor nutrition, especially in alcoholics, the presence of neuropathy in an herbicide-exposed population cannot be attributed necessarily to the herbicide without consideration of these other factors. Rigorously defined and examined comparison groups are therefore especially important in the analysis of peripheral neuropathies. The studies cited below have at least provided some form of comparison group.

Moses and colleagues (1984) studied 226 men identified by union records as occupationally exposed to 2,4,5-T in Nitro, West Virginia. A cohort of 117 subjects with either current evidence or a history of chloracne over the previous 30 years defined the exposed group. The comparison group consisted of 109 factory workers from the same occupational environment, but without chloracne. A neurologic evaluation of a smaller group of 90 volunteers was not specifically described, but the workers with chloracne experienced significantly more symptoms of muscle pains, decreased libido, and erection/ejaculation difficulties than the nonchloracne subjects. Furthermore, 18.3 percent of chloracne subjects, compared to none of the controls, had decreased pin sensation ($p < .01$). The authors did not indicate how many workers had skin lesions at the time of neurologic examination or whether the decreased sensation was in the same distribution as the skin rash and could be explained by hypertrophic skin. This study is further compromised because the analytical and clinical methods were vaguely described,

no EMG examinations were performed, and the groups were not necessarily comparable for other factors known to be associated with peripheral neuropathy, such as alcoholism and diabetes mellitus (which were considered for the study's assessments of liver function). Finally, participation was voluntary, and it is not clear what proportions of the total chloracne and nonchloracne group were represented.

When Suskind and Hertzberg (1984) examined nerve conduction velocities among a larger number of exposed (N = 204) and nonexposed workers (N = 163) from the same West Virginia plant, they found no differences between the two groups. This second study was a long-term follow-up assessment, 30 years after the time of exposure. Exposure to 2,4,5-T during the manufacturing process was determined from occupational histories.

Singer and colleagues (1982) examined 56 plant workers from Arkansas who were exposed to 2,4,5-T and 2,4-D and who had no history of diabetes or neurologic disease. They compared these men to 25 unexposed subjects from completely different work environments—a group of laboratory personnel and a group of brake workers. Alcohol consumption of more than four drinks per day was exclusionary for controls, but not subjects, and it is not clear whether the groups were of comparable ages. EMG was performed to detect peripheral neuropathy, and nerve conduction velocities were found to be significantly slowed in exposed subjects compared to controls.

Sweeney and colleagues (in press) performed the most rigorous evaluation of peripheral chronic neuropathy in a study of chemical workers exposed 15 years earlier to TCDD during the production of TCDD-contaminated chemicals. A referent group of randomly selected unexposed neighborhood comparison subjects, matched on age, sex, and race, was also studied. Serum TCDD levels were assessed and documented to be different in the exposed workers (median = 220 ppt, N = 281) than in the comparison group of unexposed referents (median = 7 ppt, N = 260). Peripheral nerve function was assessed by nerve conduction tests, thermal thresholds, and physical examinations, with strict definitions applied to the term peripheral neuropathy. There were no significant differences in the prevalence of peripheral neuropathy between the two groups. Serum TCDD levels were not associated with the incidence of peripheral neuropathy.

Environmental Studies

Major environmental disasters in Seveso, Italy, and Missouri have served as sources for study of the neurologic effects of TCDD exposure. Boeri and colleagues (1978) conducted peripheral nerve examinations seven months after the accidental explosion in Seveso and reported descriptive differences between 470 volunteer subjects in zone A (high-exposure group) and 152

volunteer residents from the town of Bovisio in zone R (low-exposure group). Cranial and peripheral nerve symptoms and signs were generally more prevalent among the highly exposed group. No statistical analyses were performed, except on EMG examinations, and these data showed no significant differences.

As a follow-up to the above screening, Filippini and colleagues (1981) compared 308 Seveso residents with 305 nonexposed residents from nearby towns. The authors found no increased risk of peripheral neuropathy among the exposed residents. However, within the subgroup of exposed subjects who showed clinical signs of significant exposure (chloracne or elevated hepatic enzymes) the risk ratio was 2.8 (CI 1.2-6.5). Similarly, for Seveso residents with known risk factors for peripheral neuropathy (alcoholism, diabetes, and inflammatory diseases) an elevated risk ratio was observed (2.6, CI 1.2-5.6).

The above-mentioned assessments of subacute morbidity have been complemented by reports of chronic follow-up. Twenty months after the explosion, Gilioli and colleagues (1979) examined 35 laboratory technicians working in the area of heavy TCDD contamination and 35 controls matched for age and sex. Electromyographic examinations resulted in statistically significant differences in some variables; the values for the exposed cases, however, were always within the range of published norms.

Focusing specifically on a subgroup of 193 subjects (88 percent were under 15 years of age) with chloracne, Assennato and colleagues (1989) performed follow-up electrophysiologic examinations in 1982-1983, 1983-1984, and 1985. The comparison group (individually matched by age and sex) was randomly selected from a nearby unexposed town. They did not observe an increased prevalence of abnormal findings in the exposed group at any point in the follow-up study, suggesting that whatever effects might have been seen after the Seveso accident were no longer distinctive between the two groups. Similarly, Barbieri and colleagues (1988) found that six years after the Seveso accident, there was no increased prevalence of peripheral neuropathy among 152 residents with chloracne or a history of lesions, compared to 123 age- and sex-matched unexposed control subjects.

The other major environmental study involved a self-selected group of Missouri residents living in areas sprayed with TCDD (Stehr et al., 1986; Webb et al., 1987). Exposure was based on TCDD soil measurements. A pilot study examined 68 subjects at high risk of exposure with potential exposure to 20-100 ppb of TCDD for at least two years, or levels greater than 100 ppb for at least six months. The low-risk comparison group was formed of 36 volunteers with little or no reported history of exposure to TCDD. No statistically significant differences in neurologic dysfunction were documented in the high-exposure patients, although the percentage of patients with abnormalities was higher in every neurologic test for that

group. Hoffman and colleagues (1986) found no clinical evidence for PNS damage in their study of 154 residents of the Quail Run mobile home park, one of the Missouri sites with extremely high TCDD levels. A group of 155 individuals residing for at least six months in another mobile home park with no TCDD contaminants in the soil formed the comparison group.

Vietnam Veterans Studies

The Air Force Health Study (1984) compared 1,208 Air Force Ranch Hands to a group of 1,238 Air Force personnel assigned to cargo missions in Southeast Asia. The groups were matched for age, race, and occupational codes. Assessments included neurologic symptom evaluation, physical examination, and nerve conduction velocity tests. No neurological differences were observed at follow-up between Ranch Hands and the comparison population when controlling for diabetes and alcohol consumption (AFHS, 1984; 1987). Peripheral and cranial nerve dysfunction was strongly correlated with alcohol consumption and serum glucose levels. There was no interaction between the records-based exposure index and peripheral nerve deficits in the earlier reports. The serum TCDD analysis did not produce evidence of an association between peripheral neuropathy and TCDD exposure (AFHS, 1991). The assessments, however, were restricted to questionnaires and physical examination data, and no electrophysiologic evaluations were performed.

To assess whether Vietnam service per se increased the risk of chronic peripheral neuropathy, the CDC (1988) evaluated 2,490 male Vietnam veterans serving one term of enlistment with a minimum of 16 weeks of active Vietnam duty and 1,972 non-Vietnam veterans. Symptom history, neurologic examination, nerve conduction velocity, thermal sensitivity, hearing acuity, and visual acuity were monitored. There were more symptoms of peripheral neuropathy reported among Vietnam veterans than among non-Vietnam veterans, but there was no increased objective evidence of peripheral neuropathy (CDC, 1988).

Summary of Peripheral Nervous System Disorders

Although many case reports suggest that an acute or subacute peripheral neuropathy can develop with exposure to TCDD and related chemicals, reports with comparison groups do not offer clear evidence that TCDD exposure is associated with chronic peripheral neuropathy. The most rigorously conducted studies argue against a relationship between TCDD or herbicides and chronic neuropathy.

As a group, the studies concerning peripheral neuropathy have been conducted with highly varying methodologies and have lacked uniformity of operational definitions of neuropathy. They have not applied consistent

methods to define a comparison population or to determine exposure or clinical deficits. Timing of follow-up may be important since many, but not all, reports that find neuropathy were short-term assessments (months after exposure). Careful definition of neuropathy and standardization of protocols will be essential to future evaluations.

Conclusions for Peripheral Nervous System Disorders

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and disorders of the peripheral nervous system.

Biologic Plausibility

Studies in laboratory animals do not support an association between exposure to TCDD or herbicides and disorders of the peripheral nervous system.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the studies that have been reviewed and inadequate control for important confounders, it is not possible for the committee to quantify the degree of risk likely to be experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

NOTE

* The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

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11

Other Health Effects

A variety of health outcomes are evaluated in separate sections in this chapter. Many of these health outcomes have not been addressed as thoroughly in the epidemiologic literature as the health outcomes previously described in Chapters 8-10. The sections in this chapter of other health outcomes include chloracne, porphyria cutanea tarda, other metabolic and digestive disorders (diabetes mellitus, alterations in hepatic enzymes, lipid abnormalities, and gastrointestinal ulcers), immune system disorders (immune modulation, autoimmunity), and a number of circulatory and respiratory disorders.

CHLORACNE

After traumatic injuries, skin disorders are among the most common health problems encountered in combat. The tropical environment and living conditions in Vietnam resulted in a variety of skin conditions ranging from bacterial and fungal infections to a condition known as "tropical acne" (Odom, 1993). However, the only dermatologic disorder consistently reported to be associated with Agent Orange or its components, including the contaminant, TCDD (2,3,7,8-tetrachlorodibenzo*p*-dioxin), is chloracne. Therefore, this discussion will focus on chloracne and its link to TCDD.

Chloracne is characterized by persistent comedones, keratin cysts, and inflamed papules. Lesions are often associated with hyperpigmentation and may result in a characteristic scarring pattern. The maculopapular rash of chloracne characteristically occurs in a facial butterfly distribution,

frequently associated with back, chest, or periorbital lesions (Crow and Puhvel, 1991).

Although severe cases of juvenile acne may mimic adult chloracne in appearance and distribution, it is generally agreed that exposure to chlorinated aromatic compounds causes the overwhelming preponderance of chloracne cases in adults (Crow and Puhvel, 1991). Chloracne was first described in relation to occupational exposure among chemical industry workers by Von Vettman in 1887 (AFHS, 1991b) and was first linked to the specific chemical trichlorophenol in 1957 (Crow and Puhvel, 1991). A large number of chloracne cases have been reported in industrial workers throughout this century. Among the numerous industrial chemicals known to cause chloracne, the most potent appears to be TCDD. However, as noted later in this discussion, individual host factors appear to play an important role in determining disease expression. Even at relatively high doses, not all exposed individuals develop chloracne, whereas others with similar or lower exposure demonstrate the condition.

The natural history of chloracne is quite variable. Longitudinal studies of exposed cohorts suggest that the lesions typically regress and heal over time. However, historical reports indicate that a chronic form of the disease can persist up to 30 years after an exposure (Suskind and Hertzberg, 1984). As with many dermatologic conditions, chloracne can reasonably be suspected on the basis of a careful medical history or appropriate questionnaire information. A key element in diagnosis is the characteristic anatomic distribution. Because acne is such a common dermatologic condition, in any analysis attempting to link acne or chloracne with an environmental or occupational exposure, it is critical that adequate attention be paid to the clinical characteristics, time of onset, and distribution of lesions, as well as careful comparison with an appropriate control group. Definitive diagnosis may require histologic confirmation from a biopsy specimen.

The toxicology of TCDD in animals, animal models, and humans has been well described; the major issue of current concern is the precise dose-response relationship between TCDD exposure by various routes (dermal, inhalation, ingestion) and the occurrence of chloracne. Recent reports have suggested a genetic basis for susceptibility to chloracne in animals (see Chapter 4). If the genetic and biochemical basis for this susceptibility can be defined in humans, it may lead to new tests to detect susceptible sub-populations among exposed individuals.

Chloracne can be viewed both as a toxic outcome from exposure to TCDD and as a potential clinical marker of TCDD exposure. It is the latter that has generated the most controversy. For the purposes of this section, the primary focus is the linkage of chloracne to TCDD exposure. Dose-response relationships between TCDD exposure and chloracne are addressed briefly. The inadequacies of chloracne as a human biomarker of dioxin

exposure are discussed in more detail in Chapter 6. A major unresolved issue is whether TCDD exposure below the level required to cause chloracne may cause other adverse health consequences such as cancer.

Epidemiologic Studies of Chloracne

Occupational Studies

Several reports have detailed the mortality experience and incidence of chloracne among a group of 2,192 employees of Dow Chemical, Michigan Division, who were potentially exposed to TCDD through the manufacture of higher chlorinated phenols beginning in the 1930s (Cook et al., 1980; Bond et al., 1987; Bond et al., 1989a,b). Nearly 16 percent of 2,072 workers whose medical records were reviewed were identified as having chloracne based on clinical criteria (Bond et al., 1989a). Approximate historical exposure categories were reconstructed from employment records in production areas where TCDD exposure was considered most likely. Unfortunately, accurate quantitative industrial hygiene or other exposure measures for TCDD were not available. Even with this limitation, however, a plausible dose-response relationship was identified between probable exposure to TCDD and the relative risk (RR) for chloracne. Relative risk estimates were as high as 5.5, compared to a referent nonexposed worker population. Younger age at time of employment and duration of exposure were also significant risk factors.

Another large occupational cohort reported from the United States involved workers at the Monsanto Company plant in Nitro, West Virginia (Moses et al., 1984; Suskind and Hertzberg, 1984). Exposures at this plant included both accidental exposures following a trichlorophenol (TCP) process accident in 1949 and exposures occurring during regular operations concerning the production of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) from 1948 to 1969 (Suskind and Hertzbert, 1984). Like the Dow cohort, quantitative exposure data were not reported. However, among 204 workers considered exposed based on proximity to an accident or work history, more than 53 percent had clinical evidence of persistent chloracne (Suskind and Hertzberg, 1984). Interestingly, a self-reported history of chloracne was recorded among 86 percent of the exposed workers versus none of the clearly nonexposed workers (Suskind and Hertzberg, 1984). Not all reports were substantiated by company or medical record review. Age at exposure did not appear to influence the rate of chloracne. No attempt was made to define a dose-response relationship.

Poland and colleagues (1971) described a study of 73 male employees in a 2,4,5-T manufacturing facility with potential exposure to TCDD in a follow-up to an earlier report of Bleiberg and colleagues (1964) on the same

plant. Chloracne was identified in 18 percent of these workers (Poland et al., 1971). Although a series of clinical and laboratory evaluations was carried out on this small cohort, no exposure data or estimates were reported. Finally, Fitzgerald and colleagues (1989) reported a medical follow-up of 377 individuals potentially exposed to TCDD from an electrical transformer fire in a Binghamton, New York, office building. Although a variety of complaints were reported in this population, no individuals were diagnosed with chloracne.

An industrial accident in Great Britain resulted in the development of chloracne in 79 workers (May, 1973). Exposure information was unavailable and the total population of potentially exposed was not identified. Individuals with documented chloracne served as a presumably highly exposed cohort for subsequent mortality analyses. A 10 year follow-up of these workers was reported by May (1982). Three subject groups were formed: group A, 31 employees with no dioxin exposure; group B, 54 employees with possible dioxin exposure; and group C, 41 dioxin-exposed workers with chloracne. Medical employment histories were obtained by self-report. In group C, 22 of 41 workers still had mild forms of chloracne at follow-up.

Workers involved in the manufacture of 2,4,5-T were examined in a mortality study 20 to 30 years after the initial exposure (Suskind, 1985). Included in the study were 204 exposed workers, 163 nonexposed workers, and 51 workers of questionable exposure. Of the 204 exposed workers, 86 percent developed chloracne at some time after exposure and 53 percent still had chloracne on examination 20 to 30 years postexposure. No workers in the other groups developed chloracne.

A number of reports of occupational exposure to TCDD have also come from sites in Europe. Pazderova-Vejlupkova and colleagues (1981) reported a 10 year follow-up of 55 individuals exposed to TCDD in a 2,4,5-T manufacturing facility in Czechoslovakia. Study subjects were those available from a cohort of 80 workers who became ill following the occupational exposure (total worker population was approximately 400). Of the 55 symptomatic workers studied, 95 percent were reported to have chloracne of varying severity. A number of other clinical and laboratory abnormalities were reported, but no useful exposure data or estimates were included. Zober and coworkers (1990) reported a mortality follow-up study of several cohorts of workers exposed to TCDD during an accident in a plant in Germany. Three cohorts were identified, each with differing probable exposures based on the amount and reliability of exposure information, but no quantitative exposure estimates were available. Chloracne was found in all 69 workers from one cohort, 17 of 84 workers in a second cohort, and 28 of 94 workers in a third cohort.

The results of a surveillance program of 200 employees in Germany with high occupational exposure to TCDD were reported by Beck and colleagues

(1989). Adipose tissue was obtained from 45 exposed workers and from 21 Hamburg residents in a referent group. In the referent group, TCDD levels ranged between 2 and 20 parts per trillion (ppt) in fat, while there were values in the exposed group reaching 2,252 ppt. When the association between TCDD concentration and the development of chloracne was examined, there were workers with extremely high concentrations who showed no evidence of chloracne, whereas other workers with very low concentrations had chloracne. The authors suggested that skin contact as the main route of absorption may explain the individual variability in the development of chloracne and the lack of a linear association between fat levels and risk of chloracne. Thus, the appearance of chloracne does not seem to be a sensitive indicator of systemic TCDD dose. Several other European studies reported low-level exposure from pesticide application or industrial exposures (Riihimaki et al., 1983; Van Houdt et al., 1983; Jennings et al., 1988). Accurate exposure information was unavailable for all studies, and chloracne was rarely observed.

Environmental Studies

The best-documented environmental exposure to TCDD occurred near a chemical factory in Seveso, Italy, in 1976. Numerous epidemiologic studies have analyzed population groups in the surrounding region to define the potential adverse health impact of this disaster. Potential exposure zones at Seveso were identified using TCDD levels in soil samples taken immediately after the accident (Bisanti et al., 1980). Three exposure zones were established: zone A was the closest to the factory and most polluted; zone B was located southeast of zone A and slightly contaminated; and zone R, which surrounded both zones A and B, was found to be the least contaminated. Within the first three months after the accident, 50 cases of chloracne (34 among children aged 0-14 years) were identified; 46 cases occurred in zone A residents, no cases were reported in zone B, one case was identified in zone R, and 3 cases occurred in nearby cities. A total of 187 cases of chloracne were ultimately identified, including 164 children under the age of 15 and 23 adults. As with any environmental or ecological study, precise exposure data for individual subjects were not available. Caramaschi and colleagues (1981) investigated the distribution of 164 cases of chloracne among children following the accident. Of 146 of the original 164 children identified with chloracne, the highest frequency of chloracne was in zone A. In the majority of cases, the disease resolved within seven years after the incident (Assennato et al., 1989a).

In a follow-up study of the residents of Seveso nine years after the accident, levels in serum taken at the time of the accident were analyzed (Mocarelli et al., 1991). Of the 30 samples, 10 were taken from residents of

zone A with chloracne, and 10 with no evidence of chloracne, and 10 from residents of the least contaminated zone. TCDD levels as high as 56,000 ppt were observed—the highest level ever reported in humans. All participants who had TCDD levels greater than 12,000 ppt ($N = 7$) had chloracne, although some with lower levels also developed chloracne. Children developed chloracne more frequently than adults, but this was not related to serum TCDD levels. The authors concluded that there had been minimal, if any, adverse health effects of TCDD observed in this population to date, with the exception of chloracne; and that chloracne was an indicator, but neither a sensitive nor a dose related indicator of TCDD exposure. No evidence of chloracne was found among cleanup workers following the Seveso accident (Assennato et al., 1989b).

Although high levels of soil contamination were identified in several residential areas in Times Beach, Missouri, no cases of chloracne were identified in any of the reported pilot studies or epidemiologic surveys (Webb, 1984; Stehr et al., 1986; Webb et al., 1987).

Vietnam Veterans Studies

As indicated in other sections of this report, the anticipated exposure level in Vietnam veterans was substantially lower than that observed in occupational studies and in environmental disasters such as Seveso. Therefore, chloracne might be expected to be a relatively uncommon outcome in these studies. The Iowa Agent Orange Survey of 10,846 Iowa veterans who responded to a questionnaire, concluded from preliminary data that no definitive evidence could be found to link Agent Orange exposure and long-term adverse health effects, including chloracne (Wendt, 1985). However, this study was performed long after the potential exposure occurred, and methodologic considerations seriously limited the ability, in many instances, to identify chloracne even if it had existed. Of note, other studies did identify an apparent excess of acne or chloracne potentially attributed to TCDD in Vietnam. In the Centers for Disease Control Vietnam Experience Study (CDC, 1988), chloracne was reported more often by Vietnam veterans compared to Vietnam era veterans who were interviewed (OR = 3.9). Chloracne was also more often reported by Vietnam veterans compared to Vietnam era veterans who were examined, 1.9 percent and 0.3 percent, respectively (OR = 7.3). Quantitative exposure indices to TCDD and dose-response characteristics were not identified. Using an innovative exposure estimation algorithm, Stellman and colleagues (1988) reported a potential dose-response relationship between Agent Orange exposure (low, medium, high) and "adult acne" among Vietnam veterans. All health information was determined by self-administered questionnaire, and no attempt was made to validate these conditions by medical history or physical examination.

A significant excess in acne was reported among Air Force Ranch Hand personnel potentially exposed to Agent Orange, compared to a nonexposed group (Wolfe et al., 1990). The odds ratio for acne appearing after Vietnam experience was 1.6 (CI 1.1-2.1) when comparing Ranch Hands to the nonexposed group. Among the 84 percent of Ranch Hands and 75 percent of comparison subjects who underwent a medical physical evaluation nearly 20 years after the potential herbicide exposure, no individuals from a total of 995 Ranch Hands and 1,299 comparisons examined, were observed to have lesions of chloracne or postinflammatory scars suggesting prior chloracne. Attempts to document medical records or physical examination results more proximal to the Vietnam exposure period were not discussed. In a follow-up report on serum TCDD analysis of the 1987 examination results for the Air Force Ranch Hand study, no cases of chloracne were identified, and no dermatologic endpoints were consistently related to the current body burden of TCDD (AFHS, 1991b).

Summary for Chloracne

In summary, chloracne has been linked to TCDD exposure in numerous epidemiologic studies of occupationally and environmentally exposed populations. The data on Vietnam veterans potentially exposed to Agent Orange and other herbicides are less convincing.

Conclusions for Chloracne

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and chloracne.

Biologic Plausibility

The formation of chloracne lesions after administration of TCDD is observed in some species of laboratory animals. Similar observations have not been reported for the herbicides.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to

that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam. Because TCDD-associated chloracne becomes evident shortly after exposure, there is no risk of new cases occurring long after service in Vietnam.

PORPHYRIA CUTANEA TARDA

Porphyria cutanea tarda (PCT) is an uncommon disorder of porphyrin metabolism manifested in-patients by thinning and blistering of the skin in sun-exposed areas, in addition to hyperpigmentation (excess pigment in skin) and hypertrichosis (excess hair growth) (Muhlbauer and Pathak, 1979; Grossman and Poh-Fitzpatrick, 1986). The disease is caused by a hereditary or acquired deficiency in uroporphyrinogen decarboxylase (UROD), a cytoplasmic enzyme in the pathway of hemoglobin synthesis (Sweeney, 1986). In the hereditary form of the disease, no precipitating exposure is necessary for the appearance of excess uroporphyrin and coproporphyrin in the urine and the development of clinical symptoms.

