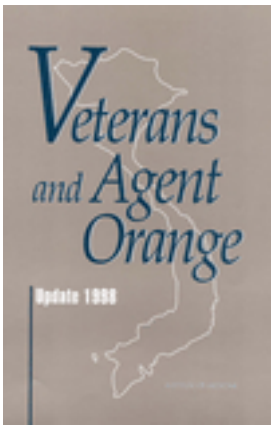


Veterans and Agent Orange: Update 1998

Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Second Biennial Update), Institute of Medicine

ISBN: 0-309-52468-7, 624 pages, 6 x 9, (1999)

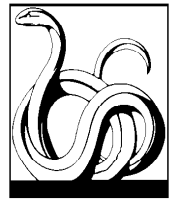
Veterans and Agent Orange

Update 1998

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

Division of Health Promotion and
Disease Prevention

INSTITUTE OF MEDICINE



NATIONAL ACADEMY PRESS
Washington, D.C.

NATIONAL ACADEMY PRESS • 2101 Constitution Avenue, NW • Washington, D.C. 20418

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.

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.

Support for this study was provided by the Department of Veterans Affairs (contract no. V101(93)P-1331).

International Standard Book Number 0-309-06326-4

Printed in the United States of America.

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 logotype by the Institute of Medicine is based on a relief carving from ancient Greece, now held by the Staatliche Musseen in Berlin.

COMMITTEE TO REVIEW THE HEALTH EFFECTS IN VIETNAM VETERANS OF EXPOSURE TO HERBICIDES (SECOND BIENNIAL UPDATE)

David Tollerud, MD, MPH (*Chair*),[1,2] Professor, School of Public Health, MCP Hahnemann University

Michael Aminoff, MD,[2] Professor, Department of Neurology, University of California at San Francisco School of Medicine

Steven Goodman, MD, MHS, PhD, Associate Professor, Department of Oncology, Division of Biostatistics, Johns Hopkins University School of Medicine

Robert Herrick, PhD, CIH, Lecturer on Industrial Hygiene, Department of Environmental Health, Harvard School of Public Health

Irva Hertz-Picciotto, PhD, Associate Professor, Department of Epidemiology, University of North Carolina, Chapel Hill

David Hoel, PhD, Distinguished University Professor, Medical University of South Carolina

Andrew Olshan, PhD,[1,2] Associate Professor, Department of Epidemiology, University of North Carolina, Chapel Hill

Trevor Orchard, MBBCh, MMSc, Professor, University of Pittsburgh, Rangos Research Center

Howard Ozer, MD, PhD, Professor, Department of Medicine, MCP Hahnemann University

Kenneth Ramos, PhD,[2] Professor, Department of Physiology and Pharmacology, Texas A&M University College of Veterinary Medicine

Noel Rose, MD, PhD,[2] Professor, Department of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health

Susan Woskie, PhD, CIH, Associate Professor, Department of Work Environment, University of Massachusetts, Lowell

[1]Member of the committee responsible for *Veterans and Agent Orange* (1994).
[2]Member of the committee responsible for *Veterans and Agent Orange: Update 1996.*

Project Staff

DAVID A. BUTLER, Study Director
SANJAY S. BALIGA, Research Associate
JAMES A. BOWERS, Research/Project Assistant
KATHLEEN R. STRATTON, Director, Division of Health Promotion and Disease Prevention
DONNA D. DUNCAN, Division Assistant
SHARON GALLOWAY, Financial Associate

Staff Consultants

JANE DURCH, Senior Program Officer, Institute of Medicine
CAROL MACZKA, Director of Toxicology and Risk Assessment, National Research Council
FLORENCE POILLON, Contract Editor

Preface

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 Agent Orange and other herbicides (Public Law 102-4, enacted on February 6, 1991). The creation of the first NAS Institute of Medicine (IOM) committee, in 1992, underscored the critical importance of approaching these questions from a non-partisan scientific standpoint. The original Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides 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 in order 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 committee members on recent research results and studies still under way. This information provided a meaningful backdrop for the numerous scientific articles that the committee considered.

Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam (abbreviated as *VAO* in this report) reviewed and evaluated the available scientific evidence regarding the association between exposure to dioxin or other chemical compounds contained in herbicides used in Vietnam and a wide range of health effects. The report provided information for the Secretary of Veterans Affairs to consider as the Department of Veterans Affairs carried out its responsibilities to Vietnam veterans. It also described areas in which the available scientific data were insufficient to determine whether an association exists and provided the committee's recommendations for future research.

Public Law 102-4 also tasked the NAS to conduct biennial updates that would review newly published scientific literature regarding statistical associations between health outcomes and exposure to dioxin and other chemical compounds in these herbicides. The first of these, *Veterans and Agent Orange: Update 1996* (*Update 1996)* was published in March of that year. The focus of this second updated review is on scientific studies published since the release of *Update 1996*. To conduct the review, the IOM established a committee of 12 members representing a wide range of expertise to take a fresh look at the studies reviewed in *VAO* and *Update 1996* along with the newest scientific evidence. In order to provide a link to the experience and expertise developed by the previous committees, five of the members of the committee responsible for this report were recruited from the committee responsible for *Update 1996*; two of these individuals also served on the *VAO* committee. All committee members were selected because they are leading experts in their fields, have no conflicts of interest with regard to the matter under study, and have taken no public positions concerning the potential health effects of herbicides in Vietnam veterans or related aspects of herbicide or dioxin exposure. Biographical sketches of committee members and staff appear in Appendix C.

The committee worked on several fronts in conducting this updated review, 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 NAS, 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 evidence. It also convened two open meetings to provide the opportunity for veterans and veterans service organizations, researchers, policymakers, and other interested parties to present their concerns, review their research, and exchange information directly with committee members. The first of these was held in conjunction with the committee's second meeting in June 1997 in Washington, D.C.; the second in Irvine, California, in October, 1997. To solicit broad participation, the committee sent announcements to individuals, organizations, and listserves known to have an interest in this issue. The oral presentations and written statements submitted to the committee are described in detail in Appendix A. In order to address one area of interest identified by the Department of Veterans Affairs, the committee convened a workshop on the combination and reanalysis of existing data on the health effects of herbicide and dioxin exposure. The workshop, which took place in August 1997, brought together experts in these methodologies with researchers who have developed and analyzed datasets evaluating the health of Vietnam veterans and individuals exposed to herbicides or dioxin. The results of this effort will be addressed in a separate report.