The acquired form of the disease appears in association with excessive iron intake, alcoholism, and exposure to hexachlorobenzene (Cam and Nigogosyan, 1963; Strik and Doss, 1978; Axelson, 1986). In evaluating associations between herbicides and PCT, the relationship to hereditary and acquired development of the disease should be considered. It is postulated that heterozygotes for the disease with depressed levels of UROD are at increased risk for PCT if external exposures to agents that lower UROD are encountered. For example, abnormalities in urinary porphyrin excretion may be found in predisposed heterozygotes who do not have clinical symptoms but are susceptible to induction upon exposure to specific agents (Goerz and Merk, 1985). This induced form of porphyria, regardless of cause, is not associated with neuropsychiatric manifestations or with abdominal pain. Both hereditary and acquired PCT can be treated successfully by phlebotomy or restriction of alcohol intake.

In cell culture and in rodents (mice and rats), TCDD causes a toxic porphyria resembling PCT in humans (De Verneuil et al., 1983; Cantoni et al., 1984; Smith and De Matteis, 1990). TCDD inhibits UROD, which is universally deficient in the liver tissue of patients with human PCT. Immunoquantitation of hepatic UROD in animals when UROD catalytic activity is rendered deficient by TCDD, is normal, suggesting strongly that the compound binds the enzyme protein and inhibits rather than destroys the enzyme. TCDD induces heme biosynthesis by the liver, and the reduced UROD activity in this situation probably leads to the porphyria in animals.

There are several case reports suggesting the appearance of PCT in

chemical workers exposed to TCDD. However, in most of the reported cases, multiple chemicals were involved in the workplace, complicating interpretation of an association between TCDD exposure and the occurrence of PCT (Bleiberg et al., 1964; Jirasek et al., 1974). Follow-up after removal of workers from the contaminated environment showed a resolution of abnormal urinary porphyrin excretion.

Epidemiologic Studies of Porphyria Cutanea Tarda

Occupational Studies

Bleiberg and colleagues (1964) reported the appearance of increased urinary uroporphyrin excretion in 11 of 29 workers in a chemical factory manufacturing 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T. Three of these individuals had some clinical evidence of PCT. Chloracne was diagnosed in the same plant but did not correlate with porphyrin abnormalities. In a follow-up of this same manufacturing facility six years later, Poland and colleagues (1971) restudied 73 workers and found no abnormalities in urinary porphyrin excretion or in the appearance of clinical PCT. Moreover, restudy of some of the original workers from 1964 showed normal porphyrin excretion. Although Bleiberg's chemical analysis of porphyrin excretion has been questioned, it seems equally plausible that markedly reduced contamination of herbicides by TCDD in this manufacturing plant accounted for the disappearance of abnormal uroporphyrin and coproporphyrin excretion and of clinical PCT. It is noteworthy that PCT occurred in a different group of workers from those with chloracne, suggesting that these are separable clinical manifestations of chemical exposure, most likely in specifically predisposed individuals.

In a report at the Twelfth International Symposium on Chlorinated Dioxins, Calvert and colleagues (1992b) reported no difference in porphyrinuria or the occurrence of PCT between 281 workers who were involved in the production of TCP and exposed to TCDD at least 15 years earlier, and 260 unexposed workers who resided in the same community as the workers. In addition, serum TCDD levels were not associated with uroporphyrin or coproporphyrin levels. However, careful urinary porphyrin studies were not conducted during the period of acute exposure.

Environmental Studies

PCT has not been reported in individuals exposed to TCDD as a result of the 1976 chemical plant explosion in Seveso, Italy, unless a hereditary UROD deficiency existed. Doss and colleagues (1984) reported that exposure to TCDD triggered the manifestation of clinical PCT in a brother and

sister with the underlying genetic UROD abnormality. During the 1976 Seveso accident, it was reported that coproporphyrinuria without clinical symptoms occurred in 16 of 30 individuals (Caputo, 1989). In 1977, 60 Seveso residents were tested for elevated porphyrins: none of the 60 residents developed PCT; however, 13 exhibited secondary coproporphyrinuria. In three of the 13 persons with secondary coproporphyrinuria, elevated porphyrin levels persisted upon retesting in 1980, but were attributed by the authors to liver damage and alcohol consumption; elevated levels returned to normal in the remaining 10 cases (Doss et al., 1984).

In a study of the Quail Run mobile home park in Missouri, 154 residents who were exposed to dioxin as a result of the spraying of waste oil that was contaminated with TCDD were compared to 155 individuals who lived in an unexposed area with no detectable levels of TCDD in the soil (Hoffman et al., 1986; Stehr-Green et al., 1987). Mean levels of urinary uroporphyrins were elevated among the exposed group, although there were no cases of clinical PCT diagnosed in either exposed or unexposed individuals.

Vietnam Veterans Studies

The baseline study of the U.S. Air Force Ranch Hands (1984) showed no difference in uroporphyrin or coproporphyrin levels in the urine between Ranch Hands and a control group of Air Force personnel who were not occupationally exposed to herbicides. There were no indications of the clinical appearance of PCT in Ranch Hands. In the first follow-up study (AFHS, 1987), two porphyrin analyses were not consistent with findings from the earlier baseline survey. Mean uroporphyrin levels were greater for the comparisons (17.9 mg/24 hours) than for Ranch Hands (16.9 mg/24 hours), whereas mean coproporphyrin levels were higher for Ranch Hands (119.1 mg/24 hours) than the comparison group (115.6 mg/24 hours). The clinical significance of such small changes in these mean levels is uncertain.

Summary for PCT

The occurrence of clinical PCT is rare and may be influenced by genetic predisposition of individuals demonstrating low enzyme levels of protoporphyrinogen decarboxylase. The cases reported have occurred relatively shortly after exposure to specific chemicals, including TCDD, and improve after removal of the agent. Simultaneous exposure to alcohol and other chemicals, such as hexachlorobenzene, probably increases the risk and severity of PCT. Abnormal porphyrin excretion without clinical illness may occur more commonly than clinical evidence of PCT.

There is no suggestion of an increase in PCT in studies of Vietnam

veterans or the Ranch Hand group, possibly because of comparatively low dioxin exposure even in Ranch Hand studies, or a fortuitous absence of genetically predisposed individuals who could develop PCT after TCDD exposure. Further studies of PCT incidence in Vietnam veterans would not be called for since the biologic and clinical evidence indicate that the rare appearance of PCT occurs soon after heavy TCDD exposure and improves with time. Moreover, the association of PCT with alcoholism makes it difficult to interpret studies of TCDD and PCT that do not simultaneously assess alcohol consumption.

It is possible that a rare individual with asymptomatic hereditary PCT was not worsened by exposure to TCDD. Whether such individuals were present in the military cannot be determined, although patients with overt symptomatic disease would likely be excluded from military service. In any individual case, evaluation of potential exposure to chemicals other than TCDD, such as ethanol, estrogens, or hexachlorobenzene, would be necessary to attribute abnormalities to dioxin or herbicide exposure specifically.

The epidemiologic evidence associating PCT and TCDD is sparse because PCT is rare and because of methodological problems. However, case studies and animal studies show that PCT may be associated with TCDD exposure in genetically predisposed individuals.

Conclusions for PCT

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and PCT in genetically susceptible individuals.

Biologic Plausibility

There is some evidence that TCDD administration can be associated with porphyrin abnormalities in laboratory animals, although PCT has not been reported. Porphyria has not been reported in animals exposed to herbicides.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important

confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam. Because TCDD-associated PCT becomes evident shortly after exposure, there is no risk of new cases occurring long after service in Vietnam.

OTHER METABOLIC AND DIGESTIVE DISORDERS

Several other metabolic and digestive disorders (diabetes mellitus, hepatic enzymes, lipid abnormalities, and gastrointestinal ulcers) have been reported in the scientific literature as possibly associated with TCDD or herbicide exposure. Assessment of these disorders in association with exposure to herbicides and TCDD involves the medical evaluation of a wide array of clinical signs and symptoms, laboratory parameters, and other diagnostic tools. These diagnostic criteria and their use in the clinical evaluation of these four health parameters are described below.

Diabetes Mellitus

Diabetes mellitus is a syndrome of disordered metabolism and hyperglycemia due to an absolute or relative deficiency of insulin secretion, a reduction in its biologic effectiveness, or both. There are two major types of diabetes: type I (insulin-dependent) and type II (non-insulin dependent). Type I diabetes occurs most commonly among juveniles but occasionally among adults, whereas type II diabetes occurs predominantly in adults and only occasionally in juveniles. More than 90 percent of the estimated 7 million diabetics in the United States are classified as type II diabetics (Karam, 1992). Many of these patients initially exhibit few or no symptoms, although polyuria (increased urination) and polydipsia (excessive thirst) may be present.

Diabetes is associated with high levels of serum glucose. Plasma glucose levels in excess of 140 mg/dl after an overnight fast are generally suggestive of an abnormal glucose tolerance, a prerequisite for the diagnosis of diabetes. The presence of obesity or a strongly positive family history for mild diabetes suggests a high risk for the development of type II diabetes.

A 1981 study suggesting a potential association between exposure to TCDD and the risk of diabetes in humans has prompted further investigation (Pazderova-Vejlupkova et al., 1981). There have been no reported animal studies suggesting that TCDD or other herbicides are associated with changes resembling human diabetes. There is also no biological reason to suspect these agents as a cause of diabetes.

Epidemiologic Studies of Diabetes Mellitus

Occupational and Environmental Studies

Pazderova-Vejlupkova and colleagues (1981) reported an increased number of abnormal glucose tolerance tests in a 10 year follow-up of 55 workers involved in the production of TCP between 1965 and 1968 who were also exposed to TCDD. Two-fifths of the workers exhibited a pathological change in glucose tolerance tests; one-fifth exhibited a diabetic glucose tolerance test, and another one-fifth had a flat glucose tolerance test.

Sweeney and colleagues (in press) reported briefly on serum glucose and TCDD levels in 281 exposed workers at a TCP production plant and 260 nonexposed neighborhood referents at the Twelfth International Symposium on Chlorinated Dioxins. These findings suggest that serum TCDD levels may be positively and significantly related to fasting serum glucose levels and to an increased risk of diabetes; however the authors also note the strong confounding effects of age and body mass index in interpreting these data.

Two other studies have not found evidence of an increased risk of diabetes or glucose intolerance with TCDD exposure (Moses et al., 1984; Suskind and Hertzberg, 1984). Other studies that have examined mortality among individuals occupationally and environmentally exposed to TCDD have not found a significantly increased risk of death from diabetes (Bertazzi et al., 1989a; Cook et al., 1987; Henneberger et al., 1989).

Vietnam Veterans Studies

In recent studies of Air Force Ranch Hand veterans, fasting glucose and two-hour postprandial glucose levels were measured in 930 Ranch Hands and 1,198 controls (AFHS, 1991b). A significant relationship between elevated levels of blood glucose and TCDD serum levels was observed. Although the correlation was statistically significant, the glucose values were not outside the normal range.

An increased risk of diabetes was noted among Ranch Hands with the highest serum TCDD levels in reference to the comparison group. Among Ranch Hand veterans who developed diabetes, the data suggest an earlier onset of the disease compared to controls and a possible greater severity of the disease in the Ranch Hand group. The problem of interpreting the observed association in epidemiologic studies between serum TCDD and glucose levels is complicated by the role of body fat/obesity as both a risk factor for diabetes and a major determinant of the storage and metabolism of TCDD in the body (see Chapter 6, and the example below in the section on lipid abnormalities). Therefore, this confounding possibility would need

to be excluded before the data are accepted as indicating an association between serum TCDD levels and diabetes. In the absence of reliable data and statistical models for the metabolism of TCDD in humans, however, even if current measurements of body fat or other measures of obesity are available, considerable uncertainty will remain in the interpretation of associations seen in epidemiologic studies carried out many years after exposure.

Summary for Diabetes Mellitus

Limited information suggests the possibility of increased glucose intolerance and diabetes in chemical production workers and Ranch Hand veterans exposed to TCDD, but the data are inconclusive. Additional information on the pharmacokinetics of dioxin metabolism, particularly with regard to total body fat, is necessary in order to interpret epidemiologic studies indicating an association between TCDD exposure and serum glucose levels.

Hepatic Enzymes

Increases in the serum activity of certain hepatic enzymes, including aspartate aminotransferase (AST or SGOT), and alanine aminotransferase (ALT or SGPT), as well as gamma-glutamyltransferase (GGT), *d*-glucaric acid, and others, are commonly noted in many kinds of liver disorders. The relative sensitivity and specificity of these enzymes for liver disease vary, and several different tests may be required to suggest a diagnosis. The only regularly reported abnormality in liver function associated with TCDD exposure in humans is elevation in GGT. Estimates of the serum activity of this enzyme provide a sensitive indicator of alcohol and drug hepatotoxicity, infiltrative lesions of the liver, parenchymal liver disease, and biliary tract obstruction (Berkow and Fletcher, 1987). Elevations are noted with many chemical and drug exposures without evidence of liver injury. The confounding effects of alcohol ingestion (frequently associated with increased GGT) make interpretation of changes in GGT in exposed individuals difficult (Calvert et al., 1992a). Moreover, elevation in GGT may be considered a normal biologic adaptation to chemical, drug, or hormone exposure.

In animal species that exhibit sensitivity to TCDD, the liver represents one of the primary metabolic organs; studies show that TCDD is transported to the liver where it is stored and metabolized (Piper et al., 1973; Lakshman et al., 1986). TCDD is metabolized by enzymes in the liver to form derivatives that can dissolve in water and therefore be more easily eliminated from the body than TCDD itself, which is water insoluble. Changes in hepatic enzyme levels after TCDD exposure in animals have been observed although there is considerable variation among species (see Chapter 4).

Epidemiologic Studies of Hepatic Enzymes

Occupational and Environmental Studies

There are several reports of changes in liver enzymes among workers occupationally exposed to TCDD and in residents of Seveso, Italy. Elevated levels of GGT have been reported in industrial accidents and in studies of chemical workers. May (1982) observed increased GGT levels in 41 workers with chloracne examined 10 years after exposure as a result of a TCP accident in comparison to a group of 31 unexposed workers. Elevated GGT levels were also reported among 105 workers with chloracne exposed to TCDD in a factory explosion in Nitro, West Virginia, compared to 101 workers without chloracne (Moses et al., 1984).

In an assessment of hepatic and gastrointestinal effects among the National Institute for Occupational Safety and Health (NIOSH) cohort of 281 workers involved in the manufacture of TCP and 260 unexposed comparisons, Calvert and colleagues (1992a) noted significantly higher mean GGT levels for workers compared to the unexposed referent group (p = .03, means not provided). In addition, workers had a significantly elevated risk for out-of-range GGT levels compared to the referents (OR = 2.3, CI 1.2-4.4).

Mocarelli and colleagues (1986) observed alterations in serum GGT in a series of laboratory tests conducted from 1976 to 1982 among children who were aged 6 to 10 years at the time of the industrial accident in Seveso, Italy. A slight increase in GGT levels in exposed boys who lived in the area of highest TCDD contamination (zone A) compared to those who lived in the control area was noted after the accident, although the values were within reference limits and declined over time. Caramaschi and colleagues (1981) reported more abnormal GGT levels among children with chloracne in Seveso who presented themselves to clinics before July 1977, compared to children without chloracne. The degree of severity of chloracne was positively correlated with soil concentrations of TCDD at the sampling point of each child's residence.

A medical evaluation of 41 individuals exposed to TCDD-contaminated waste oils in Times Beach, Missouri, who had participated in a study of TCDD levels in adipose tissue found no association between adipose tissue TCDD levels and GGT levels or alterations in any other liver enzymes (Webb et al., 1989).

Slightly abnormal levels of alanine aminotransferase (ALT) were observed by Mocarelli and colleagues (1986) among male children in Seveso compared to nonexposed male children, although there was no clinical evidence of liver disease and the observed abnormalities disappeared over time. No abnormal findings in ALT levels were observed among the NIOSH cohort

of TCP production workers (Calvert et al., 1992a). Also, Hoffman and colleagues (1986) found no difference in ALT levels between exposed residents at Quail Run mobile home park and individuals not exposed, although when duration of residence in the mobile home park was used as a surrogate of TCDD exposure, it was found to be positively and significantly associated with serum ALT levels.

The increase in urinary *d*-glucaric acid observed in animal studies may be another manifestation of biochemical induction of liver enzymes (Lucier et al., 1986), although reports of changes in *d*-glucaric acid excretion after human exposure to TCDD are inconsistent. Elevated levels have been observed among chemical production workers and among residents of Seveso (May, 1982; Martin, 1984; Ideo et al., 1985). In 1978, two years after the Seveso accident, *d*-glucaric acid excretion was higher among Seveso residents than those from a neighboring community. At all ages, exposed subjects showed higher urinary *d*-glucaric acid levels than controls. Until 1979, Seveso area adults and children showed higher levels of *d*-glucaric acid than controls (Ideo et al, 1985). Among children with chloracne, Ideo and colleagues (1982) found significantly higher levels of *d*-glucaric acid as compared to children without chloracne from the same exposure zone. Elevations in urinary *d*-glucaric acid were not associated with TCDD exposure among residents of Quail Run mobile home park (Hoffman et al., 1986) or workers involved in TCP production (Calvert et al., 1992a).

Vietnam Veterans Studies

Serum TCDD levels were found to be positively and significantly associated with elevated GGT levels in the 1991 Ranch Hand serum TCDD analysis (AFHS, 1991b). Increased levels of ALT were also reported in association with higher TCDD levels, although there was no reported association between serum TCDD and elevations in urinary *d*-glucaric acid. Because of the confounding effect of obesity or alcohol ingestion on these measurements, a specific association with dioxin exposure is difficult to determine.

Summary for Hepatic Enzymes

Among these studies, changes in liver function in humans exposed to TCDD are limited to an increase in GGT, whereas results are inconsistent regarding ALT and *d*-glucaric acid excretion. These metabolic "adaptations" to chemical exposure have been seen in industrial workers as well as Ranch Hand veterans. Any study suggesting an association between TCDD exposure and changes in hepatic enzymes or occurrence of liver disease must consider known associations with alcohol, hepatitis, or other known

toxic chemical exposures. Given the long observation period since TCDD exposure occurred in most studies and consideration of other known risk factors, it seems very unlikely that there is any association between TCDD or herbicide exposure, at levels seen to date in humans, and liver dysfunction.

Lipid Abnormalities

Hyperlipidemia, or elevation in cholesterol, triglycerides, or the lipoprotein carriers of these lipids [very low-density lipoproteins (VDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL)] has been described in relation to hereditary and dietary factors. The clinical manifestations of these changes relate primarily to the incidence of ischemic cardiovascular disease and other forms of atherosclerosis. Measurement of lipid and lipoprotein levels is essential to the diagnostic evaluation of hyperlipidemia; however, the measurements should be adjusted for diet and gender, because the concentration of lipids varies with nutritional, endocrine, and gender differences. There is frequently a substantial delay time between onset of lipid changes and clinical manifestations of disease, often 20 to 40 years (Stanbury et al., 1978).

Recent studies comparing the hepatotoxic effects of TCDD in Ah-responsive and nonresponsive mice have demonstrated a mild to moderate hepatic lipid accumulation in some mice strains. Fatty liver induction following exposure to TCDD and related compounds has also been demonstrated in a number of other animal species including the rat, chicken, and man. Sublethal doses of TCDD in rats have been reported to produce an increase in total hepatic lipid content. Specifically, triglycerides and free fatty acids were increased while sterol esters were decreased.

In light of the results from animal studies that indicated hepatic lipid accumulation following TCDD treatment and the frequent association of increased serum lipids with elevated hepatic fat, measurements of serum lipids have been made in individuals exposed to TCDD.

Epidemiologic Studies of Lipid Abnormalities

Occupational and Environmental Studies

Among Czechoslovakian TCP workers studied by Jirasek and colleagues (1974), lipid metabolism was altered in more than 50 percent of the 55 individuals tested; the majority of individuals with altered lipid levels showed elevated serum triglyceride and cholesterol levels. There was mixed chemical exposure in these individuals. In a 10 year follow-up study of this same group, Pazderova-Vejlupkova and colleagues (1981)

reported that all workers tested had a return of lipid levels to normal after removal from the environment.

Moses and colleagues (1984) reported a modest increase in serum triglycerides in workers who had also displayed chloracne versus those who had not exhibited chloracne from a Nitro, West Virginia, chemical plant. The last occupational exposure to TCDD for these individuals had been at least 10 years prior to the study, and for the majority more than 20 years had passed. No significant changes were observed in serum cholesterol levels that could be attributed to TCDD exposure.

Similarly, a study of British TCP workers 10 years after exposure to TCDD showed a slight elevation in triglycerides and a modest decrease in serum cholesterol compared to unexposed controls (May, 1982). In a separate study of workers from this same plant, Martin (1984) reported a slight increase in triglycerides among those individuals that had exhibited chloracne as an indicator of exposure to TCDD, compared to unexposed controls, but also found serum cholesterol to be slightly decreased among this same group. Although the mean values for serum triglycerides and cholesterol in TCDD-exposed individuals were higher than in nonexposed controls, these values were still within the normal laboratory range.

No effect on either serum triglycerides or cholesterol was observed in children who resided in highly contaminated areas of Seveso, Italy, compared to children living in uncontaminated areas (Mocarelli et al., 1986). Furthermore, no changes were noted after they were moved to uncontaminated areas. In a follow-up study, Assennato and colleagues (1989a) compared serum cholesterol and triglycerides among a subgroup of subjects who developed chloracne between 1976 and 1985. Mean cholesterol and triglyceride values were significantly higher in 1976 compared to the 1982-1983 data.

Vietnam Veterans Studies

In the follow-up of the Ranch Hand cohort reported in 1984 and 1987, no alterations in lipid values were recognized in the total group (AFHS, 1984; Wolfe et al., 1990). However, the serum dioxin analyses of the 1987 follow-up examination data found that the ratios of cholesterol to HDL cholesterol and to triglycerides were both positively correlated with serum TCDD levels in the Ranch Hands (AFHS, 1991b).

Flanders and colleagues (1992), however, suggest that this particular pattern of results, observed in this case when serum TCDD levels and triglycerides are measured concurrently, could have been the result of "reverse causation." That is, the outcome (triglyceride levels) may have affected the measured exposure level (serum TCDD), rather than the exposure to TCDD (as measured by serum TCDD levels) exerting an effect on triglycerides.

Summary for Lipid Abnormalities

The variable results do not allow clear-cut attribution of lipid abnormalities to TCDD exposure. It is possible that levels of TCDD were increased in those with hypercholesterolemia (elevated levels of serum cholesterol) because of the concentration of the agent in lipids or because of obesity. In industrial exposures, increases have been modest and variable. A positive association was noted between serum TCDD levels and triglycerides among Ranch Hands. Further research on the pharmacokinetics of TCDD and its relation to percentage body fat will be important in understanding the significance of these associations.

The problem of interpreting the observed association in epidemiologic studies between serum TCDD and lipid levels is complicated by the role of body fat/obesity as both a risk factor for lipid abnormalities and a major determinant of the storage and metabolism of TCDD in the body (see Chapter 6, and the example above in the section on lipid abnormalities). Body fat must be taken into account in analyses of this kind to ensure the validity of the results. In the absence of reliable data and statistical models for the metabolism of TCDD in humans, however, even if current measurements of body fat or other measures of obesity are available, considerable uncertainty will remain in the interpretation of associations seen in epidemiologic studies carried out many years after exposure.

Gastrointestinal Ulcers

There are usually no specific physical signs in-patients with gastrointestinal ulcers. The symptoms of gastric ulcer are often nonspecific; the typical pattern of epigastric pain is variable, and many patients with this symptom may not have an ulcer when examined endoscopically, whereas patients with ulcers demonstrated by endoscopy may report no pain. Endoscopy is generally recommended only in-patients whose symptoms persist despite treatment. Acid secretory values are of relatively little clinical value in diagnosis of ulcer disease (Samiy et al., 1987). The onset of symptomatic ulcer disease may be initiated by nonsteroidal anti-inflammatory drugs (NSAIDs) or alcoholism. In many patients, the disease is associated with the presence of *Helicobacter pylori (H. pylori)*.

Several investigators, in their review of the scientific literature on the health effects of herbicide exposure, have reported a positive association between herbicides and/or TCDD exposure and gastrointestinal ulcer (Agent Orange Scientific Task Force, 1990; Jenkins, 1991). Based on their conclusions, the human epidemiologic studies investigating the relationship between exposure and ulcers were reviewed.

Epidemiologic Studies of Ulcers

Occupational and Environmental Studies

A greater frequency of upper gastrointestinal tract ulcers was reported in Nitro, West Virginia, 2,4,5-T production and maintenance workers exposed to TCDD, as compared to a group of unexposed workers (Suskind and Hertzberg, 1984). Additional studies have not confirmed this finding in chemical plant workers exposed to TCDD (Bond et al., 1983; Calvert et al., 1992a). In Seveso, among children with chloracne, gastrointestinal tract symptoms were reported more frequently than among children from that area who did not have chloracne (Caramaschi et al., 1981).

Vietnam Veterans Studies

The serum TCDD analysis of the Ranch Hand Study did not find an increase in the risk of upper gastrointestinal ulcer in Vietnam veterans with elevated TCDD levels (AFHS, 1991b).

Summary for Ulcers

The risk of ulcers in exposed populations has not been sufficiently studied to exclude an association with TCDD or herbicides. However, detection of a specific association is unlikely, given the frequency of ulcer disease and the varied factors (e.g., alcoholism, NSAIDs, *H. pylori* infection) that are known to be related to the onset of symptomatic ulcer disease. Furthermore, given the length of time that has elapsed since veterans last exposure to TCDD in Vietnam, it is unlikely that new cases of ulcer disease would occur.

Conclusions for Other Metabolic and Digestive Disorders

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and diabetes mellitus, changes in hepatic enzymes, lipid abnormalities, or gastrointestinal ulcers.

Biologic Plausibility

The liver is the site of TCDD storage and metabolism in laboratory animals. Some of the herbicides have also induced liver toxicity in laboratory

animals. There have been no reports of an association between TCDD or herbicide exposure and diabetes in laboratory animals. Hyperlipidemia has been reported in laboratory animals following exposure to TCDD, but not following exposure to the herbicides. Specific digestive disorders have not been reported.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

IMMUNE SYSTEM DISORDERS

Immunotoxicology is the study of the effects of xenobiotics (chemical compounds that are foreign to the human body) on the immune system. The consequences of alterations of the immune response to a foreign antigen are outlined more fully below. Following a method recently outlined by the CDC for categorizing effects on the immune system, immune suppression, immune enhancement (reviewed collectively here as immune modulation), and autoimmunity are discussed. The evidence for alterations of immunity in humans by the herbicides of interest is also categorized. Although alterations in the immune system can be related to increases in the incidence of infection and neoplasm (immune suppression) and immune-mediated diseases (immune enhancement and autoimmunity), there is no observed increase in infectious (perhaps an increase in the incidence of some types of neoplasms) or immune-mediated disease in the populations examined. Instead, alterations are observed in measures of immune function or populations of immune cells. Efforts are under way in animal studies to correlate such parameters with increased susceptibility to disease, but these analyses have not yet been completed.

Immune Modulation

The immune system is involved with the defense of the host (the body) against foreign invaders. It confers resistance to infection by bacteria, viruses, and parasites; functions in the rejection of allografts (tissue transplants); and may eliminate spontaneously occurring tumors (Katz and Benacerraf,

1972). Proper function of the immune system is exquisitely sensitive to disruptions in physiologic homeostasis. The immune response is highly complementary, and several different mechanisms may be employed to eliminate an antigen (Jerne, 1974). Therefore, a toxicant can affect one facet of the immune system without altering the ability of the host to survive challenge by an infectious agent.

In most circumstances, the protection of the host against infection and neoplasms is accomplished in the absence of extensive destruction of the surrounding tissues due to tolerance of self-antigens through mechanisms currently not understood. However, a number of diseases involve hyperresponsiveness of the immune system to either foreign allergens (e.g., allergy) or self-antigens (autoimmunity). Allergic responses have been noted to numerous environmental components including ragweed and domesticated animals. Allergy is the result of formation of allergen-specific immunoglobulin E (IgE) antibodies, which bind to the surface of mast cells and lead to mast cell degranulation upon subsequent exposure to antigen, and allergen-specific T cell activation. The alterations discussed below are only in immune parameters and not in disease incidence. In fact, there was no observed increase in hypersensitivity disease in any of the studies reviewed.

Suppression of the immune system leads to increased susceptibility to infection and neoplasia. However, the degree of immune suppression necessary to result in increased disease is unknown at this time and is the subject of intense scientific interest. Immune deficiency may result from genetic abnormalities (e.g., a deficiency in the enzyme adenosine deaminase, leading to severe combined immune deficiency), congenital malformations, surgical accidents, pregnancy, stress, disease [e.g., human immunodeficiency virus (HIV-I) can lead to AIDS], and exposure to immunosuppressive agents (Purtilo et al., 1972; Jose and Good, 1973; Folch and Waksman, 1974; Heise et al., 1976; Monjan and Collector, 1977; Cohen, 1978; Cohen et al., 1978; Heise and Palmer, 1978). Immune suppression can also occur in-patients with autoimmune disease (discussed further below); for example, in systemic lupus erythematosus, the suppression of complement levels and leukocyte function has been noted. Impaired host defenses can result in severe and recurrent infections with opportunistic microorganisms. As noted above, the immune system may prevent or limit tumor growth, and a high incidence of tumors may follow immune suppression (Penn and Starzl, 1972; Penn, 1985).

Epidemiologic Studies of Immune Modulation

Occupational and Environmental Studies

Pilot studies of Missouri residents living in the Times Beach area were

conducted by Knutsen (1984) and by Webb and colleagues (1987). In the Knutsen study, trends toward immune suppression were noted, but the changes did not reach statistical significance. In the Webb study, there was no difference in (1) the prevalence of immune disorders, (2) the total duration of the response to delayed hypersensitivity skin test (DTH), or (3) lymphocyte proliferative response. The pilot study indicated that living in an area with TCDD-contaminated soil had no effect on immune function (Stehr et al., 1986; Webb et al., 1987).

A larger study involving the examination of persons in the Quail Run mobile home park, who were potentially exposed to TCDD through contaminated oil in the soil, indicated a decrease in T cell numbers; suppressed cell-mediated immunity as measured by the generation of a cutaneous DTH response to recall antigens; and an increase in the percentage of persons in the exposed population with decreased T4/T8 ratios (Hoffman et al., 1986; Knutsen et al., 1987; Stehr-Green et al., 1987; Andrews et al. 1986). Of note, T cell subset changes were in a different pattern from those of Webb and colleagues (1987), and very small changes were observed. There was a greater than expected number of anergic persons in the control population. Further examination of these results indicated that the amount of antigen used in the test was suboptimal, and the data from two out of four of the readers (i.e., assessors of the immunologic response) were eliminated, thereby calling into question the data from the remaining two readers. On retesting, anergic subjects failed to confirm this anergy (Evans et al., 1988). This may be due to (1) an increase in immunity resulting from exposure to the antigen in the initial test, (2) recovery from immune suppression related to TCDD exposure, or (3) the gathering of incorrect information in the initial test (Allison and Lewis, 1986). A decrease in the level of the thymic peptide thymosin was also measured in persons thought to be exposed to TCDD (as determined by length of residence in a TCDD-contaminated area) after controlling for age, sex, and socioeconomic status (Stehr-Green et al., 1989).

An immunologic assessment of 41 persons from Missouri with documented levels of TCDD in adipose tissue was conducted by Webb and colleagues (1989). The data were analyzed by multiple regression based on adipose tissue TCDD level and clinical dependent variables. Increased TCDD levels were correlated with increased numbers of T cells, primarily increases in CD8+ and T11+ cells, with no change in CD4+ cells. There was an increase in serum IgG but not IgA. No change in proliferative responses to mitogen was noted. No adverse clinical disease was associated with TCDD levels in these subjects. In this study, no anergy or alterations in proliferative responses to mitogens were found despite a clustering of decreased T4/T8 ratios in persons with the highest adipose TCDD level. On the other hand, some immune parameters were increased (as discussed below).

Pocchiari and colleagues (1979) reported studies on the immune status of 45 children that were exposed to TCDD following an explosion at a chemical factory in Seveso, Italy. No abnormalities were found in many of the immune parameters tested, including serum immunoglobulin levels, levels of circulating complement, and mitogenic responses. A study of the immune function of persons involved in cleanup operations after this accident showed no alterations in immune function (Ghezzi et al., 1982). A study conducted six years after the explosion in a different cohort of TCDD-exposed children reported an increase in complement protein levels, which correlated with chloracne; an increased number of peripheral blood lymphocytes; and increased lymphocyte proliferative responses to mitogens (Sirchia, 1980; Tognoni and Bonaccorsi, 1982).

No association with TCDD exposure and Kaposi's sarcoma in AIDS patients has been found (Hardell et al., 1987).

Vietnam Veterans Studies

Only one study that was conducted suggested that exposure to herbicides, specifically Agent Orange, in Vietnam resulted in immune suppression. This study was conducted by Stellman and colleagues (1988) and showed that a cluster of nonspecific self-reported symptoms, including faint aches, fatigue, and colds, was associated with combat exposure and herbicide handling in Vietnam. This study, however, indirectly measured herbicide exposure (i.e., self-reported location of the person at a particular place and time) and did not specifically measure immune responsiveness. In addition, self-reported disease data are subject to recall bias that may confound these results.

Another study was conducted by the New Jersey Agent Orange Commission (Kahn et al., 1992). Three study groups were included in this study: herbicide handlers in Vietnam ($N = 10$), "nonexposed" Vietnam veterans ($N = 10$), and Vietnam era veterans ($N = 7$). The groupings of individuals were confirmed by measurements of serum TCDD levels, although the authors indicate that some outliers in the nonexposed group may have been exposed to base perimeter spraying. The skin response to recall antigens was uninterpretable due to anergy in 4 of 17 controls and 0 of 10 exposed veterans. There were some alterations in the lymphocyte subpopulations, but the inconsistency of control values with expected laboratory control levels, and the lack of differences between the exposed and the nonexposed Vietnam veterans, reduced the relevance of these data to the present report.

A co-twin control study of veterans from the Vietnam war involving self-reported physical problems was also conducted (Eisen et al., 1991). No significant effect on the immune system was observed in this study. Problems

with interpreting the data from this study include the potential for misclassification of exposure due to the indirect measures of TCDD and the self-reporting of medical problems.

In a study of immune system parameters of Vietnam veterans conducted by the Agent Orange Advisory Committee of Texas, out of 12 immune parameters examined, an increase in the percentage of cells in a subset of T cells (called "rosette-forming cells") that were active was increased (Newell, 1984). The physiologic relevance of this parameter is unknown. The study did not control for confounders such as smoking.

Studies to date of individuals involved in Operation Ranch Hand, which include a records-based and serum TCDD measure of exposure, and specific tests for immune function, have not revealed any indication of overt immune suppression (Wolfe et al., 1985, 1990). In fact, most studies have indicated no effect on the immune parameters examined. The few parameters that were altered indicated an increase and are discussed further below. Studies of Vietnam veterans in the CDC Vietnam Experience Study (VES) also suggested that there was no effect on immune parameters in the cohorts studied (CDC, 1988).

A recent reanalysis of the 1987 results (AFHS, 1991b, vol. 6) from follow-up of the Ranch Hands using serum dioxin levels as a measure of exposure, indicated that there is a dose-dependent increase in the maximum proliferative response of peripheral blood lymphocytes to the mitogen phytohemagglutinin. The authors of the report do not feel that this is indicative of an overall alteration in the cell-mediated immune responsiveness of these individuals due to the fact that (1) the enhancement was slight, (2) the enhancement may be due to confounders not assessed by the investigators, and (3) an enhancement of the proliferation of lymphocytes in response to allogeneic lymphocytes (a mixed leukocyte reaction) was not observed in these patients. However, this increase was dose-dependent and significant, and therefore should not be dismissed. Significant dose-dependent associations between increasing concentrations of TCDD in the serum and increasing IgA concentrations were also found. The authors suggest that these increased IgA levels may represent a chronic inflammatory response as a result of TCDD exposure, but the concentrations of IgA in the serum were also found to increase with the amount of alcohol consumption reported, which confounded the results. In other sections of the 1991 reanalysis, it was shown that there was a dose-dependent increase in the erythrocyte sedimentation rate (also observed in the 1982 baseline study AFHS, 1984), white blood cell count, and platelet count. Increases in these parameters confirm the possibility that there is an ongoing chronic inflammatory response occurring in these individuals as a result of TCDD exposure.

Autoimmunity

In general, the immune response is directed to foreign antigens. However, in some instances, antibodies can be demonstrated that react with endogenous antigens i.e., autoantibodies. These "natural autoantibodies" are usually of the IgM class, are not associated with any disease, and may actually have beneficial or regulatory function(s).

Autoimmune disease is the pathological consequence of autoimmunization, either inadvertent or deliberate. Among the effector mechanisms of autoimmune disease are autoantibodies that activate the complement cascade or interact with "killer" mononuclear cells to induce antibody-dependent cell-mediated cytotoxicity. Other autoimmune diseases are caused by cytotoxic T cells acting directly on their targets or by injurious cytokines released by activated T cells.

It is important to distinguish the mere presence of autoimmunity from autoimmune disease. Autoimmunity, as indicated by the presence of autoantibodies, is relatively common, whereas autoimmune disease is a relatively rare occurrence. Yet the presence of autoantibodies, particularly in high titers and with high affinity, is the first step in diagnosing autoimmune disease in humans. A definite diagnosis of autoimmune disease, however, depends on careful correlation of history and clinical findings with detailed immunologic investigations.

It is convenient to consider two major categories of autoimmune disease, although the distinction between the two groups is often blurred. So-called systemic autoimmune disease is directed to antigens that are widely distributed throughout the body; the disease correspondingly affects multiple organ systems. The prototype of a systemic autoimmune disease is systemic lupus erythematosus (SLE). The other group of autoimmune diseases targets antigen unique for particular organs of the body, and the pathological phenomenon is confined to that organ. The prototype of an organ-specific autoimmune disease is autoimmune thyroiditis. The evidence associating TCDD with systemic autoimmune disease is considered next, and in the following section its possible role in organ-specific autoimmune disease is discussed.

Epidemiologic Studies of Autoimmunity

Occupational and Environmental Studies

An assessment of immunological abnormalities among 18 workers involved in the manufacture of 2,4,5-T and exposed to TCDD during an industrial accident 17 years earlier, found no change in many of the immune parameters examined (Jennings et al., 1988). However, an increase was

noted in the number of persons with immune complexes (low titer), the number of antinuclear antibodies (in one assay but not in another), and the number of Leu 7+ cells (natural killer cells) along with decreased T4/T8 ratios. In this study, exposure was defined by being in the vicinity during the explosion or cleanup, and there was no measure of the body burden of TCDD. The findings from this study are compatible with, but not necessarily diagnostic of SLE or a related connective tissue disorder.

Vietnam Veterans Studies

Early results of the Ranch Hand study do not show any evidence of an increase in the signs and symptoms associated with connective tissue diseases. Changes in the immune profiles of Ranch Hand veterans were also not observed (Wolfe et al., 1990). The CDC Vietnam Experience Study failed to show evidence of connective tissue diseases or of abnormal immune responses (CDC, 1988).

In a more limited study of Vietnam veterans reported by Newell (1984), no medical problems associated with connective tissue disease were found, but as stated, a small decrease in the total number of T cells and an increase in the number of so-called active T cells were observed in exposed veterans. The deviations from normality were not of sufficient magnitude to be biologically significant.

Two autoimmune diseases, chronic thyroiditis and insulin-dependent diabetes, are suspected in connection with exposed Vietnam veterans. An extensive assessment of endocrine function including a series of thyroid function tests, was carried out in connection with the Ranch Hand study (AFHS, 1991b). These studies failed to show any difference in thyroid function between exposed and control veterans. Diabetes was increased in a population of the Ranch Hands (as discussed in the earlier section on diabetes); however it is unclear at this time if this is an autoimmune disease or adult-onset diabetes related to increased body fat.

Summary for Immune Disorders

The effects of herbicide exposure on the level of several immune parameters have been presented. The data are divided into the categories: immune modulation and autoimmunity. Parameters of cellular function or number were measured, not incidence of disease. Currently, the level of alteration in immune parameters necessary to increase the incidence of disease is unknown.

These data correlate with some of the data observed in animal studies, but much more information is required to determine the mechanism and clinical significance of this increase in immune parameters. Furthermore,

since so many immune parameters have been assessed in these studies, there is a high probability that at least a few positive results would be noted based on chance alone, which would undermine the interpretation of the few positive results.

Conclusions for Immune Disorders

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and immune modulation or autoimmunity.

Biologic Plausibility

Experiments in laboratory animals have demonstrated a variety of effects of TCDD on immunologic parameters. Similar effects have not been demonstrated for the herbicides.

Increased Risk in Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

CIRCULATORY DISORDERS

The studies to be reviewed in the following section on circulatory diseases cover a wide variety of diverse circulatory conditions. Cerebrovascular diseases, including stroke, are not included in the following section but are covered in Chapter 10. The studies that are reviewed here are divided into two categories—morbidity studies and mortality studies. In the morbidity studies, a variety of methods were used to assess the circulatory system, including assessing symptoms or history, performing physical examination of the heart and peripheral arteries, and assessing Doppler measurement of peripheral pulses, electrocardiogram results, and chest radiographs. Doppler measurements and physical examination of the pulses in

the arms and legs are used to detect decreased strength of the pulses, which can be caused by conditions such as atherosclerosis, a thickening and hardenin g of the arteries (blood vessels that carry oxygenated blood from the heart to the rest of the body). The electrocardiogram can be used to detect heart conditions and abnormalities such as arrhythmias (abnormal heart rhythms), heart enlargement, and previous heart attacks. Chest radiographs can be used to assess whether the heart is enlarged, which can result from heart failure and other heart conditions.

Very limited information is available on the cardiovascular toxicity of TCDD and herbicides in animals. The available studies are described in Chapter 4.

Epidemiologic Studies of Circulatory Disorders

Occupational Studies

The occupational studies assessing circulatory outcomes can be divided into mortality and morbidity studies. Zack and Suskind (1980) observed no excess mortality for diseases of the circulatory system including heart disease in a study of 121 male workers who developed chloracne following the accident at the TCP production plant in Nitro, West Virginia. In a larger Monsanto cohort, Zack and Gaffey (1983) observed an SMR = 1.3 for excess of arteriosclerotic heart disease, including chronic heart disease, and significantly decreased SMR = 0.6 for all other diseases of the circulatory system. Other occupational mortality studies used different diagnostic codes (or did not specify the International Classification of Disease [ICD] code) to report on various circulatory outcomes, none of which was significantly elevated in the exposed group. These included the following: overall circulatory deaths among pesticide applicators (Blair, 1983); ischemic heart disease among soil/forest conservation workers (Alavanja et al., 1989); hypertensive and ischemic heart disease deaths among 2-methyl-4-chlorophenoxyacetic acid (MCPA) manufacturers/sprayers (Coggon et al., 1986); and circulatory diseases among production workers of phenoxy herbicides and chlorophenols (Coggon et al., 1991).

In occupational studies that assessed circulatory morbidity (Moses et al., 1984; Suskind and Hertzberg, 1984), no significant differences were found in the circulatory variables examined between exposed and unexposed groups. Suskind and Hertzberg (1984) compared 204 workers involved in 2,4,5-T production with 163 nonexposed workers from the same plant. No exposure-related differences were observed in self-reports of hypertension, coronary artery disease, or angina, or in measured blood pressure, plasma lipids, electrocardiogram (EKG), or chest radiographic results. Additionally, in Dow workers included in a medical surveillance no differences

in reports of high blood pressure occurred between those exposed to TCP or TCDD and those unexposed (Bond et al., 1983).

Moses and colleagues (1984) evaluated circulatory morbidity in 226 workers exposed to TCDD in a 2,4,5-T production plant. Workers were categorized based on current evidence or past history of chloracne (N = 117) versus no current or past chloracne (N = 109). There were no differences between the two groups with regard to age-adjusted angina or reported myocardial infarction, or physical examination of the cardiovascular system. Limitations in interpretation of these studies arise from possible misclassification due to the inaccuracy of chloracne history as a means of assessing exposure.

Environmental Studies

The major environmental studies that address circulatory mortality are those describing mortality among residents in the area of Seveso, Italy, following the 1976 accident that released TCDD (Bertazzi et al., 1989a,b). The highest soil levels of TCDD were generally found in zone A, followed by lower levels in zone B, and the lowest levels in zone R. In one study that defined the population groups by zone of exposure and gender (Bertazzi et al., 1989b), the authors reported significantly increased mortality for several cardiovascular outcomes (comparing exposed groups with the reference population) as follows: chronic ischemic disease for the first five year follow-up period 1976-1981 in males in zones A (RR = 5.2, CI 1.3-21) and R (RR = 1.7, CI 1.2-2.5); cerebrovascular disease among males in zone A for the second five year period 1982-1986 (RR = 4.8, CI 1.5-15), hypertensive vascular disease among females in zone R for the first five year period 1976-1981 (RR = 4.8, CI 1.3-4.4); and all circulatory disease among females in zone A in the second five year period 1982-1986 (RR = 2.9 CI 1.1-7.8). No statistically significant increase in cardiovascular mortality was reported when comparing subjects (males or females) from zone B with the reference population.

In the other study (Bertazzi et al., 1989a), which was not stratified by zone of exposure, results showed significant increases in relative risk for mortality due to chronic ischemic heart disease in males in the first five year follow-up (RR = 1.8, CI 1.2-2.5) from 1976 to 1981 and in the full ten year follow-up period, 1976-1986, (RR = 1.6, CI 1.2-2.1) and an increase in hypertensive vascular disease deaths among females in the first five year period (RR = 2.4, CI 1.4-4.3).

These studies are limited for the purpose of assessing relationships between circulatory deaths and TCDD exposure. Because increases in chronic ischemic disease and hypertensive vascular disease mortality were observed in the first five years following the accident, stressors related to the accident

itself could not be eliminated as a cause. The second study (Bertazzi et al., 1989a) was performed in part because of criticisms raised about possible inaccuracies in defining zones of exposure, but itself was limited because of dilution of the exposed cohort in combining all three exposed groups. Variability in diagnosis of specific circulatory cause of mortality, which varied across gender and zones, also limits this study.