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 expo-

sure to herbicides. The committee also heard from the public through telephone calls, letters, and emails.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their participation in the review of this report: Patricia Buffler, University of California, Berkeley; Graham Colditz, Harvard University; John Doull, University of Kansas; Kristine Gebbie, Columbia University; David Kriebel, University of Massachusetts, Lowell; Gilbert Omenn, University of Michigan; Jonathan Samet, Johns Hopkins University; David Strogatz, University of Albany, SUNY. While the individuals listed above have provided constructive comments and suggestions, it must be emphasized that responsibility for the final content of this report rests entirely with the authoring committee and the institution.

David A. Butler served as the study director for this project and deserves credit for drafting sections of the report. The committee would also like to acknowledge the excellent work of IOM staff members Sanjay Baliga and James Bowers. Carol Maczka of the Academy's National Research Council provided invaluable help on the toxicology chapter of the report. Thanks are also extended to Sharon Galloway, who handled the finances for the project; Florence Poillon and Jane Durch, who provided excellent editorial skills; Susan Fourt, who conducted database searches; Michael Edington, who supervised the report through the editorial and publication phases; and Donna Thompson, who provided administrative support to the project. The knowledge and experience of Michael Stoto and Catharyn Liverman, who served as staff members on the original committee, were helpful in this effort.

The committee also benefited from the assistance of several scientists and researchers who generously lent their time and expertise to help give committee members insight on particular issues, provide copies of newly released research, or answer queries concerning their work. Special thanks are extended to Drs. Bruce Armstrong (New South Wales Cancer Council, Australia), Michael DeVito (U.S. EPA), Keith Horsley (Commonwealth Department of Veterans' Affairs, Australia), Han Kang (U.S. Department of Veterans Affairs), Stephen Katz (National Institutes of Health, DHHS), Edward McCarthy (Johns Hopkins University), Joel Michalek (Armstrong Laboratory, USAF), and Jerry Rice (International Agency for Research on Cancer).

David Tollerud
Chairman

Contents

1

Executive Summary

Because of continuing uncertainty about the long-term health effects of exposure to the herbicides used in Vietnam, Congress passed Public Law 102-4, the "Agent Orange Act of 1991." This legislation directed the Secretary of Veterans Affairs to request the National Academy of Sciences (NAS) to conduct a comprehensive review and evaluation of scientific and medical information regarding the health effects of exposure to Agent Orange, other herbicides used in Vietnam, and the various chemical components of these herbicides, including dioxin. A committee convened by the Institute of Medicine (IOM) of the NAS conducted this review and in 1994 published a comprehensive report, entitled *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (henceforth called *VAO*) (IOM, 1994).

Public Law 102-4 also called for the NAS to conduct subsequent reviews at least every two years for a period of ten years from the date of the first report. The NAS was instructed to conduct a comprehensive review of the evidence that has become available since the previous IOM committee report and to reassess its determinations and estimates of statistical association, risk, and biological plausibility. On completion of *VAO*, a successor committee was formed that produced *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996).

The present IOM report is the second updated review and evaluation of the newly published scientific evidence regarding associations between diseases and exposure to dioxin and other chemical compounds in herbicides used in Vietnam. For each disease, the IOM was asked to determine, to the extent that available data permitted 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 the disease among those exposed to herbicides during Vietnam service; and (3) whether there is a plausible biological mechanism or other evidence of a causal relationship between herbicide exposure and the disease.

In addition to bringing the earlier scientific evidence up to date, the committee has addressed five specific areas of interest identified by the Department of Veterans Affairs (DVA). These are: (1) the relationship between exposure to herbicides and the subsequent development of diabetes; (2) the issue of the latency between exposure to herbicides and development of adverse health outcomes; (3) the classification of chondrosarcomas of the skull; (4) herbicide exposure assessment for Vietnam veterans; and (5) the potential for using data combination methodologies to informatively reexamine existing data on the health effects of herbicide or dioxin exposure.

In conducting its study, the IOM 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. 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 conclusions in this updated report are based on cumulative evidence from the scientific literature reviewed in *VAO* and *Update 1996*. This present update is intended to supplement rather than replace the two previous reports; therefore, much of the information on studies reviewed in those reports has not been repeated. Most chapters begin with brief summaries of the scientific data in *VAO* and *Update 1996*, followed by a more thorough discussion of the newly published data and their interpretation. The reader is referred to relevant sections of the previous reports for additional detail and explanation.

Chapter 2 provides an overview of the methods and conclusions of *VAO* and *Update 1996*. In addition, it provides a summary of the recent activities of several federal government agencies that are relevant to the health effects of Agent Orange and other herbicides used in Vietnam. Chapter 3 provides an update of the recent experimental toxicology data on the effects of the herbicides and of 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, commonly referred to as TCDD or "dioxin"), a compound found as a contaminant in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). These data serve as the basis for the biological plausibility of potential health effects in human populations. Chapter 4 describes the methodological considerations that guided the committee's review and its of evaluation. Chapter 5 addresses exposure assessment issues. Chapter 6

provides a general review of the epidemiologic studies used to assess the potential association between herbicides and specific health outcomes. The chapter is organized to reflect similarities and differences in the nature of exposure among three types of study populations: occupationally exposed, environmentally exposed, and Vietnam veterans. Chapter 8 reviews the methods used to study latency, or time-related effects—a topic of special interest to the DVA—and evaluates the evidence on latency for the cancers under study.

Health outcomes are addressed in the remaining chapters: Chapter 7 focuses on cancer outcomes; Chapter 9, on reproductive effects; Chapter 10, on neurobehavioral disorders; and Chapter 11, on other (noncancer) health effects including respiratory, immune system, metabolic, digestive, and circulatory disorders. Many of the same epidemiologic studies were used to assess different types of health outcomes (see Chapter 6).

The committee focused most of its efforts on reviewing and interpreting epidemiologic studies, in order to evaluate the extent to which the scientific literature does or does not suggest that particular human health effects are associated with exposure to herbicides or dioxin. The committee weighed the strengths and limitations of the scientific data in *VAO* and *Update 1996*, as well as the newly published scientific data, and reached its conclusions by interpreting the new evidence in the context of the whole of the literature. Each disease has been placed into one of four categories, depending on the strength of evidence for an association (see "Conclusions About Health Outcomes," below). The committee used the same criteria to categorize health outcomes as used in the two previous reports.