Vietnam Veterans Studies

Several studies have examined morbidity or mortality due to circulatory diseases among Vietnam veterans. The studies that addressed circulatory mortality reported on various outcomes using different ICD codes. None showed significant increases in circulatory mortality, with the exception of cerebrovascular disease mortality in one study (Kogan and Clapp, 1985). The Australian Vietnam veteran study (Fett et al., 1987) found no significant differences in circulatory disease mortality. In a study that compared U.S. Army Vietnam veterans with U.S. Army Vietnam era veterans or all Vietnam era veterans, and compared U.S. Marine Vietnam veterans with U.S. Marine Vietnam era veterans or all Vietnam era veterans (Watanabe et al., 1991), there were no significant differences in proportionate mortality ratios for circulatory diseases. A proportionate mortality study comparing Wisconsin Vietnam veterans with Wisconsin Vietnam era veterans (Anderson et al., 1986) reported no differences in all circulatory system disease mortality or arteriosclerotic disease mortality.

The Centers for Disease Control VES study (CDC, 1987, 1988) showed significantly reduced mortality due to circulatory diseases. In a study comparing Massachusetts Vietnam veterans with Vietnam era veterans who did not serve in Vietnam (Kogan and Clapp, 1985), the authors reported a significantly decreased proportionate mortality ratio (PMR) for circulatory diseases (PMR = 0.8, p = .03), excluding cerebrovascular diseases. Cerebrovascular diseases were significantly increased among Vietnam veterans (see Chapter 10). In a mortality study comparing women Vietnam veterans to women Vietnam era veterans, the authors reported no increases in deaths from circulatory diseases (Thomas et al., 1991).

Other studies have examined morbidity due to circulatory diseases among Vietnam veterans. In the CDC VES study, Army Vietnam veterans were compared to Army Vietnam era veterans who had not served in Vietnam (CDC, 1988). A significant difference in self-reported hypertension between the groups was reported. However, there was no significant difference in hypertension measured as part of a physical examination in the study. At physical exam all cardiovascular tests showed no difference between Vietnam and Vietnam era veterans except for left ventricular hypertrophy

which was associated with an increased risk (OR = 1.8 CI 1.0-3.3) among Vietnam veterans (CDC, 1988).

A study of monozygotic twins who were in U.S. military service during the Vietnam era showed that service in Southeast Asia was not associated with increased self-report of heart trouble (Eisen et al., 1991). In another study, no differences were reported in the two chest x-ray abnormalities related to the heart, namely, cardiomegaly and prominent pulmonary vasculature in comparing 422 Vietnam veterans from the Agent Orange Registry at the Albuquerque Veterans Administration (VA) Medical Center with 105 Air Force flight staff who did not serve in Vietnam (Pollei et al., 1986).

Stellman and colleagues (1988) assessed the effects on self-reported health outcomes not only of the Vietnam experience, but also of herbicide handling and Agent Orange exposure among nonherbicide handlers. In that study, 2,858 American Legion veterans who had served in Southeast Asia from 1961 to 1975 and returned questionnaires were compared to veterans who had served elsewhere during the same period. The authors reported a significant increase among Vietnam veterans compared to those serving elsewhere in self-report of physician-diagnosed heart disease occurring no earlier than one year prior to discharge from military service (OR = 1.5, age-adjusted). Among those who served in Southeast Asia, herbicide handlers did not differ significantly from those who did not handle herbicides in frequency of age-adjusted heart disease. Among nonherbicide handlers, the level of Agent Orange exposure, based on a score derived from self-reported service locations and military records of spraying locations, and the level of combat experience, were not significantly associated with heart disease. For the self-reported variable of physician-diagnosed hypertension, Vietnam veterans did not differ from Vietnam era veterans. Among those who served in Southeast Asia, herbicide handlers reported significantly more hypertension than those who did not handle herbicides (OR = 1.7). Among nonherbicide handlers, the level of Agent Orange exposure was not significantly associated with hypertension, but the level of combat was. These results suggest that the Vietnam experience, but not herbicide handling in Vietnam, was associated with self-report of physician-diagnosed heart disease and that herbicide handling and level of combat experience among nonherbicide handlers were associated with self-report of physician-diagnosed hypertension. Conclusions to be drawn are limited by the potential for misclassification of exposure and the lack of validation of self-reported diagnoses. It is unclear if adequate adjustments were made for modifiers such as cigarette smoking and obesity.

Two major groups of U.S. Vietnam veterans were known to have handled herbicides during military service in Vietnam, the Air Force Ranch Hands and the Army Chemical Corps. The latest available mortality study comparing Ranch Hands with other U.S. Air Force veterans involved in cargo

missions in Southeast Asia but not exposed to herbicides presents cumulative mortality through December 31, 1989 (AFHS, 1991a). The authors report statistically increased mortality from circulatory deaths among nonflying enlisted personnel (19 deaths observed, 11.3 expected; SMR = 1.7, CI 1.0-2.6). They further state that this increase is of concern because Ranch Hand nonflying enlisted personnel have higher current TCDD levels than Ranch Hands in other categories. In addition, they state that current and extrapolated initial TCDD levels were shown to be significantly associated with diabetes and increased cholesterol, HDL cholesterol, and triglyceride abnormalities. To assess the possibility that the increase in circulatory deaths could be related to TCDD through an association with diabetes and serum lipids, a review of individual medical records of all the circulatory deaths in Ranch Hands is being carried out. Potential confounders such as cigarette smoking were not addressed in this report, although results among living participants in the Air Force Health Study show that Ranch Hands had a higher frequency of current smokers (36 percent) than the comparison group of other U.S. Air Force veterans involved in cargo missions in Southeast Asia but not exposed to herbicides (31 percent). In addition, the specific causes of circulatory deaths in the Ranch Hand and comparison group would be of interest because the category of circulatory diseases is broad and contains diagnoses that are unrelated to either diabetes, lipid abnormalities, or cigarette smoking.

The latest Ranch Hand cardiovascular assessment results have been reported (AFHS, 1990), comparing the health status of Ranch Hands with other U.S. Air Force veterans involved in cargo missions in Southeast Asia but not exposed to herbicides. The parameters examined were the following: questionnaire data on the presence of three cardiovascular conditions—essential hypertension, heart disease, and myocardial infarction; verification of those three conditions by review of medical records; physical examination data on systolic and diastolic blood pressure, heart sounds, funduscopic examination, carotid bruits, and pulses including radial, femoral, popliteal, dorsalis pedis, and posterior tibial; and eight EKG abnormalities.

The report indicated that the health of the Ranch Hands and comparison group was similar with regard to reported and verified heart disease and central cardiac function (i.e., systolic blood pressure, heart sounds, and EKG results). Ranch Hands had a marginally higher mean diastolic blood pressure, but the frequency of elevated diastolic blood pressures was not significantly different between the groups. Ranch Hands also had a marginally higher frequency of individuals with carotid bruits. There were significant or marginally significant differences in femoral pulses, dorsalis pedis pulses, and three aggregate pulse indices. Significantly more abnormal pulses had been found at the baseline examination in 1983 but not in 1985, when both manual and Doppler examinations were used.

In addition to the 1987 Ranch Hand cardiovascular assessment summarized above, further analyses of the 1987 circulatory outcomes were performed to assess associations with serum TCDD levels (AFHS, 1991b, vol. 5). According to these analyses, there was no consistent evidence of an adverse TCDD effect among nondiabetics. There were significant increases in risk of essential hypertension and adjusted mean systolic and diastolic blood pressures in Ranch Hands with high current TCDD levels (> 33 ppt) compared to comparison subjects in the background category (≤10 ppt), when body fat effects were not considered. In addition, verified heart disease risk was significantly decreased for Ranch Hands in the high-TCDD category, and the frequency of elevated systolic or diastolic blood pressure was not associated with TCDD level. The authors also suggested that there were significant associations between TCDD and decreases in peripheral pulses.

Difficulties with interpreting the Ranch Hand data are summarized in Appendix C. A consistent pattern of cardiovascular abnormalities among Ranch Hands or consistent associations between TCDD levels and cardiovascular outcomes has not been reported. Effects of body fat are suggested as confounders for the blood pressure abnormalities. Peripheral pulse differences between baseline (1983) and first follow-up examination (1985) have been explained, in part, by the requirement for four-hour tobacco abstinence during the 1985 examination prior to the Doppler measurement. It is unclear whether this requirement was used before the examination of peripheral pulses in the 1987 examination.

A mortality study of 894 Army Chemical Corps personnel who served in Vietnam, compared with the U.S. male population, reported 6 observed deaths from circulatory disease, whereas 10.9 were expected (Thomas and Kang, 1990). That study is of limited use in evaluating circulatory mortality because of the small number of deaths and the wide range of unrelated diagnoses covered by the ICD codes in classifying the circulatory disease deaths.

Summary for Circulatory Disorders

The literature concerning the effects of herbicides on the human circulatory system can be divided into morbidity and mortality studies. The circulatory outcomes addressed in these studies include the following: mortality from circulatory diseases, including overall circulatory disease mortality and various subgroups of cardiovascular disease; symptoms or history of circulatory illnesses such as heart disease, hypertension, coronary artery disease, angina, or myocardial infarction; abnormalities on physical examination; electrocardiogram results; and chest radiographs.

Several factors limit the usefulness of the mortality studies. For the most part, those studies have presented no a priori hypotheses regarding

herbicides and any particular circulatory outcome. The result is that many of the studies (e.g., Blair, 1983; Boyle et al., 1987; CDC, 1987; Fett et al., 1987; Thomas and Kang, 1990; Thomas et al., 1991; and Watanabe et al., 1991;) use the ICD codes for all diseases of the circulatory system, 390 to 458 or 459, as the circulatory mortality outcome. Those ICD codes cover the following circulatory system diseases: acute rheumatic fever; chronic rheumatic heart disease; hypertensive disease; ischemic heart disease; cerebrovascular disease; diseases of the arteries, arterioles, capillaries, veins, and lymphatics; and other heart diseases. This wide range of diverse conditions makes it difficult to assess any particular circulatory outcome of interest by using mortality studies. In some studies, the ICD codes used were not stated, making comparisons with other studies difficult. Possible independent risk factors for coronary artery disease, including cigarette smoking, diabetes, lipid abnormalities, and hypertension were generally not assessed in the mortality studies. The AFHS mortality study (1991a) results are important and require further examination to assess the role of confounders such as cigarette smoking and the specific circulatory diseases accounting for the increase. In general, the lack of assessment of independent risk factors for circulatory disease limits the usefulness of the mortality studies.

Among the morbidity studies, strong rationales for examining circulatory outcomes were not given. However, the Air Force Health Study (1991b) has reported associations between serum TCDD and both diabetes and blood lipids. This suggests a rationale for examining a major circulatory disease, coronary artery disease, in those exposed to dioxins because of the possible association between risk factors for coronary artery disease and serum TCDD level.

Two occupational studies (Moses et al., 1984; Suskind and Hertzberg, 1984) showed no significant increases in symptoms or history of circulatory disease. Several studies of Vietnam veterans showed increases in self-reported hypertension. The CDC VES study (CDC, 1988) reported a significant increase in the self-report of hypertension, but physical examinations did not show any differences in increased blood pressure. In the Stellman et al. (1988) study, herbicide handlers had significantly more self-reports of physician-diagnosed hypertension. However, validation by medical record review or measurement of blood pressure was not done in this study. The Air Force Health Study (1991b) stated that there were significant increases in reported essential hypertension associated with high current TCDD levels. However, it further reported that this effect was observed after body fat and cholesterol were removed from the model (AFHS, 1991b). Further evaluation is needed to assess the appropriate choice and interpretation of models before conclusions can be drawn from this study (AFHS, 1991b).

Several studies provided electrocardiogram results (CDC, 1988; AFHS, 1990; Suskind and Hertzberg, 1984), but comparisons were difficult because

of nonuniformity in classifying the EKGs. Results were negative or inconsistent. Nonuniformity in classifying EKG abnormalities across the studies limited useful comparisons.

Chest radiograph results were presented in three studies with inconsistent findings (Suskind and Hertzberg, 1984; Pollei et al., 1986; CDC, 1988). Although all three reports showed no differences in chest radiographs, the Suskind and CDC studies did not assess herbicide or contaminant exposures. In the one study that attempted to assess such exposure (Pollei et al., 1986), the numbers in the subgroup that recalled repeated handling of herbicides were too small for adequate analysis.

Doppler measurements showed no differences in the two studies reported (AFHS, 1987; CDC, 1988). However, other reports of peripheral pulse abnormalities were in contrast to these (AFHS, 1984, 1991b, vol. 5). The timing of cigarette smoking in relation to the examination is a possible explanation that requires further assessment.

One parameter that could be assessed across several studies was blood pressure measurement from physical examination (Suskind and Hertzberg, 1984; CDC, 1988; AFHS, 1990). Similar criteria were used to define an elevated blood pressure (greater than 140/90), although one study used a higher cutoff for those 60 years of age and older (Suskind and Hertzberg, 1984). An occupational study of 2,4,5-T production workers (Suskind and Hertzberg, 1984) and the Vietnam Experience Study (CDC, 1988) showed no significant differences in measured blood pressure between exposed and unexposed. The AFHS (1991b) reported increases in adjusted mean systolic and diastolic blood pressure in association with high current TCDD levels when body fat was not considered. However, it reported that the frequency of elevated systolic or diastolic blood pressure was not associated with TCDD level. Further evaluation is needed to assess appropriate modeling of variables and the interpretation of models before conclusions can be drawn from this study (AFHS, 1991b). With regard to other abnormalities on physical examination, the AFHS reported no significant differences between Ranch Hands and the comparison group in heart sounds, marginally higher frequencies of carotid bruits, and significant or marginally more significant differences in peripheral pulses (AFHS, 1990). It further reported significant associations between TCDD and decreases in peripheral pulses (AFHS, 1991b). Further evaluation is needed to assess appropriate choice and interpretation of models, and the timing of cigarette smoking in relation to the examination of peripheral pulses, before conclusions can be drawn from these studies (AFHS, 1990, 1991b). In the morbidity studies, a wide range of outcomes was assessed, and the presentation of results varied across studies. Symptoms or history of heart disease was difficult to assess across studies since each study gathered unique noncomparable data. Data derived from physical examination differed among the studies.

Conclusions for Circulatory Disorders

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and the following circulatory outcomes: circulatory disease mortality and various subgroups of cardiovascular disease; symptoms or history of circulatory illnesses such as heart disease, hypertension, coronary artery disease, angina, or myocardial infarction; abnormalities on physical examination, electrocardiogram results, and chest radiographs.

Biologic Plausibility

Limited information from animal studies is available on the potential association between circulatory diseases and exposure to TCDD or the herbicides.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

RESPIRATORY DISORDERS

The studies to be reviewed in the following section on respiratory diseases covered a wide variety of diverse respiratory conditions. In the morbidity studies, a variety of methods were used to assess the respiratory system, including assessing symptoms, performing physical examination of the chest, and assessing lung function tests and chest radiographs. Lung function tests, also called pulmonary function tests, included tests commonly used to detect airflow obstruction (which can occur in conditions such as asthma, chronic bronchitis, and emphysema) and restriction or decrease in lung volumes (which can occur in lung scarring and inflammation). The tests that measure reduced in airflow obstruction include FEV_1 (amount of air which can be forcefully exhaled in one second), FEV_1/FVC

ratio (ratio of the amount of air that can be forcefully exhaled in 1 second to the total amount of air that can be forcefully exhaled), FEF_{25-75} (rate of airflow in the middle range of total volume), and FEF_{max} (rate of airflow at the highest lung volume). The test that measures restriction is the FVC, or forced vital capacity, the total amount of air that can be forcefully exhaled. Chest radiographs, which were used in several studies, can assess whether inhaled agents have damaged the lungs, usually seen as opacities in the lungs indicating scarring, or inflammation, or a combination of the two.

Little research has been conducted on the possible effects of herbicides and TCDD on the respiratory tract in animals. Studies that are available have focused on enzyme induction, primarily cytochrome P450 in lung tissue (see Chapter 4).

Epidemiologic Studies of Respiratory Disorders

Occupational Studies

The occupational studies assessing respiratory outcomes can also be divided into mortality and morbidity studies. Mortality from respiratory disease was not elevated in studies of production workers of phenoxy herbicides and chlorophenols (Coggon et al., 1991), pesticide application (Blair, 1983), soil/forest conservationists (Alavanja et al., 1989), and MCPA manufacturing/spraying (Coggon et al., 1986).

In one study examining health outcomes among 204 workers involved in 2,4,5-T production as compared to 163 nonexposed workers from the same plant, results showed that the exposed group was older, had more retirees and terminated employees, and differed in education (Suskind and Hertzberg, 1984). Exposed and unexposed workers had similar frequencies of smokers, but for both smokers and ex-smokers, the pack-years were greater among those exposed, possibly reflecting age differences between the groups. There were also more reported and observed cases of chloracne among the exposed. No exposure-related differences were seen in chest radiograph findings. Among current smokers, the frequencies of abnormal forced expiratory volume (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and forced mid-expiratory flow rate (FEF_{25-75}) were significantly higher among those exposed than those unexposed. After adjustment for the number of pack-years smoked, the number of abnormal pulmonary function values were increased among the exposed group. Means predicted for FEV_1, FEV_1/FVC, and FVC, adjusted for smoking, were significantly lower among the exposed. For FEV_1, the mean was significantly lower in smokers.

In another respiratory morbidity study (Calvert et al., 1991), 281 workers involved in the manufacture of TCDD-contaminated products at two plants were compared with 260 neighborhood controls. Exposed workers

had higher serum TCDD levels, but there was no difference between exposed and control groups in chest/lung physical examination results, chronic bronchitis (as defined by the American Thoracic Society), chronic obstructive pulmonary disease defined by abnormal results for FEV_1 and FEV_1/FVC ratio, or in any pulmonary function result (FEV_1, FVC, FEV_1/FVC ratio).

Environmental Studies

The limited available data concerning respiratory effects of environmental exposure to herbicides come from two mortality studies following the accidental release in 1976 of TCDD in the area of Seveso, Italy (Bertazzi et al., 1989a,b). Neither study showed significant differences in respiratory outcomes attributable to TCDD exposure.

Vietnam Veterans Studies

Several studies have examined mortality due to nonmalignant respiratory diseases among Vietnam veterans. In the CDC Vietnam Experience Study (Boyle et al., 1987; CDC, 1987), five deaths from respiratory diseases (ICD codes 460-519) were observed among the U.S. Army veterans who had served in Vietnam, compared with four deaths among U.S. Army veterans who served in the United States, Korea, or Germany during the same period. Among Australian Army Vietnam veterans (Fett et al., 1987), the number of deaths from respiratory disease was very low; only 1 of 270 deaths was observed among the Vietnam veterans and 1 among the Vietnam era veterans who had served in Australia. In a study with larger numbers of observed deaths due to respiratory disease (531 among Army Vietnam veterans and 93 among Marine Vietnam veterans), Watanabe and colleagues (1991), reported no significant differences in PMRs for respiratory diseases when comparing U.S. Army Vietnam veterans with U.S. Army Vietnam era veterans and all Vietnam era veterans or when comparing U.S. Marine Vietnam veterans with U.S. Marine Vietnam era veterans and all Vietnam era veterans. In another proportionate mortality study comparing Wisconsin Vietnam veterans with Wisconsin Vietnam era veterans, the authors reported an increase in PMR among Vietnam veterans due to pneumonia (Anderson et al., 1986). Eight deaths were observed, compared with four expected, for a PMR of 2.0 (CI 1.1-4.0), but the authors warn that small numbers make the PMRs unstable. Since the observed deaths covered the period from 1964 through 1983, the authors raise the possibility that the pneumonia deaths may have been due to complications of combat-related wounds. The PMRs for all respiratory diseases taken together showed no significant differences

when Vietnam veterans and Vietnam era veterans were compared (PMR = 1.0, CI 0.5-1.8).

Other studies have examined morbidity due to respiratory diseases among Vietnam veterans. In the VES study of U.S. Army veterans compared to U.S. Army Vietnam era veterans who had not served in Vietnam (CDC, 1988), there were no differences between the groups in pulmonary abnormalities found on chest x-rays or on pulmonary function tests, both unadjusted and adjusted for current smoking status. A study of monozygotic twins who served in the U.S. military during the Vietnam era showed that service in Southeast Asia was not associated with increased self-report of respiratory conditions (Eisen et al., 1991). Veterans who had served in Southeast Asia from 1961 to 1975 were compared to veterans who served elsewhere during the same period (Stellman et al., 1988). The authors reported no significant differences between the groups in self-report of physician-diagnosed chronic bronchitis occurring no earlier than one year prior to discharge from military service. Among those who served in Southeast Asia, herbicide handlers did not differ from those who did not handle herbicides in frequency of chronic bronchitis. Among nonherbicide handlers, the level of Agent Orange exposure, based on a score derived from self-reported service locations and Air Force and Army records of spraying locations, was not significantly associated with chronic bronchitis. In another study (Pollei et al., 1986) no differences were reported in six types of chest x-ray abnormalities between 422 Vietnam veterans from the Agent Orange Registry at the Albuquerque Veterans Administration medical center compared to 105 Air Force flight staff who did not serve in Vietnam. There were no obvious differences in distribution of chest x-ray abnormalities in a subset of 27 Vietnam veterans who recalled handling Agent Orange repeatedly, although the number of those with abnormalities was small.

A mortality study of 894 Chemical Corps personnel who served in Vietnam compared to the U.S. male population (Thomas and Kang, 1990) reported one observed death from respiratory disease, whereas 1.5 were expected. That study is of limited use in evaluating respiratory mortality primarily because of the small number of observed deaths.

The latest Ranch Hand pulmonary assessment, examined the following parameters: questionnaire data on the presence of five reported respiratory illnesses—asthma, bronchitis, pleurisy, pneumonia, and tuberculosis; physical examination data on asymmetric expansion, hyperresonance, dullness, wheezes, rales, and a composite including all the others; chest x-rays read as normal or abnormal; and pulmonary function test results including FVC, FEV_1, FEV_1/FVC ratio, and FEF_{max} (AFHS, 1990, 1991b). There was no suggestion of an herbicide effect on the five reported respiratory illnesses based on results from the 1987 follow-up exam. Regarding the pulmonary assessment, the investigators reported that the "health of the two groups

was reasonably comparable based on the clinical and laboratory variables, although the Ranch Hands had a significantly higher percentage of thorax and lung abnormalities on examination than the comparisons, based on the unadjusted analysis, and a marginally higher percentage after adjustment for covariates" (AFHS, 1991b:22).

In addition to the 1987 Ranch Hand pulmonary assessment (AFHS, 1990), further analysis of the 1987 pulmonary outcomes was performed to assess associations with serum TCDD levels (AFHS, 1991b, vol. 7). According to these analyses, there was no evidence of a TCDD effect on the five verified respiratory illnesses or on chest x-ray results. There were significantly increased risks or marginally significant increased risks for each of the physical examination variables in at least one adjusted analysis. Initial serum TCDD was significantly associated with decreases in FVC, FEV_1, and FEF_{max}, and an increase in FEV_1/FVC ratio. Models assessing current serum TCDD levels and adjusted for covariates also showed these significant associations. The authors suggested that differences in mean spirometric indices were not clinically significant and might be due in part to associations between body fat and serum TCDD levels. The most recent updated reports of Ranch Hand mortality data have included no information about nonmalignant respiratory disease mortality (AFHS, 1991b; Michalek et al., 1990).