TOXICOLOGY SUMMARY

The results of cellular and animal studies published since the release of *Update 1996* that investigated the toxicokinetics, mechanism of action, and disease outcomes of TCDD, plus the herbicides themselves are reviewed in Chapter 3.

TCDD elicits a diverse spectrum of biological sex-, strain-, age-, and species-specific effects, including carcinogenicity, immunotoxicity, reproductive or developmental toxicity, hepatotoxicity, neurotoxicity, chloracne, and loss of body weight. To date, the scientific consensus is that TCDD is not genotoxic and that its ability to influence the carcinogenic process is mediated via epigenetic events such as enzyme induction, cell proliferation, apoptosis, and intracellular communication.

There is evidence that the mechanism by which TCDD induces tumor promotion may involve oxygen radicals. In support of this, other studies have shown that TCDD induction of cancer-causing processes appears to result in a release of oxygen radicals and subsequent oxidative DNA damage that could lead to muta-

tion and cancer. This is also evidence that TCDD tumor promotion may be due to its ability to interfere with intercellular communications.

Low doses of TCDD administered to experimental animals alter the reproductive development and fertility of the progeny. Studies in male rats and hamsters have shown that decreased daily sperm production and cauda epididymal sperm number are some of the most sensitive effects of in utero and lactational TCDD exposure. However, in utero and lactational TCDD exposure does not appear to alter sperm transit time through the whole epididymis. Studies have been conducted to determine whether in utero and lactational TCDD exposure decreases male rat accessory sex organ weights during postnatal development and whether this effect involved decreases in hormone production or metabolism. Results suggest that in utero and lactational TCDD exposure selectively impairs rat prostate growth and development. TCDD exposure in gestating animals results in malformations of the external genitalia, including complete to partial clefting of the phallus. Additionally, functional reproductive alterations in female progeny are observed after TCDD exposure. Moreover, TCDD-mediated inhibition of angiogenesis has been suggested as an important contributor to the embryotoxicity of TCDD.

Animal studies and test-tube studies continue to emphasize the importance of alterations in neurotransmitter systems as underlying mechanisms of TCDD-induced behavioral dysfunction. TCDD can affect the metabolism of serotonin, a neurotransmitter in the brain able to modulate food intake. This biochemical change is consistent with observations of progressive weight loss and anorexia in experimental animals exposed to TCDD. In certain brain cells, there is evidence that TCDD may increase the uptake of calcium.

TCDD exposure causes a broad range of immunologic effects in experimental animals. Recent studies support earlier data that TCDD decreases innate immunity and host resistance to pathogenic microorganisms; impairs cell-mediated immune responses, such as the generation and lytic activity of cytotoxic T cells; and suppresses humoral immunity by inhibiting B-lymphocyte differentiation into antibody-producing cells. Despite considerable laboratory research, the mechanisms underlying the immunotoxic effects of TCDD are still unclear. TCDD immunotoxicity appears to be mediated primarily through aryl hydrocarbon receptor (AhR) dependent processes, but some components of immunosuppression have been shown to act independently of the Ah receptor.

Several recent studies have examined the effects of TCDD on specific disease outcomes in animals. Liver enlargement has, for example, been shown to occur following high subchronic doses. The mechanism by which TCDD affects the liver is still under investigation. Recently, TCDD has been shown to inhibit DNA synthesis of liver cells, decrease certain receptors in liver cell membranes, and inhibit liver enzymatic activity. TCDD has also been shown to affect blood serum hormone levels, an outcome thought to be partially due to the action of TCDD on the pituitary gland. TCDD has also been shown to affect the develop-

ment of skin cells by binding to the AhR. This effect is antagonized by retinoids. Several reports published during the reference period describe developmental deficits in the cardiovascular system of TCDD-treated animals. Evidence suggests that the endothelial lining of blood vessels is a primary target site of TCDD-induced cardiovascular toxicity.

Much research over the past two years has focused on the elucidation of the molecular mechanism of TCDD toxicity. Recent studies confirm earlier findings that the toxic effects of TCDD are caused by the binding of TCDD to the aryl hydrocarbon receptor. TCDD binding to this receptor triggers other effects that result in a toxic sequelae. Structural and functional studies of AhR and its partner protein Arnt indicate that similar protein receptors exist in a number of different species and interact with a number of other proteins to influence receptor function. TCDD may influence the ways in which genes are expressed by binding to the AhR. Researchers have recently bred mice that lack the AhR protein, and it is anticipated that these mice will allow more informative studies of TCDD effects in the future.

The toxicity of the herbicides used in Vietnam remains poorly studied. In general, the herbicides 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T, cacodylic acid, and picloram have not been identified as particularly toxic substances since high concentrations are often required to modulate cellular and biochemical processes. New reports suggest that 2,4-D may affect the membrane sheath around nerve cells. Other studies support the view that 2,4-D may disrupt cellular processes in the liver, and reports of kidney and muscle damage have been published. A case-control study of dogs exposed to 2,4-D, in addition to other pesticides used in yard work, reported an increase in lymphomas associated with exposure. Some animal studies suggest that 2,4,5-T may alter nerve and muscle function. 2,4,5-T may also induce mutations at different stages of cell development and hinder a cellular process that is involved in the elimination of harmful carcinogens.

Limited evidence from bioassays published during the past two years suggests that cacodylic acid may promote urinary, bladder, kidney, liver, and thyroid gland cancer in some species of animals.

EXPOSURE ASSESSMENT

Assessment of individual exposure to herbicides and dioxin is a key element in determining whether specific health outcomes are linked to these compounds. The committee responsible for producing *VAO* found, however, that the definition and quantification of exposure are the weakest methodologic aspects of the epidemiologic studies. Although different approaches have been used to estimate exposure among Vietnam veterans, each approach is limited in its ability to determine precisely the intensity and duration of individual exposure.

A separate effort by another Institute of Medicine committee is facilitating

the development and evaluation of models of herbicide exposure for use in studies of Vietnam veterans. That committee authored and disseminated a Request for Proposals for exposure assessment research in 1997 (IOM, 1997) and has begun to carry out scientific oversight of the research.