Summary for Respiratory Disorders

Among the morbidity studies, strong rationales for examining respiratory outcomes were not given. However, in the case of occupational exposures or exposures of military personnel who performed spraying, the respiratory tract could be viewed as a target organ for aerosol or other particulate deposition. Several studies provided spirometry data including FEV_1, FVC, and FEV_1/FVC ratio (Suskind and Hertzberg, 1984; CDC, 1988; AFHS, 1991b; Calvert et al., 1991). Results of chest physical examinations were reported in some studies (AFHS, 1991b; Calvert et al., 1991). Although chest radiograph results were provided in several studies, there was no uniform reporting system used, such as the International Labor Organization classification system. For example, in one study (AFHS, 1991b), chest films were classified as either normal or abnormal. In another (CDC, 1988), clinical descriptions were provided by radiologists. In another (Pollei et al., 1986), scoring for six specific abnormalities was performed by three radiologists. Questionnaires used to assess smoking histories and various respiratory symptoms, and the resultant symptoms reported from questionnaire data, also varied across studies. The lack of working hypotheses about respiratory disease outcomes associated with herbicides and the nonuniformity

in methods and reported results make it difficult to interpret much of the morbidity data, especially symptom report and radiographic data.

Any study examining respiratory outcomes such as symptoms of chronic bronchitis, decrements in lung function, or radiographic abnormalities must take into account the effects of cigarette smoking. Smoking data were generally not available in the mortality studies, but smoking was taken into account to various degrees in most of the morbidity studies.

Interpretation of many of the mortality studies is limited by the small number of deaths observed. These studies also tend to use the ICD codes for all respiratory diseases, codes 460 to 519, as the respiratory mortality outcome. These codes include all diseases of the respiratory tract. For example, the following are among the diseases covered by these codes: acute respiratory infections, other diseases of the upper respiratory tract, pneumonia, influenza, chronic bronchitis, emphysema, asthma, pleurisy and pneumoconiosis. The combination of this wide range of diverse conditions and the small number of total deaths makes it difficult to assess any particular respiratory outcome of interest using mortality studies. In some studies, the ICD codes used were not stated, making comparisons with other studies difficult.

Conclusions for Respiratory Disorders

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides* (2,4-D; 2,4,5-T and its contaminant TCDD; cacodylic acid; and picloram) and the following respiratory outcomes: mortality from respiratory diseases; symptoms or history of respiratory illnesses such as chronic bronchitis, bronchitis, asthma, pleurisy, pneumonia, tuberculosis, and respiratory conditions; abnormalities on lung or thorax physical examination; pulmonary function test results; and chest radiographs.

Biologic Plausibility

Limited information from animal studies is available on the potential association between respiratory diseases and exposure to TCDD or the herbicides.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental,

and veterans studies that have been reviewed, inadequate control for important confounders in these studies, and the lack of information needed to extrapolate from the level of exposure in the studies reviewed to that of individual Vietnam veterans, it is not possible for the committee to quantify the degree of risk likely to have been experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

NOTE

[*] The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

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12

Research Recommendations

The committee's review of the existing literature has identified some health outcomes having likely or possible associations with herbicide and/or TCDD exposure and other outcomes for which the data are simply insufficient (Chapters 8 through 11). Even where an association with herbicide exposure is likely, the magnitude of the risk remains uncertain, especially for Vietnam veterans. Given this scientific uncertainty, the second part of the committee's charge—to assess the need for continued research efforts—is timely and appropriate.

As stated in Public Law 102-4, the committee must consider the available scientific evidence, the value and relevance of scientific information that could result from additional studies, and the cost and feasibility of carrying out such studies. Consistent with this mandate, two considerations have guided the committee's research recommendations. First, as discussed in Chapter 6, the interpretation of existing epidemiologic studies is compromised by the frequent lack of appropriate individual measures of exposure to herbicides or TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). This is especially true for studies of Vietnam veterans, yet the committee believes that there may be substantial numbers of veterans who have been exposed to levels of herbicides equivalent to workers in the occupational studies that the committee reviewed. However, as outlined in Chapter 6, the committee believes that it is possible to develop better exposure measures for Vietnam veterans, for use in any future research efforts. Second, as Chapters 8 through 11 have shown, substantial scientific uncertainty about the health effects of herbicides and TCDD remain, especially with regard to Vietnam

veterans, despite the number of existing studies. Thus, the committee concluded that a series of epidemiologic studies of Vietnam veterans could yield valuable information if a new, valid exposure reconstruction model could be developed.

The committee's research recommendations focus on studies in Vietnam veterans rather than on general toxicologic research or on epidemiologic studies of occupationally or environmentally exposed populations. The substantial amount of research on the toxicology and epidemiology of herbicides and herbicide components already under way in the United States and abroad, although not targeted specifically to Vietnam veterans, is likely to contribute to the knowledge of potential health effects in veterans.

ANALYSES OF EXISTING DATA AND RESEARCH PROGRAMS

Much can be learned by reanalysis of existing data and by more in-depth analysis of data from ongoing research programs that investigate the health of Vietnam veterans. Studies of particular interest include the Air Force Ranch Hand study and the Department of Veterans Affairs (DVA; formerly the Veterans Administration) studies of other highly exposed Vietnam veterans such as members of the Army Chemical Corps.

Studies of Highly Exposed Vietnam Veterans

Recommendation 1. The committee endorses continued follow-up of the Air Force Ranch Hand cohort and its comparison group, and recommends that members of the Army Chemical Corps and an appropriate comparison group be followed in a similar study. An independent, nongovernmental scientific panel should be established to review and approve a new, expanded research protocol for both study populations, and to commission and direct a common analysis of the results.

The Ranch Hand cohort includes the Vietnam veterans with the highest documented exposure to herbicides, and the committee endorses continuation of the planned follow-up, including both mortality and morbidity studies. Because they will be available at three points in time, the serum TCDD measurements from this study are particularly important to better understand TCDD metabolism and to assess its use as a biomarker to further analyze health effects. Studies of individual characteristics and other factors affecting TCDD metabolism are particularly important and should be encouraged. This knowledge will lead to more appropriate analyses of the Ranch Hand data and other studies that use serum TCDD gathered years after exposure as a biomarker.

A major limitation of the Ranch Hand study, however, is the small size

of the cohort—1,261 men at the start of the study, 995 who completed the 1987 physical exam (Michalek et al., 1990). Studies of other groups, in addition to the Ranch Hand, that may also have been highly exposed to herbicides in Vietnam would be valuable. One such group is the Army Chemical Corps, which has been studied by the DVA (Thomas and Kang, 1990).

Members of the Army Chemical Corps were responsible for the storage, preparation, and handling of herbicides and other chemicals. Although they were exposed to a wider range of chemicals, the level and intensity of herbicide exposure may have been similar to those of the Ranch Hand cohort. In their first study, Thomas and Kang (1990) identified 894 members of this group and obtained vital status for 745. According to a recent abstract, Dalager and Kang (1993) have now identified 2,954 Army veterans who held chemical operation positions in Vietnam. Continued follow-up of the members of this group would substantially increase the size of the highly exposed population of Vietnam veterans and would yield greater statistical power to detect less common health outcomes. Prompt collection and storage of biological samples and determination of serum TCDD levels for these additional highly exposed veterans would enhance future epidemiologic studies of health outcomes.

Further study of both groups is likely to yield information on a number of health outcomes for which additional research is needed. Priorities for specific health outcomes are discussed after recommendation 6. However, to be useful for this purpose, a common protocol must be followed for both groups. Thus, it is important that a new and expanded protocol for both study populations be developed to include sufficient data for assessing health outcomes.

The high participation rate of veterans in the Ranch Hand study (84 percent) is a credit to both the Air Force and the Ranch Hands themselves. However, the study could benefit from improved methods of analysis and presentation of results both for existing data and for data obtained in the future. Difficulties with interpreting these analyses, as exemplified in the recent reproductive effects report from the Ranch Hand study (AFHS, 1992), along with specific suggestions for further analyses, appear in Appendix C.

As a committee of the National Academy of Sciences noted in a review of the proposed protocol for the Ranch Hand study (NRC, 1980), public perception of the federal government's interest in the outcome of the study results suggests the need for studies of the health of Vietnam veterans to be conducted by a nongovernmental organization. Ranch Hand's excellent participation rate argues that components of the Department of Defense (DOD) or the DVA continue to conduct the follow-up examinations of the Ranch Hand and Army Chemical Corps cohorts. However, an independent, nongovernmental scientific panel is needed to oversee the analyses of the

resulting data in order to satisfy the public's concern about impartiality and scientific credibility. Such a panel could commission others to perform the analyses or arrange to conduct the analyses itself. The panel should also ensure that data from the study are made available to others for secondary analyses within a reasonable time frame and with appropriate safeguards for individual confidentiality.

FEASIBILITY OF NEW EPIDEMIOLOGIC STUDIES OF VIETNAM VETERANS

As discussed in Chapter 6, one of the major problems with the interpretation of existing studies is the frequent lack of appropriate measures of exposure to herbicides or TCDD; however, the committee finds that it may be possible to develop better exposure measures for Vietnam veterans. In particular, Chapter 6 proposes measures that are not dependent on serum TCDD levels (which the committee finds inappropriate for the full range of herbicide exposures) but instead recommends the use of less formal sources of historical information about base perimeter spraying and other relevant exposures, as discussed below in Recommendation 4. Thus, the committee concludes that certain further research efforts using new measures of exposure to herbicides in Vietnam are both necessary and potentially feasible. However, each of the possible measures that the committee has considered involves some degree of nondifferential misclassification bias, and the effect of this bias on risk estimates would likely be to underestimate true effects if they existed, possibly to the point that they would not be detected. In particular, the committee recommends that the following steps be taken prior to undertaking new epidemiologic studies of Vietnam veterans, for the reasons described below.

Recommendation 2. The Department of Defense and the Department of Veterans Affairs should identify Vietnam service in the computerized index of their records.

Chapter 3 notes that Vietnam service is not a "flagged item" on the computerized index of military personnel records archived at the National Personnel Records Center in St. Louis, Missouri, which is maintained by the General Services Administration under an agreement with the Department of Defense. Therefore, the computerized index of the record system does not allow for searches or selection of records of individuals who have served in Vietnam. The lack of an indicator of Vietnam service complicates every epidemiologic study based on military records and leads to methodologic inconsistencies among studies in defining the population under consideration. Adding this indicator to the computerized data base would facilitate future mortality studies based on computerized records, thereby increasing

accuracy and decreasing cost, and would also simplify other epidemiologic studies of health outcomes in Vietnam veterans. All servicemen and women who were stationed in Vietnam or in the Vietnam theater during the Vietnam era should be identified in the records.

Recommendation 3. Biomarkers for herbicide exposure should be developed further.

Considerable uncertainty remains about the use of current or future serum TCDD levels as indicators of past exposure to TCDD in Vietnam veterans. Further research on the toxicokinetics of TCDD is needed to permit more accurate extrapolation from current serum TCDD measurements to past exposures. The multiple serum measurements from the Ranch Hand study group and other highly exposed groups should be particularly useful in this research.

An individual's serum TCDD level, however, is at best an indicator of past exposure to TCDD; it does not necessarily reflect general exposure to all herbicides used in Vietnam. Further refinement of currently available TCDD biomarkers and improved understanding of TCDD metabolism so that these measures can be appropriately included in statistical analyses, as well as development of new biomarkers for exposure to herbicides per se, would also be useful.

Recommendation 4. A nongovernmental organization with appropriate experience in historical exposure reconstruction should be commissioned to develop and test models of herbicide exposure for use in studies of Vietnam veterans.

Exposure assessment has been a weak aspect of most epidemiologic studies of Vietnam veterans. The military reports and personal testimony reviewed by the committee suggest that a sufficient range of exposure to herbicides may exist among Vietnam veterans for valid epidemiologic studies of certain health outcomes, and the committee believes that it is possible to develop valid exposure reconstruction models for epidemiologic studies using methods of historical exposure reconstruction. Such models would estimate the likelihood that each individual veteran was exposed to herbicides in Vietnam and possibly quantify their degree of exposure. These models (described in more detail in Chapter 6) would incorporate information from existing military records about troop movements and herbicide spraying (including, but not limited to, the HERBS and Services HERBS tapes). The models must also include less formal sources of historical information about ground and perimeter spraying, such as records of herbicide shipments to individual bases from the major shipping depots, and consideration of the types of terrain, typical foliage, and military mission of the bases or troops located there. Supplemental information gathered from

surveys of military officers with first-hand knowledge of herbicide operations in Vietnam, such as the 1971 survey conducted by the Army (U.S. Army, 1972) could also be incorporated into the exposure model.

Historical exposure reconstruction is a well-developed specialty in occupational health research that requires substantial professional judgment. The committee recommends that the DVA arrange for a nongovernmental organization with appropriate experience in historical exposure reconstruction to develop and test potential models of herbicide exposure for use in studies of Vietnam veterans. This group will need access to DOD and DVA records to carry out this work.

Recommendation 5. The exposure reconstruction models developed according to Recommendation 4 should be evaluated by an independent, nongovernmental scientific panel established for this purpose.

Herbicide exposure reconstruction models for Vietnam veterans must be evaluated thoroughly before epidemiologic studies based on these models proceed. The committee has identified three possible approaches to such an evaluation, which are discussed in more detail in Chapter 6: (1) internal consistency checks, (2) comparisons of model exposure measures with serum TCDD measurements, and (3) assessment of the association between exposure reconstruction measures and health outcomes shown in occupational or environmental studies to be associated with herbicides. Scientific judgment is required in interpreting the results of such an evaluation, so the committee cannot specify explicit criteria for acceptance or rejection of the new exposure reconstruction models in advance of their development and testing.

The committee recommends that an independent, nongovernmental scientific panel be established to review the results of the proposed evaluation studies and judge the validity and feasibility of the exposure reconstruction models. This panel should have expertise in historical exposure reconstruction and epidemiology. To maintain the public and scientific credibility of the study, the panel members should be nongovernmental and independent of the organization that develops the exposure reconstruction models.

Recommendation 6. If the scientific committee proposed in Recommendation 5 determines that a valid exposure reconstruction model is feasible, the Department of Veterans Affairs and other government agencies should facilitate additional epidemiologic studies of veterans.

A number of possible epidemiologic studies could provide additional information on the health effects of exposure to herbicides in Vietnam beyond what is already known. Highest research priority should be given to those health effects for which additional study is likely to change the balance of the evidence for or against an association. This includes

a. health outcomes for which current evidence is limited/suggestive of an association (lung and respiratory cancers, prostate cancer, and multiple myeloma);
b. health outcomes for which current evidence is insufficient or inadequate to determine whether an association exists, but which, in the committee's judgment, are plausible based on animal toxicologic data (such as nasal/nasopharyngeal cancer) or for which there are known associations with related chemical compounds in humans (such as liver cancer and polychlorinated biphenyls);
c. health outcomes for which the typical age at onset has not yet been reached by members of the Vietnam veteran cohort (such as prostate cancer).

The committee also recommends that priority be given to additional research on reproductive outcomes that would help clarify the possible effects of herbicides. Since Vietnam veterans are expected to have relatively few additional children because of their age, reanalyses of existing reproductive data, especially those based on registries, with the new exposure reconstruction measures proposed in this chapter, would be especially relevant. In particular, the committee believes that extensive reanalysis of the Ranch Hand reproductive data could shed additional light on these questions (see Chapter 9 Appendix C).

Although there is sufficient evidence of an association between occupational or environmental exposure to herbicides and non-Hodgkin's lymphoma, Hodgkin's disease, and soft tissue sarcomas, the existing information on dose-response relationships is incomplete, especially with regard to Vietnam veterans. If a valid exposure reconstruction method can be developed, it might be applied to the exposure data available from existing case-control studies to provide additional dose-response evaluations. Further refinement of the clinical and pathological definitions of soft tissue sarcomas in epidemiologic studies would also help to determine which of the specific cancers in this class are associated with herbicides and/or TCDD.

The exposure reconstruction models to be developed could be used in either case-control or cohort studies. The type of study design will depend on the health outcome being investigated. Rare health effects, for instance, will likely require case-control studies, as described in Chapter 5.

The cost of these epidemiologic studies will depend on the study design. A design based on an exposure reconstruction model applied to computerized troop location records using existing mortality data or health outcome data could be relatively inexpensive. Adding detailed record review by experts or analysis of clinical or morbidity data would substantially increase costs. A study design involving serum TCDD measurements would also increase cost, if current costs for the biochemical assays are not reduced.

Although the number of women Vietnam veterans may be too small to provide adequate statistical power in a study by themselves, the committee believes that women Vietnam veterans should be included in Vietnam veteran studies whenever appropriate.

The committee recognizes that the recommendations for development of a historical exposure reconstruction model and its use in epidemiologic studies might seem at variance with the Centers for Disease Control (CDC) (Pirkle, 1993), White House Agent Orange Working Group (AOWG) (Young et al., 1986), and Office of Technology Assessment (OTA) (Gibbons, 1987) conclusions with regard to the congressionally mandated Agent Orange Study. The committee has come to a different conclusion for four reasons: First, the CDC-AOWG-OTA conclusions were based in large part on serum TCDD measurements, which the committee feels are insufficient for validating exposure to herbicides used in Vietnam, as explained in Chapter 6. Second, the arguments underlying the earlier conclusion that individuals in combat units were widely dispersed and that troop movement data are incomplete imply that exposure measurements may be imprecise, not that they are invalid. However, these arguments do suggest that historical reconstruction of exposure will have some degree of nondifferential misclassification bias, and the effect of this bias on risk estimates would likely be to underestimate true effects if they existed, possibly to the point that they would not be detected. Third, the committee is proposing the use of more, but less formal, information on exposure than was considered in 1986. This includes the development and use of informal information on perimeter spraying, which might account for more meaningful herbicide exposure than the aerial spraying documented on the HERBS tapes. Finally, the committee does not know whether the approach it proposes will prove valid or whether new methods will identify a sufficient number of highly exposed Vietnam veterans for an epidemiologic study. In the committee's judgment, however, the likelihood that this approach will be successful is sufficient for it to be recommended.

MANDATED RESEARCH EFFORTS

For the purposes of further research on the health effects of Vietnam service, Public Law 102-4 mandates that the DVA establish four specific programs: (1) a scientific research feasibility studies program; (2) a system to compile and analyze clinical data obtained in connection with the examination and treatment of certain Vietnam veterans; (3) a tissue archiving system; and (4) a program of blood testing for certain Vietnam era veterans. These programs are subject to initiation, continuance, or discontinuation depending on the findings of this Institute of Medicine report, as well as logistical and funding considerations, and the committee is charged with

making recommendations about specific mandates. The DVA has no specific plans for any of these research efforts beyond the minimal descriptions given in the law, so the committee is able to comment on them in only the broadest terms.

With regard to the need for a series of scientific "studies on the feasibility of conducting additional scientific research on" health hazards resulting from exposure to TCDD and herbicides used in Vietnam, the research mandate in section 8 of Public Law 102-4, the committee feels that a series of epidemiologic studies of veterans could yield valuable information if a new, valid exposure reconstruction model can be developed. The committee finds value in continuing the existing Ranch Hand study and expanding it to include Army Chemical Corps veterans. As discussed above, however, these studies should be carried out with some independence from government agencies.

Section 6 of Public Law 102-4 requires the DVA to "compile and analyze, on a continuing basis, all clinical data" that (1) are obtained in connection with examinations and treatment furnished by the DVA to certain veterans, and (2) are likely to be scientifically useful in determining the association between disabilities experienced by these veterans and exposure to TCDD or herbicides. Such a system, called the Agent Orange Registry (see Chapter 2), currently exists. Section 7 of the law calls for the establishment of a system for the collection and storage of voluntarily contributed samples of blood and tissue of veterans who served in Vietnam.

Stored biological samples and clinical data offer some promise for future research efforts. They could, for instance, form the basis for future nested case-control studies of particular diseases. For example, as Vietnam veterans age, studies of the incidence of prostate cancer could compare TCDD levels in stored serum for men with and without the disease. The benefit of such a program is that the cost of storing serum (approximately $50 per veteran per year) is substantially less than that of measuring its TCDD level (almost $1,000 per sample with current technology). It is also possible that new scientific assays or other measurement methods developed in the next 10 to 20 years could be applied to stored serum and tissue, and the resulting analyses might shed light on some of the remaining uncertainty.

To be scientifically valid, however, a study based on stored biological samples or clinical data must be designed with a sampling plan appropriate for the hypotheses to be tested (see, e.g., NRC, 1991). Stored samples are valuable, for instance, in identified high-exposure cohorts, such as the Ranch Hands, the Army Chemical Corps, and perhaps other study groups, so that health effects can be compared to their level of TCDD exposure. By contrast, storage of biological samples or medical records from self-selected individuals, or from those who feel that they are suffering from disabilities

due to herbicide exposure, is unlikely to yield scientifically valid information on the health effects of herbicides; any epidemiologic study based on these data would suffer from misclassification bias (see Chapter 5).

Over the last 15 to 20 years, the sampling and storage of human biologic specimens for later analysis in scientific studies have received increasing attention. A 1993 conference on human tissue monitoring and specimen banking, sponsored by the U.S. Environmental Protection Agency and the University of North Carolina School of Public Health, Chapel Hill, explored state-of-the-art banking techniques, design, and ethical considerations. Human tissue banks in the United States and abroad have become a valuable resource for obtaining baseline or historical measurements of toxic substances, and for monitoring and assessing trends in human exposure to environmental or occupational chemicals. Results obtained to date indicate that human tissue monitoring may become increasingly informative, provided that difficult issues of system design, quality control, and ethics are resolved.

Balancing the strengths and weaknesses of stored biological samples and clinical data for research purposes, the committee feels that systems of this sort have scientific value, but only to the extent that they are components of specific, well-designed studies. In the absence of a clear study design to guide such activities—and without resolution of the design, quality control, and ethical issues—the committee does not recommend the establishment of the clinical data and tissue archiving systems described in sections 6 and 7 of the law at this time.

The final mandate in Public Law 102-4 (section 9) on which the committee must comment calls for testing the serum TCDD levels of Vietnam veterans who apply for medical care or file a disability compensation claim. The purpose of this mandate is not stated in the legislation. If research purposes are contemplated, the committee's discussion about tissue archiving systems applies, and such a program would not be recommended at this time. It is also possible that this program is intended to provide information on individual exposure to TCDD and/or herbicides to aid in individual compensation decisions. The committee cannot make recommendations for DVA policy, but notes that the finding in Chapter 6 that individual TCDD serum levels are usually not meaningful (because of common background exposures to TCDD, poorly understood variations among individuals in TCDD metabolism, relatively large measurement errors, and exposure to herbicides that did not contain TCDD) might apply to this mandate.

OTHER DIOXIN (TCDD)/HERBICIDE STUDIES

Basic toxicologic research on TCDD will further enhance knowledge of its possible human health effects. The committee recommends that basic

research be undertaken to investigate possible differences in sensitivity among humans to TCDD-mediated toxicity. Additionally, mechanistic studies focused on identifying possible predisposing molecular or genetic factors for TCDD-associated effects would be highly significant for risk assessment purposes.