Although definitive data are presently lacking, the available evidence suggests that Vietnam veterans as a group had substantially lower exposure to herbicides and dioxin than did the subjects in many occupational studies. Participants in Operation Ranch Hand and members of the Army Chemical Corps are exceptions to this pattern, and it is likely that there are others who served in Vietnam who had exposures comparable in intensity to members of the occupationally exposed cohorts. Although it is currently not possible to identify this heavily exposed fraction of Vietnam veterans, the exposure assessment research effort presently under way may allow progress to be made on this important question.

CONCLUSIONS ABOUT HEALTH OUTCOMES

Chapters 7, 9, 10, and 11 provide a detailed evaluation of the epidemiologic studies reviewed by the committee and their implications for cancer, reproductive effects, neurobehavioral effects, and other health effects. As detailed in Chapter 4, the committee used the epidemiologic evidence it reviewed to assign each of the health outcomes being studied to one of the four categories listed in Table 1-1. The definitions of the categories and the criteria for assigning a particular health outcome to them are described in the table, and the specific rationale for each of the findings is detailed in the appropriate health outcomes chapter (Chapters 7, 9, 10, and 11).

Consistent with the mandate of Public Law 102-4, the distinctions between categories are based on "statistical association," not on causality, as is common in scientific reviews. Thus, standard criteria used in epidemiology for assessing causality (Hill, 1971) do not strictly apply. 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; these must rest with the Secretary of Veterans Affairs.

Health Outcomes with Sufficient Evidence of an Association

In *Update 1996*, the committee found sufficient evidence of an association between exposure to herbicides and/or TCDD and four diseases: soft-tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, and chloracne. The recent scientific literature continues to support the classification of these diseases in the category of sufficient evidence. Based on the recent literature, there are no additional diseases that satisfy this category's criteria—that 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 commit-

TABLE 1-1 Updated (1998) Summary of Findings in Occupational, Environmental, and Veterans Studies Regarding the Association Between Specific Health Outcomes 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

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/bronchus, larynx, trachea)
Prostate cancer
Multiple myeloma
Acute and subacute transient peripheral neuropathy
Spina bifida in the children of veterans
Porphyria cutanea tarda

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
Breast cancer
Female reproductive cancers (cervical, uterine, ovarian)
Urinary bladder cancer (category change in 1998)
Renal cancer
Testicular cancer
Leukemia
Spontaneous abortion
Birth defects (other than spina bifida)
Neonatal/infant death and stillbirths

continued

TABLE 1-1 *Continued*

Inadequate/Insufficient Evidence to Determine Whether an Association Exists (continued)

- Low birthweight
- Childhood cancer in offspring
- Abnormal sperm parameters and infertility
- Motor/coordination dysfunction
- Chronic peripheral nervous system disorders
- Metabolic and digestive disorders (diabetes, changes in liver enzymes, lipid abnormalities, ulcers)
- Immune system disorders (immune suppression and autoimmunity)
- Circulatory disorders
- Respiratory disorders
- Skin cancers

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:

- Gastrointestinal tumors (stomach cancer, pancreatic cancer, colon cancer, rectal 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.

tee regards evidence from several small studies that are free from bias and confounding, and that show an association that is consistent in magnitude and direction, as sufficient evidence for an association. The evidence that supports the committee's conclusions for the three cancers is detailed in Chapter 7 and for chloracne in Chapter 11.

Health Outcomes with Limited/Suggestive Evidence of Association

In *Update 1996*, the committee found limited/suggestive evidence of an association for six classes of diseases, three cancers—respiratory (larynx, lung/bronchus, and trachea) cancer, prostate cancer, and multiple myeloma—and three other health outcomes—spina bifida in the children of veterans, acute and subacute (transient) peripheral neuropathy, and porphyria cutanea tarda. The recent

scientific literature continues to support the classification of these diseases in the limited/suggestive category of sufficient evidence. Based on the recent literature, there are no additional diseases that satisfy this category's criteria.

For outcomes in this category, the evidence must be suggestive of an association with herbicides, but the association 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.

Since the last update, there have been several studies of respiratory cancer among occupationally exposed groups and Vietnam veterans. Newly published studies of phenoxy herbicide production workers (Kogevinas et al., 1997) and workers exposed as a result of an industrial accident (Ott and Zober, 1996) show small but statistically significant excesses of lung cancer mortality. Results in both studies indicate higher estimated risk for individuals with higher estimated exposure. One other occupational study (Ramlow et al., 1996) reports a relative risk indistinguishable from 1. A study of rice farmers in Italy (Gambini et al., 1997) found lower lung cancer incidence than observed in the general population, a result similar to that found in studies of U.S. farmers, which may reflect lower incidence of smoking in this occupational group. New data from the Seveso accident (Bertazzi et al., 1997) do not indicate any increase in lung cancer mortality in this environmentally exposed group, but an insufficient number of years have passed since exposure to draw conclusions about any effect that the accidental exposure may have had. Increases in respiratory cancers were seen in new studies of U.S. and Australian Vietnam veterans, although there is evidence that cigarette smoking was more prevalent among Vietnam veterans than among non-Vietnam veterans or the general public. In summary, the most recently published studies continue to support placing respiratory cancers in the category of limited/suggestive evidence. Although smoking undoubtedly plays a role in these cancers, the consistency of the finding across several studies argues against the notion that it is the sole explanatory factor.

New studies of production workers continue to show weak but consistent evidence of effects on prostate cancer mortality, whereas new research on agricultural workers shows no indication of increased risk. A detailed and well-conducted analysis of Australian male Vietnam veterans' mortality (Crane et al., 1997) found a statistically significant relationship between Vietnam service and prostate cancer. The committee's summary evaluation, based on all of the epidemiologic evidence, was that the data continue to support the classification of prostate cancer in the limited/suggestive category.

The evidence that supports the committee's conclusions for multiple myeloma is detailed in Chapter 7 and is not substantially changed from *Update 1996*.