It may be possible to learn more about the health effects of the herbicides used in Vietnam by studying occupationally exposed workers than by studying veterans, because the amount and intensity of exposure to TCDD and herbicides experienced by Vietnam veterans were probably small compared to the exposures of production workers, agricultural workers, and herbicide sprayers. Furthermore, records on which to base exposure measures for some occupational groups may be better than those for veterans. Thus, although the committee is not in a position to make detailed recommendations about the conduct of occupational studies, it notes the potential value of such studies to the DVA and other government agencies. In particular, the committee has found that the occupational studies being conducted by the National Institute for Occupational Safety and Health in the United States and the International Agency for Research on Cancer in Europe and elsewhere (see Chapter 7) have contributed substantially to the evidence base for some health outcomes that the committee reviewed, and the committee endorses continuing follow-up and analysis of these cohorts. Making available the individual level exposure and outcome data from these two studies would be valuable; these data could be pooled and analyzed further with comparable exposure groups and outcome measures. Additional, carefully conducted epidemiologic studies—with adequate sample size to detect elevated associations—of the reproductive history of individuals with occupational or environmental exposure to herbicides and dioxin are also needed.

Studies of the Vietnamese population exposed to herbicides are also possible and potentially useful. These studies might include Vietnamese still in Vietnam or perhaps even those now in the United States. Accurate disease ascertainment and exposure reconstruction may pose difficult problems, however. The actual exposure of this group to herbicides would be extremely difficult to determine accurately because (1) serious problems with recall bias are likely; (2) given war conditions, documents about location during the war in Vietnam are unlikely to be completely available or reliable; and (3) the Vietnamese in question may have relocated in the intervening decades. Current serum TCDD measures may not be practical or relevant for the same reasons discussed above for U.S. veterans. Before significant resources are committed to studies of the Vietnamese population, the committee recommends that feasibility studies of both exposure reconstruction and disease outcome monitoring be conducted.

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APPENDIXES

Appendix A

Information Gathering: Literature Searches

The primary charge to this committee was to analyze the scientific and medical literature published on the health effects of herbicides used in Vietnam. The committee used the following methods to identify, collect, and disseminate the scientific and medical literature that formed the basis of its review.

COMPUTERIZED BIBLIOGRAPHIC DATA BASES

The initial focus of the committee's efforts was a comprehensive search of relevant computerized data bases. Sixteen data bases covering biomedical, toxicological, chemical, historical, and regulatory information were accessed utilizing Dialog, a commercial data base vendor, and the National Library of Medicine's (NLM's) Medical Literature Analysis and Retrieval System (MEDLARS). The majority of the data bases searched were bibliographic (Table A-1), providing citations to scientific literature. Factual data bases (Table A-2) were also searched to provide toxicological and chemical information.

To maximize retrieval, the search strategy incorporated the broad terms "phenoxy herbicides" and "dioxin," specific chemical names (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2,4-dichlorophenoxyacetic acid; 2,4,5-trichlorophenoxyacetic acid; dimethylarsinic acid; 4-amino-3,5,6-trichloropicolinic acid), abbreviations (TCDD; 2,4-D; 2,4,5-T), and synonyms (Agent Orange, picloram, cacodylic acid). The search term "herbicides/adverse effects" was also used to increase the comprehensive retrieval of the search. Accuracy

was enhanced in applicable data bases by using Chemical Abstracts Service Registry Numbers, which uniquely identify each individual chemical. Individual searches were customized to reflect the structure of each data base. For MEDLARS data bases, searching was done on the standardized terminology and alphanumeric designators for each chemical found in NLM's Medical Subject Headings (MeSH) and the MeSH tree structures.

TABLE A-1 Bibliographic Data Bases Searched

Biosis
Books in Print
Cancer Lit
Conference Papers Index
Dissertations Abstract
Embase
Enviroline
Environmental Bibliography
Federal Register
Medline
National Institute for Occupational Safety and Health
National Technical Information Service (NTIS)
Pascal
Psych Info
Toxline
World Translations Index

Although there is subject and content overlap, each data base serves a unique function, has a distinct subject emphasis, and indexes literature not available elsewhere. To serve the comprehensive goals of this study, it was decided to search all relevant data bases in their entirety for epidemiologic studies, case reports, and secondary literature (reviews, letters, news articles, etc.). The animal toxicology literature on this subject is extensive, and these studies build on and reference previous work. The committee decided to retrieve all animal toxicology citations from 1980 through the present.

Throughout the study, searches were updated, and targeted searches were done on specific topics such as porphyria cutanea tarda.

TABLE A-2 Factual Data Bases Searched

Chemical Carcinogenesis Research Information System
Developmental and Reproductive Toxicology
Hazardous Substances Data Bank
Integrated Risk Information System
Registry of Toxic Effects of Chemical Substances

OTHER SOURCES

Committee staff examined the reference lists of major review articles, books, and reports for relevant citations. Reference lists of individual articles were also scanned for additional relevant references.

Literature identification was an ongoing process throughout the study. A valuable source of additional information was the input received both in written and in oral form from veterans, interested persons, and speakers at the public hearings and scientific workshops (see Appendix B).

LITERATURE DISSEMINATION

All retrieved citations were entered into the study's bibliographic data base, which at the conclusion of the study contained 6,420 references to the health effects of the herbicides used in Vietnam. Given the large number of documents on this topic, it was necessary to determine a method that would organize the material most effectively and serve as a tool in dissemination of the literature to the appropriate committee members. A list of 41 terms was developed for indexing individual articles. Each epidemiologic study was categorized by the population group exposed (occupational, environmental, veteran, Vietnamese) and the general health outcome. Toxicology references were subcategorized by main topic (e.g., mechanism of action, chemistry, pharmacology, genotoxicity). Updated subject bibliographies were distributed to the committee throughout the study to reflect additions to the bibliographic data base. Copies of papers were requested as needed for the committee's information and analysis.

EPIDEMIOLOGIC STUDIES

At its first meeting, the committee realized that the epidemiologic studies would be a primary source of information for determining associations between individual health outcomes and exposure to herbicides. It was decided that a more detailed approach was necessary in indexing and critiquing these studies, and the committee developed an abstracting form. Information on the form began with descriptions of the study and comparison populations and of the study design. Exposure to herbicides was categorized through source, validation methods, exposure definition, and length of exposure. The form detailed the major health outcome categories, listing cancers by type and site; specific adverse reproductive outcomes; subcategories of neurobehavioral, cardiovascular, respiratory, metabolic, immunologic, and dermatologic disorders; and other health effects. The study's analytical methods were detailed on the form with information including confounding factors, methods used to control confounding, and consideration for latency.

Each epidemiologic study was abstracted by consultants to the committee, and the abstracted data for each study were entered into a computerized data base. The first use of this data base was to sort the studies by health outcome. Completed abstracting forms and studies were then sent to committee members with expertise in that field, who reviewed and verified the abstracted information. Throughout the study, this data base was used extensively to aid committee members in locating relevant studies on a variety of questions ranging from methods of exposure assessment to study design.

Appendix B

Information Gathering: Presentations to the Committee and Outside Meetings

COMMITTEE MEETINGS

The committee sought the fullest range of information and advice from the widest possible spectrum of sources. Given the nature of the controversy surrounding its charge, the committee deemed it vital to convene as many open meetings as possible to provide the opportunity for veterans and veterans organizations, researchers, policymakers, and other interested parties to present their concerns, review their research, and exchange information with committee members. In addition to these open meetings, the committee received special reports and the staff met with various outside experts to gather additional information for the committee.

Presentations

Committee Meeting (June 26, 1992)

At its first meeting on June 26, 1992, the committee heard presentations on background information for the study. In order to understand fully the congressionally mandated charge, the committee heard from Lawrence Hobson, Environmental Agents Service, Department of Veterans Affairs (DVA); William Brew, Senate Veterans' Affairs Committee; and Patrick Ryan, House Veterans' Affairs Committee. Following these presentations, Hellen Gelband, Office of Technology Assessment, provided some historical research background that examined the health effects of exposure to herbicides used in

Vietnam. Nancy Dalager, Environmental Epidemiology Service, DVA, summarized the DVA's research efforts on Vietnam veterans. William Farland, Office of Health and Environmental Assessment, Environmental Protection Agency (EPA), briefed the committee on the EPA's reassessment of dioxin. Upon invitation of the chairman, several other participants provided statements to the committee, including Admiral E.R. Zumwalt, Jr., Agent Orange Coordinating Council; Richard Christian, American Legion; and Laura Petrou, office of Senator Thomas Daschle.

Public Meeting (September 9, 1992)

The committee held a public meeting on September 9, 1992, in Washington, D.C., to solicit scientific information on the health effects of exposure to dioxin and other chemical compounds in herbicides used in Vietnam during the Vietnam era. In order to reach a broad range of interested individuals, notices of the public meeting were sent to nearly 1,000 members of veterans organizations, Congress, government agencies, academic institutions, environmental groups, chemical companies, the pulp and paper industry, medical research associations, and other groups. At the public meeting (see list), which was attended by approximately 100 people, 23 individuals presented oral testimony to the committee. Written testimony was also accepted and given equal weight to oral testimony. The committee received written testimony from 28 individuals (see summary of testimony).

Scientific Workshop (December 8, 1992)

The committee held a scientific workshop on exposure assessment on December 8, 1992, in Washington, D.C. The purpose of the workshop was to discuss and compare methods for estimating exposure to Agent Orange, other herbicides, and dioxin in epidemiologic studies. There were four panel sessions at the workshop to address (1) the military use of herbicides in Vietnam, (2) records-based measures of exposure to herbicides in Vietnam veterans, (3) validation of records-based measures of exposure to herbicides in Vietnam veterans, and (4) biomarkers for exposure assessment. Seventeen invited panelists made presentations at the workshop (see list). In addition to the panelists, approximately 45 individuals from veterans organizations, Congress, government agencies, academic institutions, environmental groups, chemical companies, the pulp and paper industry, medical research associations, and other interested groups attended the workshop.

Committee Meeting (February 8, 1993)

On February 8, 1993, the committee heard presentations on dermatological

disorders found in Vietnam veterans from Howard Maibach and Richard Odom, both faculty members in the Department of Dermatology, School of Medicine, University of California, San Francisco. The committee also held an open session to hear from five individuals about their personal experiences in different branches of military service in Vietnam. The committee heard presentations from Rockne Harmon, who spoke about the U.S. Navy River Patrol Force; William Lewis, who spoke about the U.S. Marine Corps; Jack Spey, who spoke about the U.S. Air Force Ranch Hand Operation; R.W. Trewyn, who spoke about the U.S. Army Infantry; and Linda S. Schwartz, who provided information on the experience of women with the U.S. military and civilian relief agencies in Vietnam.

Special Contributions

The committee received four reports from different groups that required special attention and are summarized below.

Report on the Vietnam Veteran Agent Orange Health Study

On April 9, 1993, the committee received a report summarizing the preliminary results from a health survey conducted by the Vietnam Veteran Agent Orange Health Study (VVAOHS). A questionnaire, distributed to Vietnam veterans residing in the United States and Puerto Rico, and one veteran residing in Australia, asked about any illnesses or symptoms veterans may have suffered since their service in Vietnam. These symptoms and illnesses include psychological effects, cancer, nervous system disorders, respiratory effects, digestive and metabolic disorders, reproductive effects, skin problems, and a variety of miscellaneous symptoms including diabetes, unexplained high fevers, and lightheadedness. As of April 1993, VVAOHS had received 658 completed questionnaires from veterans or their families. Preliminary results tabulated from 250 completed questionnaires indicate that birth defects (cleft palate, bone deformities, and learning disabilities), miscarriages, psychological and nervous system disorders, and skin rashes are frequently reported.

Reports on the National Vietnam Veterans Birth Defects/Learning Disabilities Registry and Data Base

On April 13, 1993, the committee received reports on the National Vietnam Veterans Birth Defects/Learning Disabilities Registry and Data Base, which is a collaborative effort of the New Jersey Agent Orange Commission and the Association of Children's Birth Defects. These reports were prepared independently and present two parallel interpretations of the data.

This project is an effort to collect and assess data on birth defects and learning disabilities among children of Vietnam veterans, and to identify patterns of disability and clustering of these patterns within certain veterans' subgroups. The project compares children of Vietnam veterans to children of nonveterans in an attempt to assess group differences. The project utilizes an 11-page questionnaire, distributed through both agencies. Data collection and analyses are ongoing. Additional information on this project is included in Chapter 9.

An Appraisal of Military Records Available for Research on the Health Effects of Herbicides Used During the Vietnam War

In response to a request from the Institute of Medicine, Jeanne and Steven Stellman prepared a report on their appraisal of military records available for research on the health effects of herbicides used during the Vietnam war, which the committee received on February 4, 1993. The report addresses two major issues: part 1 describes use of military records for identifying troop movement and herbicide sprays, and part 2 reviews the use of surrogate measures of herbicide exposure.

Part 1 describes the extensive military data available on the use of herbicides and the location of American troops in Vietnam during the Vietnam war. The Stellmans discuss both primary and secondary data bases, and methods for linking herbicide use and troop location data. Also included is a discussion of problems of misclassification of exposure and their implications for epidemiologic and clinical research.

Part 2 discusses surrogate measures of exposure that have been used in health research studies of Vietnam veterans. The advantages and limitations of such surrogates are reviewed. The Stellmans also discuss extensively their evaluation of the Centers for Disease Control (CDC) Agent Orange Validation Study, the use of serum TCDD (2,3,7,8-tetrachlorodibenzo*p*-dioxin) as an exposure marker, selection process for the validation study, military records data, and data analysis. The Stellmans note that the CDC study has served as the basis for the abandonment of government epidemiologic investigations that utilize the military records data base. The Stellmans explore both the credibility of the hypothesis and the results obtained in the study.

Response to the Concerns Raised in the Stellmans' February 1993 Report

In response to a request from the Institute of Medicine (IOM), the Centers for Disease Control, on April 14, 1993, reviewed the February 1993 Stellmans' report to the IOM on an appraisal of military records available for research on the health effects of herbicides used during the Vietnam war.

CDC's report is organized into five major areas that correspond to issues raised in the Stellmans' report.

1. The scientific basis for using serum TCDD to assess exposure to dioxin in Vietnam veterans: CDC notes that epidemiologic studies of human exposure to dioxin conducted since the Agent Orange Validation Study (the Ranch Hand Study and the NIOSH Workers Study) have provided additional support for the use of serum TCDD levels obtained in 1987 to assess previous TCDD exposure in Vietnam veterans.
2. Military records data: CDC discusses the Agent Orange Working Group Science Panel's Subpanel on Exposure Assessment's review of the pertinent information on exposure assessment and examination of the additional pilot data that were being developed by the DOD Environmental Services Group. The subpanel recommended "that any study of ground troops, which is dependent upon military records for the assessment of exposure to herbicides, not be conducted without an additional method to verify exposure."
3. Selection procedures for the Validation Study: CDC provides information on the selection procedures for the Agent Orange Validation Study, emphasizing how the sampling of veterans purposefully included over-sampling of veterans with high likelihood of exposure.
4. Data analysis issues: CDC describes the analyses of 1987 TCDD measurements and exposure scores. The purpose of this analysis is to determine whether any of the scores (or some combination of scores) could predict which Vietnam veterans had elevated 1987 TCDD levels, indicating probable exposure to Agent Orange.
5. Results and review of CDC's Validation Study: CDC notes that the Agent Orange Validation Study found no significant association between military records-based indirect estimates of Agent Orange exposure and serum TCDD levels.

Outside Meetings

In addition to its formal meetings, the committee actively sought information from, and explained its mission to, an array of individuals and organizations with interest or expertise in assessing the effects of exposure to herbicides used in Vietnam. Institute of Medicine staff briefed several groups on the study, met with experts, and attended conferences to gather additional information for the committee.

Briefings

IOM staff presented an overview of the history of the National Academy of Sciences and the IOM, the committee process, plans for the study,

and the institutional report review process to the Agent Orange Coordinating Council on April 14, 1992; the Senate Veterans' Affairs Committee staff and other interested congressional staff members on April 23 and May 15, 1992; and the Vietnam Veterans of America's Agent Orange Committee on November 12, 1992.

Outside Experts

IOM staff met with outside experts with interest or expertise in issues dealing with the effects of exposure to herbicides used in Vietnam, including Lawrence Hobson, Layne Drash, Frederic Conway, Hang Kang, Quentin Kinderman, and Donald England, Department of Veterans Affairs; William Brew and Michael Cogan, Senate Committee on Veterans' Affairs; John Brizzi and Sue Forrest, House Committee on Veterans' Affairs; Michael Gough and Hellen Gelband, Office of Technology Assessment; Peter Beach, Department of Health and Human Services; Alvin Young, Department of Agriculture; Don Hackenson, Environmental Services Group, Department of Defense; Theodore Colton, Boston University; James Neel, University of Michigan Medical School; Richard Christian, American Legion; Richard Thomas, National Research Council (NRC); and Shelby Stanton, consultant.

Seminars and Conferences

Committee and staff members attended several seminars and conferences to gather additional material for the study. These included the Brookings Institution Risk Seminars on Dioxin held in Washington, D.C.; the Environmental Protection Agency's *Dioxin Peer Review Workshop: Health Assessment Document* held in Vienna, Virginia; the 1992 American Public Health Association sessions *Reassessing Dioxin's Health Effects* and *The Enduring Health Effects in Southeast Asia* held in Washington, D.C.; a jointly sponsored meeting by the Toxicology Forum and German government, *Current Views on the Impact of Dioxins and Furans on Human Health and the Environment*, held in Berlin, Germany; a conference on *Male-Mediated Developmental Toxicity* held in Pittsburgh; and the seminar *Human Tissue Monitoring and Specimen Banking: Opportunities for Exposure Assessment, Risk Assessment, and Epidemiologic Research* held in Research Triangle Park, North Carolina.

Commissioned Papers

To address all the issues that the committee was required to review, several outside experts were asked to prepare background papers on

- Toxicologic Data for Cacodylic Acid
- Toxicologic Data for Picloram
  Judith Hauswirth and Barbara Neal, Jellinek, Schwartz & Connolly, Inc.
- Toxicity Profile of 2,4-D
- Toxicity Profile of 2,4,5-T
  Gail Charnley, National Research Council
- TCDD's Carcinogenicity in Animals
  Gail Charnley, National Research Council
- TCDD Reproductive Toxicity
  Gail Charnley, National Research Council
- Neurotoxicological Aspects of Dioxin
  John O'Donoghue, Eastman Kodak Company
- Environmental Presence and Persistence of Dioxins
  Christopher Uchrin, Rutgers University
- Exposure to Herbicides and Dioxin and Porphyria Cutanea Tarda (PCT)
  Neville Pimstone, University of California at Davis
- Possible Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) on Autoimmune Disease of Humans
  Noel R. Rose, Johns Hopkins University
- Evaluation of the Frequency of Birth Defects Among the Offspring of Vietnam Veterans
  Lewis Holmes, Massachusetts General Hospital

PRESENTATIONS AT THE PUBLIC MEETING

September 9, 1992

Session I

E.R. Zumwalt, Admiral, U.S. Navy (Ret.)

Ellen K. Silbergeld, University of Maryland at Baltimore

William W. Lewis, New Jersey Agent Orange Commission, Trenton

Betty Mekdeci, Association of Birth Defect Children, Orlando, Florida

Gerald Bender, Agent Orange Program, State of Minnesota Department of Veterans Affairs, St. Paul

Session II

Cate Jenkins, Environmental Protection Agency, Washington, D.C.

Turner Camp, Veterans of Foreign Wars, Washington, D.C.

Michael Harbut, Center for Occupational and Environmental Medicine, Southfield, Michigan

Steven Stellman, American Health Foundation, New York City

Jeanne Stellman, Columbia University School of Public Health, New York City (presented by Steven Stellman)

Session III

Steven J. Milloy, Institute for Regulatory Policy, Washington, D.C.

Rockne Harmon, Alameda, California, and James Harmon, Garden City, New York, Attorneys/Vietnam Veterans

Michael N. Sovick, Oklahoma Agent Orange Foundation, Lexington

James H. Burdge, Sr., Vietnam Veteran/Agent Orange Health Study, Eatontown, New Jersey

Hope Tinoco, Wife of Vietnam Veteran, Olathe, Kansas

Don Braksick, Vietnam Veteran, Leesburg, Florida

Session IV

Harold Jackson, Vietnam Veteran, Houston, Texas

Julio Gonzales, Vietnam Veteran, Chicago, Illinois

Michael Eckstein, Vietnam Veterans of America, Eatontown, New Jersey

Paulette Boland, Widow of Vietnam Veteran, Chicago, Illinois

Mike Guess, Vietnam Veteran, Jerseyville, Illinois

Jennie LeFevre, Widow of Vietnam Veteran, Shady Side, Maryland

Open Session

Steven Lamm, Consultants in Epidemiology and Occupational Health, Washington, D.C.

Chester McKee, Military Order of the Purple Heart, Washington, D.C.

SCIENTIFIC WORKSHOP ON EXPOSURE ASSESSMENT

December 8, 1992

Session 1: The Military Use of Herbicides in Vietnam

Alvin Young, Department of Agriculture

Fred H. Tschirley, Consultant

Session 2: Records-Based Measures of Exposure to Herbicides in Vietnam Veterans

Han Kang, Department of Veterans Affairs

Richard Clapp, JSI Research and Training, Inc.

Steven Stellman, American Health Foundation

Jeanne Stellman, School of Public Health, Columbia University

Richard Christian, The American Legion

Session 3: Validation of Records-Based Measures of Exposure to Herbicides in Vietnam Veterans

Edward Brann, Epidemiology Program Office

Dana Flanders, National Center for Environmental Health

John Karon, National Center for Infectious Diseases

Mark Scally, National Center for Injury Prevention and Control, Centers for Disease Control and Prevention

Session 4: Biomarkers for Exposure Assessment

Joel Michalek and Judson Miner, U.S. Air Force School of Aerospace Medicine

William Lewis, New Jersey Agent Orange Commission

Marilyn Fingerhut, National Center for Occupational Safety and Health, Centers for Disease Control and Prevention

James Pirkle, Center for Environmental Health, Centers for Disease Control and Prevention

Arnold Schecter, State University of New York

TESTIMONY PRESENTED TO THE COMMITTEE (ORAL AND WRITTEN)

Political and Controversial Issues

Gerald Bender, Minnesota Department of Veterans Affairs, St. Paul
Topic: The IOM committee is a scientific entity—not a policymaking one—and must be allowed to make recommendations based solely on established methods of scientific investigation; since certainty can rarely be achieved on scientific issues, the committee might be unable to provide the definite answers on causation desired by the public.

William Bennett, National Vietnam Veterans Coalition, Washington, D.C.
Topic: Directs the committee's attention to various extra scientific material, which sheds light on the conduct of certain studies and other information about the political controversy over Agent Orange.

Don Braksick, Vietnam Veteran, Leesburg, Florida
Topic: Personal account of government conspiracy to cover-up the long-term health effects of Vietnam veterans' exposure to Agent Orange.

Turner Camp, Medical Consultant, Veterans of Foreign Wars, Washington, D.C.
Topic: The history of the Veterans of Foreign Wars involvement in the Agent Orange controversy on behalf of its members. Also a recapitulation of resolutions pertaining to Agent Orange forwarded by its membership for action at the national level.

Joy Towles Cummings, HOPE (Help Our Polluted Environment), Salem, Florida
Topic: Personal account of a cellulose chlorine-using pulp mill, which has been polluting the Fenholloway River in Florida with dioxin for the past 38 years. The fish in the river are contaminated, as is groundwater in the area. The community claims that there is a high cancer risk in the area and many of the children have learning disabilities. The community would like to have an investigation of the health risks of residents living near or downstream of chlorine-using pulp and paper mills.