In *Update 1996* the committee identified three studies of the offspring of Vietnam veterans that were suggestive of an association between exposure to the

herbicides considered in this report and spina bifida, although a number of methodologic issues limited the interpretation of these results. Since the publication of that report, occupational studies of the offspring of fathers employed in British Columbia sawmills (Dimich-Ward et al., 1996) and the offspring of Norwegian farmers (Kristensen et al., 1997), and a multicenter case-control study of paternal occupation and risk of spina bifida conducted in the Netherlands (Blatter et al., 1997), have provided some additional support for the association with this specific birth defect, although concerns remain including control of confounding, exposure determination, and isolation of exposure to specific herbicides and TCDD.

No additional evidence has been published since *Update 1996* regarding acute and subacute transient peripheral neuropathy or porphyria cutanea tarda.

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

The scientific data for many of the cancers and other diseases reviewed by the committee were inadequate or insufficient to determine whether an association exists. For diseases 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 (cancers of the liver and intrahepatic bile duct), nasal and nasopharyngeal cancer, bone cancer, skin cancers (including basal cell carcinoma, squamous cell carcinoma, and non-melanocytic skin cancers), breast cancer, cancers of the female reproductive system (including cervix, endometrium, and ovaries), testicular cancer, urinary bladder cancer, renal cancer (cancers of the kidney and renal pelvis), and leukemias. The scientific evidence regarding each of these cancers is detailed in Chapter 7.

Based on an evaluation of all the epidemiologic evidence, including studies published since the release of *Update 1996*, the committee felt that urinary bladder cancer should be added to this category. Although there is no evidence that exposure to herbicides or dioxin is related to this cancer, relative risks in some of the largest cohorts tended to be greater than 1, weakening the committee's prior conclusion that there was positive evidence of *no* relationship. The co-exposure to TCDD and a variety of known bladder carcinogens makes it very difficult to isolate any possible additional effect of herbicides, although little total effect was seen.

A recent community based case-control study examining herbicide exposure and skin cancers drew the attention of the committee (Gallagher et al., 1996). This study, which controlled for a number of factors known to influence skin cancer rates, found increasing risk of squamous cell carcinoma with increasing lifetime exposure to herbicides. Although there are concerns regarding the study's

control of confounding and the adequacy of the exposure assessment, the committee concluded that the study was the best of its kind to date. The available evidence is insufficient to determine whether an association exists between herbicide exposure and any of the forms of skin cancer. However, the committee encourages further study of basal and squamous cell skin cancer incidence among working and Vietnam veteran populations. In any future studies, careful attention should be paid to exposure assessment, as well as to controlling for confounding from UV exposures. Efforts to examine the carcinogenicity of organic arsenicals are also encouraged.

Several reproductive effects are classified in this category, including spontaneous abortion, birth defects other than spina bifida, neonatal or infant death and stillbirths, low birthweight, childhood cancer in offspring, and abnormal sperm parameters and infertility. The scientific evidence for reproductive effects is detailed in Chapter 9. Neurobehavioral effects that are classified in this category include cognitive and neuropsychiatric disorders, motor or coordination dysfunction, and chronic peripheral nervous system disorders. The scientific evidence for these effects is detailed in Chapter 10.

Other health effects that are classified in this category include metabolic and digestive disorders, immune system disorders, circulatory disorders, and respiratory disorders. The scientific evidence for these effects is detailed in Chapter 11.

Diabetes is a health outcome of special interest to the DVA. When viewed in the context of the total literature the committee concludes that, at this time, there is inadequate/insufficient evidence to determine whether an association exists between herbicide or dioxin exposure and increased risk of diabetes. Further analyses and full publication of existing studies may justify a reevaluation of this conclusion.

Many animal studies provide potential biological mechanisms for an association between herbicide exposure and diabetes risk. Although the majority of earlier reports on humans suggest little association, the potentially more definitive 1997 report from the Ranch Hand study (Henriksen et al., 1997) raises the possibility that the highest-exposure group (highest TCDD level) may have an increased risk. Such a conclusion may be supported by a currently unpublished NIOSH study of exposed workers. It is important to note that both these studies used serum TCDD levels as the measure of exposure. At this time, questions concerning case definition and full control for obesity, or other confounders (in the Ranch Hand study) preclude determining whether or not an association exists between herbicide exposure and diabetes in these studies. The committee strongly urges that the NIOSH study be documented more completely and published in the peer-reviewed literature, so that its potentially important findings can be evaluated fully. It strongly recommends that the Ranch Hand study develop a fully adjusted multivariate model (e.g., Cox Proportional Hazard with time to diabetes as the outcome), fully controlling for baseline age and obesity (BMI) and, if possible, for family history of diabetes, central fat distribution, diabetogenic drug

exposure, and a measure of obesity at the time of Vietnam service. The committee recommends consideration be given to a combined analysis of the Ranch Hand and NIOSH studies to further examine the possibility that herbicide exposure leads to an increased risk of diabetes.

Health Outcomes with Limited/Suggestive Evidence of *No* Association

In *VAO*, the committee found a sufficient number and variety of well-designed studies to conclude that there is limited/suggestive evidence of *no* association between a small group of cancers and exposure to TCDD or herbicides. This group includes gastrointestinal tumors (colon, rectal, stomach, and pancreatic) and brain tumors. Recent scientific evidence continues to support the classification of such cancers in this category and is detailed in Chapter 7. Based on the recent literature, there are no additional diseases that satisfy the criteria necessary for this category.

For outcomes in this category, several adequate studies covering the full range of levels of herbicide exposure that human beings are known to encounter are mutually consistent in not showing a positive association between exposure and health risk at any level of exposure. These studies 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 Relationship Between the Length of Time Since Exposure and the Possible Risk of Cancer Development

The importance of latency effects and other time-related factors in determining cancer risk has long been recognized, and statistical methodologies have been developed to study this issue. A variety of practical difficulties relating to exposure assessment and other data requirements, however, have limited the use of these methods in epidemiologic studies of environmental carcinogens. In response to the request from the DVA to explore latency issues related to herbicides used in Vietnam, the committee attempts in Chapter 8 to establish a methodology to address the timing of herbicide exposure and the risk of cancer. This chapter also reviews the literature on herbicide exposure and some cancers for results that describe how the timing of exposure affects the relative risk due to exposure.

One of the committee's tasks was to assess the likelihood that exposure to herbicides used in Vietnam resulted in or will result in increased risk of disease in Vietnam veterans. Currently, any such inference would have to be based on extrapolation from the findings about disease experience of other groups exposed to TCDD or herbicides generally. Given that we know when the potential exposure to TCDD and other herbicides used in Vietnam began and ended, it would

appear reasonable to examine time-related factors for those who served in Vietnam, but to date, no adequate analysis of time-related factors for cancer occurrence in Vietnam veterans has been published. Extrapolation from other types of studies is problematic for several reasons. Brief exposures, such as occurred in Seveso, and chronic occupational exposures may not apply to Vietnam veterans because of the different exposure situation. For example, there is evidence in the literature (e.g., for respiratory cancer) that latency can vary not only among individuals, but also according to other aspects of the exposure scenario, such as the magnitude of exposure. Thus, if high exposures in an occupational setting result in a certain pattern of relative risks for a given time since first exposure, this pattern may not hold for lower level exposures such as occurred in Vietnam. Similarly, direct evidence was not presented to evaluate the impact of age at exposure to herbicides. It is possible that the age at which exposure was received could influence the pattern of latency that would be observed (e.g., exposures incurred at younger ages could be more potent, but the impact might not be seen for a longer time period; conversely, exposures at older ages might be more harmful, particularly in the short run). Unfortunately, the data are not available to evaluate the hypothesis that age at exposure is important. A major limitation of the analyses discussed in this chapter is the failure of most studies to conduct analyses of latency that also controlled for factors such as duration of exposure, age, and calendar time of exposure (or analyses of age at exposure that controlled for time since exposure), particularly for occupational cohorts with protracted exposure periods.

Another consideration is the long retention time of TCDD and other highly chlorinated herbicides. Since body burdens from any exposure, no matter how brief, result in continuing exposures to internal organs, the concept of time since exposure ended has a different meaning than for chemical agents that are eliminated quickly.

A third issue concerns the distinction between morbidity and mortality. As discussed in Chapter 8, the latency between exposure and death is composed of two parts: (1) latency until disease appears and (2) time between disease occurrence and death. For diseases with low survival rates, such as respiratory cancer, the time between disease occurrence and death is generally short; therefore, a study focusing on mortality will give a good approximation of the latency period. However, for diseases that are not always fatal or that have a long survival time such as prostate cancer, it is preferable to examine incidence rather than mortality. Thus, further data on prostate cancer incidence would be of great help, since relatively few men with prostate cancer die from it.

Overall, the data on latency do not alter the committee's conclusions with regard to the categories of evidence for individual cancer sites, but they do provide some information on how long the effects of herbicide exposures last. The evidence suggests that if respiratory cancer does result from exposure to herbicides used in Vietnam, the greatest relative risk for lung cancer may be in

the first decade after exposure, but until further follow-up has been carried out for some of the cohorts, it will not be possible to put an upper limit on the length of time these herbicides could exert their effect. For prostate cancer, the published data are largely uninformative, and conclusions must await more definitive studies, preferably using incidence rather than mortality. For non-Hodgkin's lymphoma, effects are seen in the second decade after exposure begins and continue to be observed more than 20 years after external exposure ends. Because of the long retention times of TCDD, internal exposures can continue long after external exposures cease.

Increased Risk of Disease Among Vietnam Veterans

One of the three primary charges contained in the Agent Orange Act of 1991 (Public Law 102-4, subsequently codified as 38 USC Sec. 1116) states:

> For each disease reviewed, the Academy shall determine (to the extent that available scientific data permit meaningful determinations) . . . the increased risk of the disease among those exposed to herbicides during service in the Republic of Vietnam during the Vietnam era. . . .

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. Most of the evidence on which the findings regarding disease association 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 the health outcomes. However, the lack of adequate data on Vietnam veterans per se complicates the quantification of any increased risk of disease among individuals exposed to herbicides during service in Vietnam. Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the epidemiologic studies that have been reviewed (Chapters 7, 9, 10, and 11), the inadequate control for other important risk factors, and the uncertainty about the nature and magnitude of exposure to herbicides in Vietnam (Chapter 5), the necessary information to undertake a quantitative risk assessment is lacking.

Thus, the committee cannot quantify the degree of risk likely to be experienced by those exposed to herbicides during service in the Republic of Vietnam during the Vietnam era. For those outcomes in the "Sufficient" and "Limited/Suggestive" categories, what can be said is that too little is known about the herbicide exposure of veterans to make a meaningful determination of the increased risk, if any, of these outcomes among Vietnam veterans. As discussed above, the epidemiologic analyses to date have many limitations which prevent a more quantitative exposure-response analysis. Where there is inadequate/insuffi-

cient evidence to determine whether an association exists between herbicide exposure and a particular health outcome, there is also inadequate/insufficient information to assess the increased risk, if any, of that outcome. Finally, a finding of "limited/suggestive evidence of *no* association" between herbicide exposure and a health outcome means that the evidence suggests there is no increased risk of that outcome among Vietnam veterans. These conclusions are inevitably limited to the conditions, level of exposure, and length of observation covered by the studies reviewed by the committee. There are certain diseases where the committee can draw more specific conclusions, and this information is related in the discussion of those diseases.

REFERENCES

Blatter BM, Hermens R, Bakker M, Roeleveld N, Verbeek AL, Zielhuis GA. 1997. Paternal occupational exposure around conception and spina bifida in offspring. American Journal of Industrial Medicine 32(3):283–291.

Bertazzi PA, Zochetti C, Guercilena S, Consonni D, Tironi A, Landi MT, Pesatori AC. 1997. Dioxin exposure and cancer risk: A 15-year mortality study after the "Seveso Accident." Epidemiology 8(6):646–652.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997. Mortality of Vietnam veterans: the veteran cohort study. A report of the 1996 retrospective cohort study of Australian Vietnam veterans. Canberra: Department of Veterans' Affairs.

Dimich-Ward H, Hertzman C, Teschke K, Hershler R, Marion SA, Ostry A, Kelly S. 1996. Reproductive effects of paternal exposure to chlorophenate wood preservatives in the sawmill industry. Scandinavian Journal of Work, Environment and Health 22:267–273.

Gallagher RP, Bajdik CD, Fincham S, Hill GB, Keefe AR, Coldman A, McLean DI. 1996. Chemical exposures, medical history, and risk of squamous and basal cell carcinoma of the skin. Cancer Epidemiology, Biomarkers and Prevention 5(6):419–424.