Michael Eckstein, Vietnam Veterans of America, Eatontown, New Jersey
Topic: The New Jersey State Council's Agent Orange Committee would like the IOM committee to review scientific evidence uncovered by the Environmental Protection Agency, the Pointman projects, the Agent Orange Coordinating Council, and the records of defunct state Agent Orange Commissions. Also noted that the committee should seek the records of Dow Chemical, Diamond Shamrock, and other producers of herbicides used in Vietnam.

Chester McKee, Military Order of the Purple Heart, Washington, D.C.
Topic: Requested, on behalf of the Military Order of the Purple Heart, that the committee take into account, during its deliberations, the plight of the veterans who claim to have been exposed to Agent Orange while serving in the military in Vietnam.

Lawrence Welch, Vietnam Veteran, Havelock, North Carolina
Topic: Reasons for believing that Public Law 102-4 and the Department of Veterans Affairs are in error for excluding chondrosarcoma from recognition as a soft tissue sarcoma associated with exposure to certain herbicides used in Vietnam and, therefore, from compensation.

E.R. Zumwalt, Admiral, U.S. Navy (Ret.), Arlington, Virginia
Topic: The manner in which policy decisions sometimes impact on science to produce flawed scientific results. Also provided the committee with 12 documents for its review including his report to Edward J. Derwinski (former Secretary of Veterans Affairs), court documents, congressional hearings, and federal government memoranda.

Concerns About the NRC Process

Richard Christian, The American Legion, Washington, D.C.
Topic: The American Legion's concerns about how the IOM study should be conducted openly and without conflicts of interest.

Cate Jenkins, Ph.D., Environmental Protection Agency, Washington, D.C.
Topic: Jenkins's 1991 affidavit prepared on behalf of veterans attempting to gain compensation from the manufacturers of Agent Orange, and additional data compiled to support the causal relationship between dioxin and lasting central nervous system effects. Another issue raised was potential and apparent conflicts of interest in selecting individuals to serve on the review committee.

Scientific Issues

Richard Albanese, M.D., U.S. Air Force School of Aerospace Medicine, San Antonio, Texas
Topic: Personal opinion that most herbicide studies have sample sizes that are too small to detect an herbicide effect when such an effect exists and, because of this fact and inadequate treatment of type II error, Vietnam veterans' concerns about herbicides do not receive a fair hearing and judgment.

Malcolm Barr, Toxicologist, Victoria, Australia
Topic: Personal research that has uncovered new evidence implicating certain environmental agents as potential causes of PostTraumatic Stress Disorder in vulnerable individuals with deficient detoxification mechanisms and indicating that certain phenolic herbicides used in Agent Orange could be implicated in Vietnam veterans' psychiatric disorders. He has developed a clinical toxicological model to treat veterans.

Noel Benefield, Waiuku, New Zealand
Topic: Personal submission on the association between exposure of rodents to certain chemical compounds and birth defects.

James H. Burdge, Sr., Founder, Vietnam Veteran/Agent Orange Health Study, New Jersey
Topic: The Vietnam Veteran Agent Orange Health Study, in which questionnaires are sent to veterans and data are compiled from returned questionnaires. He also encouraged the committee to be open to the veterans and their families, and listen to what they have to say about this issue.

Joe Cole, BRAVO/International Independent Agent Orange Network, Olympia, Washington

Topic: Chemicals used in Vietnam during the Vietnam era and a list of health effects thought to be associated with exposure to Agent Orange, along with toxicity data on several other chemicals.

Campbell Colton, Veterans Counselor, Division of Veterans Services, Augusta, Maine

Topic: The Report on the Health and Medical Status of Maine Veterans, May 13, 1991, and a Medical Survey on Radiation and Agent Orange Veterans of Maine, August 1990.

Robert Donnan, Vietnam Veteran, McMurray, Pennsylvania

Topic: Health effects on the Vietnamese population of exposure to Agent Orange need to be evaluated, along with a review of the literature regarding the health effects of exposure to 2,4-D.

Dale Duke, Vietnam Veteran, Albuquerque, New Mexico

Topic: Personal research on the epidemiology of multiple myeloma, along with a review of the literature. Also a personal account of his diagnosis of multiple myeloma and his claim to the Department of Veterans Affairs.

Michael Harbut, M.D., M.P.H., Physician-in-Chief, Center for Occupational and Environmental Medicine, Southfield, Michigan

Topic: Described clinical treatment of three patients (Vietnam veterans), primarily for respiratory problems, autoimmune disorders, dermatological problems, neuromuscular problems, sexual dysfunction, psychological dysfunction, and blood dyscrasia; personal opinion is that a disease syndrome exists as a result of exposure to Agent Orange.

James Kasanos, Vietnam Veteran, Otter Rock, Oregon

Topic: List of toxins used in Vietnam and a summary of reported Agent Orange symptoms and effects.

Steven Lamm, M.D., Epidemiology and Occupational Health Consultants, Washington, D.C.

Topic: Personal observation of various interpretations of the dioxin literature, as well as how the committee should take care to distinguish the differences in exposures (categorically and qualitatively) among various studies.

William W. Lewis, New Jersey Agent Orange Commission, Trenton
Topic: The research efforts of the New Jersey Agent Orange Commission, particularly the Pointman Project. Pointman I was a pilot project designed to determine whether dioxin levels could be found in Vietnam veterans who had handled herbicides. Pointman II examined exposure levels of Vietnam veterans who were not herbicide handlers.

Betty Mekdeci, Executive Director, Association of Birth Defect Children, Orlando, Florida
Topic: The Association of Birth Defect Children has been selected by the State of New Jersey to collect information for the New Jersey Agent Orange Commission on birth defects, learning disabilities, childhood cancers, and other health problems in the children of Vietnam veterans.

Steven J. Milloy, Institute for Regulatory Policy, Washington, D.C.
Topic: The interpretation of low statistical associations in the context of a "weight-of-evidence" approach to causal inference. Given the existing epidemiological studies on dioxin-like compounds, this is a linchpin in the committee's review.

Arnold Schecter, M.D., M.P.H., State University of New York
Topic: Research on Vietnam concerning Agent Orange and dioxin exposure, and possible health effects on Vietnamese (the population most heavily exposed) and on American veterans, from his work coordinating the Michigan and the Massachusetts Agent Orange studies.

Ellen K. Silbergeld, Ph.D., University of Maryland at Baltimore
Topic: The current scientific evidence for the plausibility of biological mechanisms involved in the association of herbicide exposure with certain health effects in humans. Her research shows that there is no basis in molecular biology to postulate or invent putative thresholds in the mechanistic biology and toxicology of dioxin.

Michael N. Sovick, Oklahoma Agent Orange Foundation, Lexington, Oklahoma
Topic: Lists of scientific references as evidence of the association between autoimmune and other immunological disorders (e.g., lupus erythematosus) and exposure to dioxin.

Jeanne Stellman, Ph.D., Columbia University School of Public Health, New York City

Topic: Herbicide exposure assessment and available military information, and the relationship to the HERBS tape and available exposure methodologies. The Stellmans' principal concern with the primary literature is how individual authors estimate exposure and how they relate their findings specifically to Agent Orange. Another concern is with studies utilizing surrogate measures because surrogates as used invariably assign far more men to an exposed status than were really exposed, and thereby dilute the truly exposed population.

Steven Stellman, Ph.D., M.P.H., American Health Foundation, New York City

Topic: Analysis of the available health data including the Stellmans' (see Jeanne Stellman) work. The Stellmans are co-authors of the Columbia University-American Legion Study of Vietnam Veterans, and were the exposure consultants to the Agent Orange Veteran Payment Program for the U.S. Federal Court for the Eastern District of New York. It was noted that the Centers for Disease Control's methodology for matching troop and spray locations was almost identical to the Stellmans and that it has never been clear why CDC canceled its study estimating exposure based on troop locations and, instead, began a validation study to identify dioxin levels in serum of selected Vietnam veterans.

Syntex Agribusiness, Inc., Springfield, Missouri

Topic: Comments on human exposure to dioxin and dioxin-like compounds. This is a key component in making a determination as to whether any adverse health effects in Vietnam ground troops are associated with dioxin and other chemical compounds in herbicides used in Vietnam. It is critical to establish the extent of exposure of Vietnam ground troops relative to each of the other human population groups that has been exposed to dioxin and dioxin-like compounds. To facilitate a determination of whether a particular adverse health effect is associated with dioxin exposure, one should examine whether that effect follows a dose-response relationship.

Jerome Torczyner, Foster City, California

Topic: Personal research on the possibility that "superantigens" in humans may be the cause of lymphomas and cancers experienced by Vietnam veterans.

Individual Reports of Possible Health Effects Due to Exposure

Roman Besaha, Vietnam Veteran, St. Petersburg, Florida
Topic: Personal account of health effects, thought to be due to exposure to Agent Orange, include chloracne, fatigue, stomach pains, psychological disorders, and a malignant tumor in right thigh. Also, his personal research on injured adrenal glands, which may cause psychological problems.

Paulette Boland, Widow of Vietnam Veteran, Chicago, Illinois
Topic: Personal account of late husband's death, which is thought to be due to exposure to Agent Orange. Her husband served in the "Iron Triangle," which was heavily defoliated with Agent Orange. After returning home, he developed a thalamic hemorrhage and later died from a large left thalamic tumor. Her son, who was born after her husband returned from Vietnam, died at age 10 from a rare malignant tumor of the thalamus.

John Corwin, Vietnam Veteran, Chillicothe, Ohio
Topic: Personal account of psychosocial disorders, which are thought to be due to exposure to Agent Orange.

William W. Dean, Vietnam Veteran, Apple Valley, Minnesota
Topic: Personal account of having multiple bilateral fibromatosis (MBF) tumors and his research on the biological plausibility of exposure to dioxin and the development of MBF tumors of the hands and feet (bilateral palmar Dupuytren's contractures, bilateral plantar Dupuytren's contractures).

Julio Gonzales, Vietnam Veteran, Chicago, Illinois
Topic: List of 59 chemicals that were used in Vietnam during the war for the committee to consider in its review, along with background material on each of these chemicals and a paper on the health effects of these chemicals. Also, a personal account of his diagnoses of bladder cancer, chloracne, and peripheral neuropathy, which are thought to be due to exposure to Agent Orange.

Mike Guess, Vietnam Veteran, Jerseyville, Illinois
Topic: Personal account of being diagnosed at age 39 as having multiple myeloma, which is thought to be due to his exposure to Agent Orange. He served in the Marine Corps, and was sent to serve with the 26th Marines at Que Son, in I Corps. The troops

drank the local water and ate whatever they could find. They were aware that a defoliant was being sprayed in the area, only they did not know it was Agent Orange; the spray landed on their water source as well as all of their food and belongings.

Rockne Harmon, California, and James Harmon, New York, Attorneys/Vietnam Veterans

Topic: Personal account of Rockne and James Harmon, brothers who are both Vietnam veterans. James was a platoon leader in the First Cavalry Division and served in areas heavily exposed to Agent Orange or other herbicides. He had a child who died from malignant fibrous histiocytoma. Rockne was assigned to the Navy Swift Boat squadron and served in the inland waterways of the Mekong Delta and Cambodia in areas heavily defoliated by Agent Orange or other herbicides. He has a child that has been diagnosed with retinoblastoma, which resulted in loss of an eye and serious developmental delays.

Harold Jackson, Vietnam Veteran, Houston, Texas

Topic: Personal account of his diagnosis of chronic relapsing autoimmune polyneuropathy with peripheral neuropathy, which is thought to be due to exposure to Agent Orange. He is now disabled and uses a wheelchair. He has an identical twin brother who did not serve in the military and is in perfect health.

Henry Kinsey, Vietnam Era Veteran, Rathdrum, Idaho

Topic: Personal account of the health effects of 10 veterans stationed at Camp Alpine in Fuji, Japan, thought to be due to exposure to Agent Orange. These veterans mixed chemicals from 55 gallon green drums with orange bands around them and dipped timbers into the mixture to preserve them.

Jennie LeFevre, Widow of Vietnam Veteran, Shady Side, Maryland

Topic: Personal account of late husband's health problems and eventual death from cancer of the liver, stomach, spleen, pancreas, lungs, lymph nodes, and soft tissue sarcoma, thought to be due to exposure to Agent Orange.

John Martignette, Vietnam Veteran, Bethlehem, New Hampshire

Topic: Personal account of his health problems, which are thought to be due to exposure to Agent Orange.

Terry Miller, Vietnam Veteran, Mendota, Illinois
Topic: Personal account of health effects—includes metabolic disorders, psychosocial disorders, chloracne and other skin disorders, and respiratory disorders—of Terry Miller and his family, which are thought to be due to his exposure to Agent Orange.

Verna Mullinax, Widow of Vietnam Veteran, Goldsboro, North Carolina
Topic: Personal account of her late husband's death, which is thought to be due to exposure to Agent Orange. John Mullinax served in the Ranch Hand unit of the U.S. Air Force as a flight engineer. He was responsible for loading herbicide on the aircraft and discharging it during spray operations.

Gary Porter, Vietnam Veteran, Indianapolis, Indiana
Topic: Personal account of being diagnosed with squamous cell carcinoma. He served with D Company, 151st Airborne Rangers at Bien Hoa, South Vietnam, 1968-1969. Notes that he was not asked about his military service in Vietnam while being examined for his cancer.

Shannon Puglia, Widow of Vietnam Veteran, Dingmans Ferry, Pennsylvania
Topic: Personal account of her late husband's exposure to Agent Orange. He served in III Corps, Bien Hoa Province, east of Cu Chi—in the Iron Triangle, which was one of the areas most heavily sprayed with herbicides.

Patricia Robinson, Widow of Vietnam Veteran, San Diego, California
Topic: Personal account of her late husband's death and son's delayed development stemming from neurological problems, which are thought to be due to her husband's exposure to Agent Orange.

Doris and Fred Scobey, Parents of Vietnam Veteran, Hollywood, California
Topic: Personal account of their son's death from cancer, which is thought to be due to exposure to Agent Orange. Their son served aboard an aircraft carrier, where he was part of the launch crew. Apparently, there were Agent Orange canisters attached to the bellies of the planes, and he was exposed to Agent Orange while working with the planes.

Carol Solbey, Widow of Vietnam Veteran, East Grand Forks, Minnesota
Topic: Personal account of her late husband's death from chondrosarcoma, which is thought to be due to his exposure to Agent Orange. Her son was born with birth defects and a primary immune deficiency, thought to be due to her husband's exposure to Agent Orange.

Hope Tinoco, Wife of Vietnam Veteran, Olathe, Kansas
Topic: Personal account of her husband's health problems, which are thought to be due to exposure to Agent Orange. During his tour of duty in Vietnam, John Tinoco documented that he was in many locations that had been sprayed with Agent Orange. The health problems that he has experienced since then include chloracne and other skin disorders.

Shelia Winsett, Friend of Vietnam Veteran, Jasper, Alabama
Topic: Personal account of friend's health problems, which are thought to be due to exposure to Agent Orange.

Appendix C

Methodological Observations on the Ranch Hand Study

Because the veterans of Operation Ranch Hand are among those with the highest exposure to herbicides in Vietnam, because that exposure is well documented by records and serum dioxin measurements, and because the group has been the focus of extensive epidemiological and clinical observation, the Ranch Hand study represents one of the best available opportunities for understanding the health effects of exposure to herbicides in Vietnam veterans. The committee thus reviewed all of the research reports concerning the Air Force's Ranch Hand study in great detail.

The committee is aware of the logistical difficulties involved in such a large and detailed data collection effort, and it applauds the efforts of the Air Force research team in that regard. In addition, the committee also acknowledges the efforts of the Ranch Hands themselves to participate in the follow-up study; they deserve a great deal of appreciation for their efforts. In reviewing the results of the Ranch Hand study, however, the committee identified a number of concerns with the way the data have been analyzed and presented. Ranch Hand analysts seem to have made a good faith effort to be thorough in their analyses and to address a number of potential criticisms in advance, but the report is difficult to read and interpret for a number of reasons (detailed below).

In spite of these defects, the committee recognizes the possibility that extensive reanalysis of the data would not uncover any consistent positive findings, although only actual reanalysis would determine this. The following observations are offered in a constructive spirit, with the hope that they

may serve as suggestions for future analyses and reports of the Ranch Hand data and those of similar groups.

PROBLEMS WITH THE REPRODUCTIVE EFFECTS STUDY

The comments that follow focus on the report dealing with reproductive outcomes (AFHS, 1992). The committee feels, however, that many of its observations have somewhat broader applicability to other aspects of the Ranch Hand study as well.

Tabular Presentation of Results

One general concern is that many of the tables and analyses are confusing and inadequately documented. The definitions of column and row labels in the tables relied on prior text so that the tables do not stand alone. There are *p*-values presented without adequate descriptions of the specific hypotheses being tested. Although a thorough reading of the methodology section (Chapter 1) provides the required information, interpretation of the tables would be facilitated by the inclusion of better "stand-alone" documentation in the form of footnotes and expanded row and column headings. Overall, the committee believes that some clarification, some simplification, and more emphasis on estimates of effect than on *p*-values would help the researchers communicate their important findings and would facilitate the appropriate peer review of this work.

Similarly, the committee feels that there is an overreliance on *p*-values in place of simple measures of effect such as relative risks. The executive summary, in particular, contains essentially none of the relevant quantitative data.

Exploration of an Overall Effect

A more specific concern involves the text on page i, describing overall differences between Ranch Hands and control groups. Initial findings, using unverified end points, showed that when looking at births occurring prior to the Ranch Hands' experience in Southeast Asia (pre-SEA), the comparison group had slightly elevated rates of adverse outcomes. When looking at post-SEA births, the reverse was true; that is, the rate of adverse birth outcomes was higher for Ranch hands. The 1992 report states that subsequent analyses of verified end points confirmed these findings, but the committee could find only one table reporting the findings, and this table did not provide any information specific to particular types of birth defects or several other outcomes.

The data in table 1-16 suggest that some aspect of Ranch Hand service

may have caused an increase in rates of birth defects among children born to Ranch Hands, and this should be explored further. However, it is not clear what caused the difference between Ranch Hands and the control group. For this reason, internal comparisons based on different exposure measurements among the Ranch Hands themselves are appealing. The use of multiple definitions of exposure (extrapolated baseline dioxin, current dioxin adjusted for time between SEA experience and dioxin assay, and categorized current dioxin) represents an admirable effort to explore several possible mechanisms. Presentation of the results of these analyses, however, is confusing. Some of the data were analyzed by using continuous dioxin levels, but the presentations in the tables use trichotomized values. It is not very clear whether the results reported refer to the continuous or the trichotomized values, for either past or current exposure (models 1 or 2). One possibility for future analyses would be to report (in addition to what has been done) logistic regression coefficients and odds ratios for the continuous exposure measurements, perhaps by looking at quadratic terms to address any concerns about nonlinear associations.

Exclusion and Categorization of Data Points

Most of the analyses, especially those using models 1 and 2, excluded the most relevant baseline data, namely those for Ranch Hands whose measured dioxin residues were no higher than background levels. In particular, many of the analyses were restricted to those veterans with current serum dioxin levels greater than 5 or 10 parts per trillion (ppt). This restriction essentially eliminates truly unexposed individuals from the analysis, and makes it difficult to identify positive statistical relationships. It is also arbitrary; other studies reviewed by the committee used various definitions of "background," including 3-4 ppt and 7 ppt.

In model 3, page 1-6, the authors claim that they eliminated people having between 10 and 15 ppt from the "categorized dioxin analysis in an attempt to avoid misclassification of the Unknown and Low categories." There are several potential problems with this strategy:

1. As with the omission of any data from an analysis, it is possible that this biased the analysis. The easiest way to judge whether this occurred would be to redo the analyses with those people included, perhaps as a separate category.
2. An analysis involving categories and not continuous dioxin levels could have been performed using quartiles or quintiles; 0-4, 5-9, 10-14, and 15-19 ppt, or some other categories. No subjects should be discarded from the analyses. These categorical variables could be treated as either nominal scale or ordinal scale in logistic regression models. The specific

contrasts between the highest- and the lowest-exposure categories could be accommodated in logistic regression models.

Dioxin levels were unavailable for a large proportion of the cohort. Specifically, 2,278 of 6,792, or 33.5 percent, of the births had no dioxin levels associated with them. The committee could not find any analysis comparing outcomes or other characteristics in those who did versus those who did not have dioxin levels available. The potential for bias clearly exists. At the very least, there is a severe reduction of statistical power.

Correlation of Results Within Subject

The analyses did not seem to account for the reported pair matching of comparisons to Ranch Hands. Some aspects of the study design, including the matching, were not described in enough detail in the executive summary. In addition, the analyses did not consider defects other than the "most serious" one when a child had multiple defects.

The analyses treat data on outcomes for all pregnancies fathered by the same subject as independent binary observations. Especially when restricted to "full sibling" subsets however, there are good a priori reasons to expect that the outcomes within subject are correlated. A large body of statistical methodology has been developed to accommodate such correlated binary data, stimulated by similar "intralitter" correlations in toxicology. For rare outcomes, it will probably matter very little, but it could be important for more common ones. Tests for such "extrabinomial" variation are available. Because of the high a priori likelihood that extrabinomial variation exists, however, methods that accommodate such variation should be used whenever they yield results different from those obtained by standard methods, which assume independence, even when the tests of that variation do not provide statistically significant results. The generalized estimating equation methods of Zeger and colleagues (Zeger and Liang, 1986; Zeger et al., 1988) are appropriate in such circumstances.

Interaction Terms

There is extensive use of statistical interaction terms in models in the analysis that may have rendered those models difficult or impossible to interpret. Statistical interactions are subtle, but they may be obscuring some of the findings. In general, the concern is that a model containing a main effect for a covariate (such as age of the father), a main effect for dioxin level, and an interaction term, could easily be misinterpreted. If the interaction term is statistically nonsignificant, and if it is highly correlated with dioxin level, which is a very reasonable scenario, the statistical significance

for the main effect term for dioxin is uninterpretable. In particular, it is possible that a model with both main effects present, but no interaction term, could reveal a statistically significant effect of dioxin level, but this result could spuriously seem to disappear in the model with the interaction term. This problem is of particular concern for model 2, which includes a main effect for the time between SEA experience and dioxin level, a main effect for dioxin level, and the interaction. Such a model could obscure any effect of dioxin level.

Analyses of Conception Rates

One intriguing finding in the Ranch Hand report is the increase in the average number of post-SEA conceptions with increasing dioxin exposure. To be able to interpret this result, one needs much more basic descriptive information on how these three exposure groups differ on relevant characteristics, particularly data on year of entry into SEA service. If, as seems possible, those who had the highest dioxin residues tended to enter SEA service earlier so that they had the chance for longer or repeat tours, the higher number of conceptions could be explained by the longer period since their return during which they were "at risk" for conception. It is standard practice in demography to take account of period at risk when analyzing fertility rates in different populations, and the same methods would be appropriate here.