Gambini GF, Mantovani C, Pira E, Piolatto PG, Negri E. 1997. Cancer mortality among rice growers in Novara Province, Northern Italy. American Journal of Industrial Medicine 31:435–441.

Henriksen GL, Ketchum NS, Michalek JE, Swaby JA. 1997. Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand. Epidemiology 8:252–258.

Hill, AB. 1971. Principles of Medical Statistics, 9th ed. New York: Oxford University Press.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange Health: Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Institute of Medicine. 1997. Characterizing Exposure of Veterans to Agent Orange and Other Herbicides Used in Vietnam: Scientific Considerations Regarding a Request for Proposals for Research. Washington, DC: National Academy Press.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Pearce N, Saracci R. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. American Journal of Epidemiology 145(12):1061–1075.

Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L. 1997. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 8(5):537–544.

Ott MG, and Zober A. 1996. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occuptional and Environmental Medicine 53:606–612.

Ramlow JM, Spadacene NW, Hoag SR, Stafford BA, Cartmill JB, Lerner PJ. 1996. Mortality in a cohort of pentachlorophenol manufacturing workers, 1940–1989. American Journal of Industrial Medicine 30:180–194.

2

Veterans and Agent Orange: Previous IOM Reports

BACKGROUND

Public Law 102-4, the "Agent Orange Act of 1991," was enacted on February 6, 1991. This legislation, codified as 38 USC Sec. 1116, directed the Secretary of Veterans Affairs to request that the National Academy of Sciences conduct a comprehensive review and evaluation of 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 Department of Veterans Affairs (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 the law. Finally, P.L. 102-4 called for updated reviews to be completed every two years after the initial report for a period of ten years.

To carry out the study, the IOM established the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. The results of the original committee's work were published in 1994 as *Veterans and Agent Orange* (henceforth called *VAO*) (IOM, 1994). This report contains a systematic review and evaluation of the then-available scientific evidence. Upon completion of *VAO*, a successor committee of the same name was formed that produced *Veterans and Agent Orange: Update 1996* (hereafter referred to as *Update 1996*) (IOM, 1996).

The present report follows this model, summarizing the content of the two previous reports and providing detailed reviews of the most recent research.

In conducting these studies, 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 studies provide scientific information for the Secretary of Veterans Affairs to consider as the DVA exercises its responsibilities to Vietnam veterans. *Update 1996* contains a summary of the literature addressed in *VAO* and in-depth reviews of the scientific studies and other information developed during the intervening time. The present report follows this model, summarizing the content of the two previous reports and providing detailed reviews of the most recent research.

In fulfilling its charge of judging whether each of a set of human health effects is associated with exposure to herbicides or dioxin, the committee concentrated primarily on reviewing and interpreting epidemiologic studies. The committee began its evaluation presuming neither the presence nor the absence of association. It 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 exposed; the characteristics of the evidence examined; and the approach taken to evaluate this evidence. To facilitate independent assessment of the committee's conclusions, Chapter 5 of *VAO* describes as explicitly as possible the methodological considerations that guided the original committee's review and its process of evaluation. This methodology was subsequently adopted by successor committees. It is summarized in Chapter 4 of this report.

In reviewing the literature, the committee found 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 employed to estimate exposure in Vietnam veterans and others exposed occupationally or environmentally, each approach is limited in its ability to determine precisely the degree and level of individual exposure. The available quantitative and qualitative evidence about herbicide exposure, summarized in Chapter 5, suggests that Vietnam veterans as a group had substantially lower exposure to herbicides and dioxin than the subjects in many occupational studies. Participants in Operation Ranch Hand are a known 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.

To obtain additional information pertinent to the evaluation of possible health effects of herbicide exposure, the committee decided to review studies of other groups potentially exposed to the herbicides used in Vietnam (2,4,5-trichlorophenoxyacetic acid [2,4,5-T], 2,4-dichlorophenoxyacetic acid [2,4-D], cacodylic acid, and picloram), 2,3,7,8-tertachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, TCDD, or dioxin), phenoxy herbicides, chlorophenols, and other compounds. These groups include chemical production and agricultural workers, residents of Vietnam, and people possibly exposed heavily to herbicides or dioxins as a result of residing near the site of an accident or near areas used to dispose of toxic waste. 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 the 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 levels and duration of exposure to the chemicals were greater, and the studies were of sufficient size to examine the health risks among people with varying levels of exposure.

Because of the great differences among the studies, the committee concluded that it was inappropriate to use a quantitative technique such as meta-analysis to combine their individual results into a single summary measure of statistical association. Using such a summary measure would also inappropriately focus attention on one piece of the information used by the committee when, in fact, all the factors discussed above are important to evaluating the literature.

Conclusions About Health Outcomes

VAO and *Update 1996* provide detailed reviews of the scientific studies evaluated by the committee and their implications for cancer, reproductive problems, neurobehavioral problems, and other health effects. The original report summarized the literature available in 1993; *Update 1996* examined all research available through mid-1995, but concentrated on work published since the completion of *VAO*.

The committee's statutory mandate is to determine, to the extent that available scientific data permit meaningful determinations,

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.

The original committee addressed the first part of this charge by assigning each of the health outcomes under study one of four categories on the basis of the epidemiologic evidence reviewed. The categories used by that committee were adapted from those used by the International Agency for Research on Cancer (IARC) in evaluating the evidence for carcinogenicity of various agents (IARC, 1977). Successor committees have adopted these categorizations in their evaluations.

The definitions of the categories and the criteria for assigning a particular health outcome to them are discussed below. 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 the findings reported do not imply or suggest any policy decisions; these must rest with the Secretary.

Health Outcomes with Sufficient Evidence of an Association

The original 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—and two other health outcomes, chloracne and porphyria cutanea tarda (PCT). After reviewing the whole of the literature available in 1995, the committee responsible for the first update concluded that the statistical evidence still supported this classification for the three cancers and chloracne. However, new data regarding porphyria cutanea tarda combined with the studies reviewed in *VAO* justified moving PCT to the category of *limited/suggestive evidence of an association with herbicide exposure*. Chapter 11 of *Update 1996* details this decision.

For diseases 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 regarded 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.