Inclusion of Pre-SEA Births

In one of the models, the analysis included all births whether they occurred prior to or following the subject's SEA experience. There was a main effect term in each model for "pre/post SEA," a main effect for dioxin, and the interaction term between these. The *p*-values reported in these pre/post models apparently represented tests of the interaction terms, which the committee views as of only secondary importance. The hypothesis being tested is whether the effect of dioxin (if any) is the same for pre-SEA births as for post-SEA births. Presumably, if an effect of subsequent dioxin exposure on pre-SEA births were detected, this would be a result of bias in the study design, confounding, or both. Although this hypothesis is of some interest, the report did not appear to include an estimate and test of the effect of dioxin in the post-SEA births for these models. The presentation of the *p*-values conveys the impression that the dioxin effect is nonsignificant. Although this may be true as well, that is not what the committee understood those *p*-values to be testing, and it was not completely clear from the tables exactly what the *p*-values do represent. It would be preferable to analyze only post-SEA birth, create a history variable (History of

adverse reproductive outcomes prior to SEA experience? Yes or no?), and include that variable in the logistic regression model.

Lack of Documentation of Algorithms for Adjustment for Covariates

On page 1-7 the authors say, "When appropriate, analyses were adjusted for as many as 8 covariates," but they do not state the criteria for deciding when it was appropriate. In order to interpret the results, it is necessary to know how the decision was made as to which covariates were or were not included in any particular analysis.

Table 3-21 (page 3-22) provides an example: The unadjusted model shows no significant association between (log) initial dioxin and miscarriages. In the adjusted analyses, a significant (or nearly significant) effect of service occupation is found. Since service occupation was highly correlated with dioxin levels, it is unclear how to interpret the finding of no effect of dioxin in the adjusted models. Adjustment of an exposure variable for a highly correlated variable that may, in fact, represent a surrogate measure of exposure is not generally recommended and may have obscured a positive relationship.

SUMMARY OF STUDY

In summary, several issues need to be addressed in future analyses of the Ranch Hand reproductive effects data. The technical issues raised above, particularly the restriction to those with current dioxin levels above background and the potential misapplication and/or misinterpretation of interaction terms, are of concern because of the conclusions drawn from them. The problems with the analyses could lead to apparently inconsistent findings with regard to the estimated effect of dioxin on outcomes. These apparent inconsistencies might or might not reflect the actual situation. Thus, the conclusion drawn in the report that the inconsistent findings do not support an effect of dioxin may not be valid, since the inconsistencies may simply reflect problems with the analyses. On the other hand, it is very possible that reanalysis of these data, according to the recommendations listed above, would not lead to any substantively different conclusions.

Further exploration of the overall differences between Ranch Hands and the comparisons should also be pursued. Some aspect of the Ranch Hand experience seems to have increased the risk of fathering children with birth defects, but the implications of this finding are unclear. If reanalyses of the dioxin data still show that dioxin is not associated with increased risk of birth defects, a great deal of careful exploratory analysis will be required to sort out exactly what the cause of the increased risk might be.

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Zeger SL, Liang KY. 1986. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42:121-130 .

Zeger SL, Liang KY, Albert PS. 1988. Models for longitudinal data: a generalized estimating equation approach. Biometrics 44:1049-1060 (published erratum appears in Biometrics 1989 45:347).

Appendix D

Committee and Staff Biographies

COMMITTEE BIOGRAPHIES

Harold Fallon, M.D. (Chairman), is a graduate of Yale College and Yale Medical School. He completed his residency in internal medicine at the University of North Carolina and did additional fellowship training at Yale University Department of Medicine and Duke University Department of Biochemistry. He joined the University of North Carolina in 1963 and left as Professor and Vice-Chairman of the Department of Medicine to assume the position of William Branch Porter Professor and Chairman of the Department of Medicine and Professor of Pharmacology at the Medical College of Virginia in 1973. His area of special research and clinical interests concerns liver disease. He is a member of the Institute of Medicine, former President of the American Association of Physicians, former Chairman of the Board of Internal Medicine, Regent of the American College of Physicians, and former President of the American Association for the Study of Liver Disease. In February 1993, he assumed the position of Professor of Medicine and Dean of the University of Alabama School of Medicine.

David Tollerud, M.D., M.P.H. (Vice-chairman), is Director of Occupational and Environmental Medicine at the University of Pittsburgh. He received his M.D. from Mayo Medical School and his M.P.H. from the Harvard School of Public Health. He served as a Medical Staff Fellow in the Environmental Epidemiology Branch, National Cancer Institute; Pulmonary Fellow at Brigham and Women's and Beth Israel Hospitals in Boston;

Assistant Professor of Medicine at the University of Cincinnati; and Associate Professor of Environmental and Occupational Health at the University of Pittsburgh Graduate School of Public Health. He is a Fellow of the American College of Occupational and Environmental Medicine and the American College of Chest Physicians, a member of numerous professional societies, including the American Thoracic Society, the American Association of Immunologists, the Clinical Immunology Society and the American Association for the Advancement of Science, and a member of the editorial board of the Journal of the American Industrial Hygiene Association.

Norman Breslow, Ph.D., is Professor and Chair of the Department of Biostatistics, University of Washington, and committee liaison to the IOM Board on Health Promotion and Disease Prevention. A former staff member of the International Agency for Research on Cancer (IARC), he is co-author (with Dr. N.E. Day) of two influential IARC scientific publications on the design and analysis of case-control and cohort studies in cancer epidemiology. Dr. Breslow's research interests are in statistical methods for survival and categorical data and in the treatment and epidemiology of childhood cancer, particularly Wilms tumor. He is a Fellow of the American Association for the Advancement of Science and the American Statistical Association and is a Member of the International Statistical Institute and the Institute of Medicine.

Jesse Berlin, Sc.D., is Research Assistant Professor of Biostatistics in Medicine at the University of Pennsylvania School of Medicine, where he has been since 1989. He received his doctorate in biostatistics from the Harvard School of Public Health in 1988. He has participated in both the design and analysis of a wide variety of clinical and epidemiologic investigations. Dr. Berlin's principal research interest lies in the area of meta-analysis, the quantitative analysis of results of multiple studies for the purpose of integrating the findings. He has become nationally recognized in that field and has applied his experience to developing the application of meta-analysis to epidemiologic studies. Dr. Berlin also serves as an Associate Editor for Methodology for the recently initiated *Online Journal of Current Clinical Trials*, published by the American Association for the Advancement of Science.

Karen Bolla, Ph.D., is Assistant Professor of Neurology, Psychiatry, and Behavioral Sciences at the Johns Hopkins University School of Medicine and in the Department of Environmental Health Sciences, Division of Occupational Health, the Johns Hopkins University School of Hygiene and Public Health. She is currently the Director of Neuropsychology at the Francis Scott Key Medical Institution, a Johns Hopkins Medical Institution.

Graham Colditz, M.D., Dr.P.H., is an Associate Professor of Epidemiology at the Harvard School of Public Health. He has extensive experience in the epidemiology of life-style and health, having worked on the Nurses' Health Study since 1982. He currently is Principal Investigator on general studies based on the Nurses' Health Study. These include studies of screening and mortality; benign breast disease and risk of breast cancer; and diet, activity, and risk of fractures in women. He has published studies on a range of chronic conditions that affect women including heart disease, breast and other cancers, fractures, gall stones, and obesity.

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Appendix E

Author Index, Chapters 7-11

This index is provided to aid the reader in locating all discussions of the epidemiologic studies considered in this report. Each study is discussed in reference to its study design, methodology, and exposure assessment in Chapter 7 and then is discussed and evaluated regarding specific health outcome results and the assessment of an association with herbicide exposure in Chapters 8-11. This index is organized by the first author's last name based on the reference list appearing in Chapter 7, and entries are followed by the page numbers where each epidemiologic study is discussed.

Glossary

Abnormal sperm parameters. Refers to any deviation from the normal shape or form of sperm, sperm volume, or sperm motility.

Agent Blue. Herbicide formulation consisting of cacodylic acid used during the Vietnam conflict as a contact herbicide for rapid defoliation.

Agent Green. Herbicide formulation consisting of the *n*-butyl ester of 2,4,5-T used during the Vietnam conflict for crop destruction.

Agent Orange. Herbicide formulation consisting of a 1:1 mixture of 2,4-D and 2,4,5-T used during the Vietnam conflict for general defoliation.

Agent Pink. Herbicide formulation consisting of a 60:40 mixture of the n-*b*utyl ester and the isobutyl ester of 2,4,5-T used during the Vietnam conflict for defoliation.

Agent Purple. Herbicide formulation consisting of a 50:30:20 mixture of the *n*-butyl ester of 2,4-D, and the *n*-butyl and isobutyl esters of 2,4,5-T used during the Vietnam conflict for general defoliation.

Agent White. Herbicide formulation consisting of a mixture of 2,4-D and picloram used during the Vietnam conflict for forest defoliation and long-term defoliation control.

Ah receptor. Protein located within liver cells (and possibly other cells) that interacts with TCDD and other molecules and with specific sites on DNA to regulate DNA.

Altered lipid metabolism. An interference in the body's normal processes in breaking down or building up the molecules that are fat-soluble, including fatty acids, glycerides, waxes, steroids, and certain vitamins.

Anencephaly. Absence of the cranial vault, with cerebral hemispheres completely missing or reduced to small masses attached to the base of the skull.

Anergy. Absence of an immune reaction to a specific antigen.

Aphasia. Loss or impairment of the ability to use or comprehend words usually resulting from a brain lesion.

Apraxia. Loss or impairment of the ability to execute complex coordinated movements, without impairment of the muscles or senses.

Autoimmune disease. A disease involving a humoral or cell-mediated immunity to the tissues of one's own body. A failure of the immune system to discriminate between self and respondent.

Bioassay. A procedure for estimating the nature, constitution, or potency of a material by statistically analyzing the reaction that follows its application to living matter.

C-123. Fixed-wing aircraft used in Operation Ranch Hand during the Vietnam conflict to spray herbicides.

Cacodylic Acid. Dimethylarsinic acid, a contact herbicide used during the Vietnam conflict in Agent Blue.

Cancer. A general term used to indicate a malignant tumor that expands locally by invasion or systemically by metastasis.

Cardiovascular disorders. Disorders of the heart and blood vessels or the circulation of blood through the body.

Case-control study. An epidemiologic study in which persons are selected because they have a specific disease or other outcome (cases) and are compared to a control (referent comparison) group to evaluate whether there is a difference in their reported frequency of exposure to possible disease risk factors. Also termed a retrospective study or case-referent study.

Cell-mediated immunity. Immune reaction mediated by T cells; in contrast to humoral immunity, which is antibody mediated.

Chloracne. An acne-like eruption due to prolonged contact with certain chlorinated compounds.

Cleft palate. A fissure in the midline of the palate due to failure of the two sides to fuse in embryonic development.

Cognitive disorders. Disorders of the central nervous system involving cognitive decline, including memory problems and dementia.

Cohort study. An epidemiologic study in which a defined group of persons known to be exposed to a potential disease risk factor is followed over time and compared to a group of persons who were not known to be exposed to the potential risk factor to evaluate the differences in rates of the outcome. Also termed a prospective study, follow-up study, incidence study, retrospective cohort, or historical cohort study.

Confidence interval (95%). A range of values for the effect estimate within which the true value is thought to lie with a 95% level of confidence.

Confounder (Confounding factor). A factor that is associated with both the exposure and outcome of interest and can distort the apparent magnitude of the effect of the study factor.

Diabetes mellitus. Disorder of carbohydrate metabolism characterized by inadequate utilization or secretion of insulin.

2,4-Dichlorophenoxyacetic acid (2,4-D). A chlorophenoxy acid used as an herbicide in the Vietnam conflict in Agents Orange, White, and Purple.

Dioxins. A subset of halogenated aromatic hydrocarbon compounds.

Dose-response effect. The relationship between an exposure and specified outcome in which a change in amount, intensity, or duration of exposure is associated with a change (either increase or decrease) in outcome.

Healthy worker effect. The observation that lower mortality rates are found in occupational cohorts than in the general population. This is attributed to the fact that severely ill persons are usually not in the workforce.

Hematopoietic. Pertaining or relating to the formation of blood cells.

Hodgkin's disease. Malignant lymphoma characterized by progressive enlargement of the lymph nodes, liver, and spleen, and by progressive anemia.

Humoral immunity. An immune reaction that can be transferred with immune serum (as opposed to cell-mediated immunity). In general, refers to resistance that results from the presence of a specific antibody.

Hydatidiform mole. A mass or tumor formed in the uterus resulting from the abnormal development of an ovum.

Hydrocephaly. Dilation of the cerebral ventricles, most often occurring secondarily to obstruction of the cerebrospinal fluid pathways and accompanied by an accumulation of cerebrospinal fluid within the skull. It is typically characterized by enlargement of the head, prominence of the forehead, brain atrophy, mental deterioration, and convulsions.

Hypospadias. Congenital abnormality in males in which the opening of the urethra is on the underside of the penis.

Immune modulation. Fluctuation in the body's immune function, either increase or decrease.

Incidence. The number of instances of illness during a given period of time in a specified population.

Infertility. Incapability of producing offspring.

Latency. The time interval between exposure and disease manifestation or recognition.

Leukemia. Acute or chronic disease characterized by an abnormal increase in the number of leukocytes found in the tissues of the body with or without a corresponding increase in the circulating blood.

Lymphoma. Malignant tumor of lymphoid tissue.

Matching. The process of making the study population and the comparison group comparable with respect to one or more confounding factors.

Melanoma. A malignant neoplasm, derived from cells that are capable of forming melanin in the skin.

Microcephaly. Abnormally small head circumference in relation to the size of the rest of the body, usually associated with mental retardation.

Military Assistance Command, Vietnam (MACV).	The headquarters of the American military senior general staff during the Vietnam conflict.
Motor/ coordination dysfunction.	Central nervous system disorders involving motor difficulties, characterized by problems such as weakness, tremors, involuntary movements, incoordination, and gait/walking abnormalities.
Multiple myeloma.	Cancer of specific bone marrow cells characterized by bone marrow tumors in various bones of the body.
Neuropsychiatric disorders.	Central nervous system disorders including neurasthenia (a collection of symptoms including difficulty concentrating, headache, insomnia, and fatigue), depression, posttraumatic stress disorder (PTSD), and suicide.
Non-Hodgkin's lymphoma.	A heterogeneous group of malignant lymphomas, with the only common factor being the absence of the giant Reed-Sternberg cells characteristic of Hodgkin's disease.
Null hypothesis.	The statement that the results observed in the study were no different from what might have occurred as a result of chance alone.
Odds ratio.	Measure of association in case-control studies which estimates the ratio of odds of exposure among cases compared to the odds of exposure among controls. The measure approximates the relative risk.
Peripheral nervous system disorders.	Dysfunctions involving either the somatic nerves or the autonomic system, known as neuropathies.
Picloram.	4-amino-3,5,6-trichloropicolinic acid, a systemic herbicide used during the Vietnam conflict in Agent White.
Porphyria cutanea tarda (PCT).	Characterized by liver dysfunction and photosensitive cutaneous lesions, with hyperpigmentation and scleroderma-like changes in the skin. It is the result of excess porphyrin being excreted in the urine due to a deficiency in uroporphyrinogen decarboxylase. The term "porphyria" refers to disorders involving the biosynthesis of heme, the oxygen-carrying component in blood.
Proportionate mortality ratio (PMR).	The number of the observed deaths from a particular cause in the study population per 100 total deaths in the same period.

Ranch Hand. The U.S. Air Force tactical military operation designated responsible for the aerial spraying of herbicides during the Vietnam conflict.

Relative risk. Ratio of the risk of a disease or outcome in persons exposed to a particular risk factor to the risk in unexposed persons.

Risk. Probability that an event will occur.

Sarcoma. Malignant neoplasm arising in tissue of mesodermal origin (e.g., connective tissue, bone, cartilage, or striated muscle).

Silviculture. Duties in the care and maintenance of forests including planting and thinning.

Soft tissue sarcomas. Diverse group of sarcomas arising in the soft somatic tissues that occur within and between organs.

Spina bifida. Defective closure of the body encasement of the spinal cord, through which the cord and meninges are exposed and may protrude.

Spontaneous abortion. Naturally occurring loss of a nonviable fetus.

Standardized morbidity ratio (SMbR). Ratio of the number of incident cases of a disease observed in a study population to the number of deaths expected if the study population had the same rates as the standard population.

Standardized mortality ratio (SMR). Ratio of the number of deaths observed in a study population to the number of deaths expected if the study population had the same rates as the standard population.

Stillbirth. Birth of a dead fetus.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). A halogenated aromatic hydrocarbon compound found as a contaminant in the herbicide 2,4,5-T.

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T). A chlorophenoxy acid developed during World War II as an herbicide and used in the Vietnam conflict in Agents Orange, Purple, Pink, and Green. The production process can result in TCDD contamination.

Ulcer. A break in skin or mucous membrane with loss of surface tissue, disintegration and necrosis of epithelial tissue.

Vietnam era. Designated by presidential proclamation (May 7, 1975) as the time period from August 1964 through May 7, 1975.

Vietnam era veteran. Used throughout this report to designate veterans that served in other locations than Vietnam during the time span of the Vietnam conflict.

Vietnam theater. The geographic area of the Vietnam conflict encompassing the land, air, and sea areas of Cambodia, Laos, North and South Vietnam.

Acronyms and Abbreviations

AAAS	American Association for the Advancement of Science
AFHS	Air Force Health Study
AFIP	Armed Forces Institute of Pathology
AHH	aryl hydrocarbon hydroxylase
AIDS	acquired immune deficiency syndrome
ALA	δ-aminolevulinic acid
ALL	acute lymphocytic leukemia
ALS	Assembly of Life Sciences
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOS	Agent Orange Study
AOVI	Agent Orange Victims International
AOVS	Agent Orange Validation Study
AOWG	Agent Orange Working Group
AST	aspartate aminotransferase
BIRLS	Beneficiary Identification and Record Locator Subsystem (DVA)
BLS	Bureau of Labor Statistics
°C	degree Celsius
CAS	Chemical Abstracts Service
CCR	Cancer Control Region

CDC	Centers for Disease Control (now Centers for Disease Control and Prevention)
CER	Cancer Environment Registry (Sweden)
CFR	Code of Federal Regulations
CI	confidence interval
CLL	chronic lymphocytic leukemia
CLS	Commission on Life Sciences
cm	centimeter
CML	chronic myeloid leukemia
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CP	chlorophenol
CSM	cerebrospinal malformation
CTL	cytotoxic T lymphocyte
2,3-D	2,3-dichlorophenoxyacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
DMSO	dimethyl sulfoxide
DMZ	Demilitarized Zone
DOD	Department of Defense
DRE	dioxin-responsive enhancer
DTH	delayed-type hypersensitivity
DVA	Department of Veterans Affairs (formerly the Veterans Administration)
EAS	Environmental Agents Service
EBV	Epstein-Barr virus
ECOD	ethoxycoumarin *O*-deethylase
EEG	electroencephalography
EES	Environmental Epidemiology Service
EGF	epidermal growth factor
EHAC	Veterans' Advisory Committee on Environmental Hazards
EKG	electrocardiogram
EMG	electromyography
EOI	exposure opportunity index
EPA	Environmental Protection Agency
EROD	ethoxyresorufin *O*-deethylase
ESG	Joint Services Environmental Support Group
FEF	forced expiratory flow
FEV	forced expiratory volume
FT_4	free thyroxine
FVC	forced vital capacity

GAO	General Accounting Office
GBDS	General Birth Defects Study
GGT	gamma-glutamyltransferase
GI	gastrointestinal
HAC	Herbicide Assessment Commission
HAH	halogenated aromatic hydrocarbon
HCB	hexachlorobenzene
HD	Hodgkin's disease
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
H/OCDD	hexa- to octachlorodibenzodioxin
H.R.	House resolution
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
Ig	immunoglobulin
IOM	Institute of Medicine
i.p.	intraperitoneal
IRS	Internal Revenue Service
IUGR	intrauterine growth retardation
JEM	job exposure matrix
kd	kilodalton (10^3 daltons)
kg	kilogram (10^3 grams)
km	kilometer (10^3 meters)
LDL	low-density lipoprotein
m	meter
m^2	square meter
m^3	cubic meter
MACDP	Metropolitan Atlanta Congenital Defects Program
MACV	U.S. Military Assistance Command, Vietnam
MCDF	6-methyl-1,3,8-trichlorodibenzofuran
MCPA	2-methyl-4-chlorophenoxyacetic acid
MCPP	2-(4-chloro-2-methylphenoxy)propanoic acid
MDL	multidistrict litigation
MEDLARS	Medical Literature Analysis and Retrieval System
MeSH	Medical Subject Headings (National Library of Medicine)
MFO	mixed-function oxidase
mg	milligram (10^{-3} gram)

min	minute (time)
ml	milliliters
MM	multiple myeloma
MMPI	Minnesota Multiphasic Personality Inventory
mol	mole
MOR	mortality odds ratio
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Midwest Research Institute
MTD	maximum tolerated dose
MWD	military working dog
MWMS	men without known military service
µg	microgram (10^{-6} gram)
µl	microliter (10^{-6} liter)
µmol	micromole (10^{-6} mole)
NAD	nicotinamide-adenine dinucleotide
NAS	National Academy of Sciences
NaTCP	sodium trichlorophenol
NEPACCO	Northeast Pharmaceutical and Chemical Corporation
ng	nanogram (10^{-9} gram)
NHL	non-Hodgkin's lymphoma
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nM	nanomolar (10^{-9} molar)
nmol	nanomole (10^{-9} mole)
NRC	National Research Council
NSAID	nonsteroidal anti-inflammatory drug
NSVG	National Survey of the Vietnam Generation
NTP	National Toxicology Program
NVV	non-Vietnam veteran
OCDD	octachlorodibenzodioxin
OTA	Office of Technology Assessment
PBB	polybrominated biphenyl
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCT	porphyria cutanea tarda
PEPCK	phosphoenolpyruvate carboxykinase
pg	picogram (10^{-12} gram)
PH	phenoxy acid herbicide
PMN	polymorphonuclear neutrophil
pmol	picomole (10^{-12} mole)

PMR	proportionate mortality ratio
PNS	peripheral nervous system
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
pre-SEA	pre-Southeast Asia
PRR	prevalence risk ratio
PTD	preterm delivery
PTF	Patient Treatment File (Veterans Administration)
PTSD	posttraumatic stress disorder
RH	Ranch Hand
RR	relative risk
rt_3	reverse triiodothyronine
S.	Senate
SCL-90	Self-Report Symptom Inventory-Revised-90
SCS	Selected Cancers Study
SES	socioeconomic status
SIR	standardized incidence ratio
SLE	systemic lupus erythematosus
SMbR	standardized morbidity ratio
SMR	standardized mortality ratio
SRBC	sheep red blood cell
SRR	standardized rate ratio
SSA	Social Security Administration
STS	soft tissue sarcoma
2,4,5-T	2,3,4-trichlorophenoxyacetic acid
T_3	triiodothyronine
T_4	thyroxine
TBDD	2,3,7,8-tetrabromodibenzo-*para*-dioxin
TCDD	2,3,7,8-tetrachlorodibenzo-*para*-dioxin
TCP	trichlorophenol
TGF α	transforming growth factor α
TMAO	trimethylarsine oxide
TNF	tumor necrosis factor
TSH	thyroid-stimulating hormone
TT_3	total triiodothyronine
TT_4	total thyroxine
UDPGT	uridine 5' -diphosphate glucuronosyltransferase
UROD	uroporphyrinogen decarboxylase

USAF	U.S. Air Force
USDA	U.S. Department of Agriculture
UTM	Universal Transverse Mercator
VA	Veterans Administration
VDL	very low-density lipoprotein
VES	Vietnam Experience Study
VV	Vietnam veterans
VVAOHS	Vietnam Veteran Agent Orange Health Study
WAIS	Wechsler Adult Intelligence Scale

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