Health Outcomes with Limited/Suggestive Evidence of an Association

The committee responsible for *VAO* found limited/suggestive evidence of an association for three cancers: respiratory cancers, prostate cancer, and multiple myeloma. The *Update 1996* committee added three health outcomes to this list:

PCT (as explained above), acute and subacute transient peripheral neuropathy, and spina bifida in the children of veterans. Transient peripheral neuropathies had not been addressed in *VAO* since, by virtue of their transient nature, they were not amenable to epidemiologic study. In response to a request from DVA, the *Update 1996* committee added them to the list of reviewed health outcomes and made its determination on the basis of evidence available from case histories. This classification is addressed in Chapter 10 of the 1996 report. A 1995 analysis of birth defects among the offspring of Ranch Hands, in combination with earlier studies of neural tube defects in the children of Vietnam veterans published by the Centers for Disease Control and Prevention (CDC), led the *Update 1996* committee to distinguish spina bifida from other adverse reproductive outcomes and classify it in the *limited/suggestive* category. Chapter 9 of the 1996 report discusses this decision in detail.

For diseases in this category, the evidence must be suggestive of an association between herbicides and the outcome considered, but the association 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.

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

Scientific data for many of the cancers and other diseases reviewed by the *VAO* and *Update 1996* committees were inadequate or insufficient to determine whether any association exists. There was one change in the health outcomes in this category between the two reports: skin cancer was moved into this category in *Update 1996* when available evidence no longer supported its classification as a condition with limited/suggestive evidence of no association.

For diseases 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 may fail to control for confounding or have inadequate exposure assessment.

Health Outcomes with Limited/Suggestive Evidence of *No* Association

For a small group of cancers, the *VAO* 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 included gastrointestinal tumors (colon, rectal, stomach, and pancreatic), skin cancer, brain tumors, and bladder cancer. The *Update 1996* committee came to the same conclusions in all but one circumstance. It concluded that studies on skin cancer published since *VAO*, considered in combination with the evidence addressed in that report, no longer

supported the classification of this health outcome in the *no-association* category.

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 have relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, levels 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 second of the committee's three statutory mandates calls on it to determine, to the extent that available scientific data permit meaningful determinations, the increased risk of disease among individuals exposed to herbicides during service in Vietnam. Although there have been numerous health studies of Vietnam veterans, many have been hampered by relatively poor measures of exposure to herbicides or TCDD, in addition to other methodological problems. Most of the evidence on which the findings regarding associations are based comes from studies of people exposed to dioxin or herbicides in occupational and environmental settings, rather than from studies of Vietnam veterans. Both the *VAO* and *Update 1996* committees found this body of evidence sufficient for reaching their conclusions about statistical associations between herbicides and health outcomes. However, the lack of adequate data on Vietnam veterans per se complicated their consideration of the second part of the statutory charge. 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. The large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the studies that have been reviewed, the sometimes-inadequate control for important confounders, and uncertainty about the nature and magnitude of exposure to herbicides in Vietnam all combine to make quantitative risk assessments problematic. Thus, the *VAO* and *Update 1996* committees found that in general, it was not possible to quantify the degree of risk likely to be experienced by veterans because of their exposure to herbicides in Vietnam. The existing evidence about herbicide exposure among various groups studied does suggest that most Vietnam veterans (except those with documented high exposures, such as participants in Operation Ranch Hand) had lower exposure to herbicides and TCDD than did the subjects in many occupational and environmental studies. However, individual veterans who

had very high exposures to herbicides could have risks approaching those described in the occupational and environmental studies.

Existence of a Plausible Biologic Mechanism or Other Evidence of a Causal Relationship

Toxicological information forms the basis of the committee's response to the third part of the statutory charge—to determine whether there is a plausible biologic mechanism or other evidence of a causal relationship between herbicide exposure and a disease. This information is summarized in general terms in separate toxicology chapters in the previous reports: Chapter 4 of *VAO* and Chapter 3 of *Update 1996*. Specific findings for each health outcome are also given in the chapters that reviewed the epidemiologic literature.

Research Recommendations

The Academy 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 epidemiologic evidence and a consideration of the quality of exposure information available in existing studies, especially of Vietnam veterans, the committee responsible for *VAO* concluded that a series of epidemiologic studies of veterans could yield valuable information if a new, valid exposure reconstruction model could be developed. The original committee also saw value in continuing the existing Ranch Hand study and expanding it to include Army Chemical Corps veterans. The committee's research recommendations emphasized 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 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 this research is not targeted specifically to Vietnam veterans, it probably will also contribute to the knowledge of potential health effects in this population.

The committee responsible for *Update 1996* did not make any further research recommendations.

IMPACT OF THE REPORTS

On July 27, 1993, the Institute of Medicine released *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* to the news media and the public. Immediately following the press conference, the Senate Committee on Veterans Affairs held a hearing on the report. Testifying at the hearing, Secretary

of Veterans Affairs Jesse Brown announced that the Department of Veterans Affairs was already compensating Vietnam veterans exposed to herbicides for soft-tissue sarcoma, Hodgkin's disease, and chloracne. Based on the findings of the IOM committee, the DVA decided to begin immediately to compensate Vietnam veterans for non-Hodgkin's lymphoma and porphyria cutanea tarda (Category I diseases) (U.S. DVA, 1994). In September 1993, Secretary Brown announced that the DVA would also begin to compensate Vietnam veterans for respiratory cancers and multiple myeloma (Category II diseases) (U.S. DVA, 1993).

Veterans and Agent Orange: Update 1996 was publicly released on March 14, 1996. The next day, DVA formed a task force to review the findings, which reported its recommendations for action on May 16 of that year. The following subsections focus on impacts of the *Update 1996* report. A more detailed discussion of the impacts of *VAO* is contained in Chapter 2 of the 1996 report.

DVA Task Force

The DVA task force made four specific recommendations to Secretary Brown in the wake of the *Update 1996* report:

1. that the Secretary establish a presumption of service connection for prostate cancer based on exposure to an herbicide agent;
2. that the Secretary establish a presumption of service connection for acute and subacute peripheral neuropathy based on exposure to an herbicide agent if manifested within one year of exposure to the agent;
3. that the Secretary support increased research efforts to learn more about the possible relationship between exposure to herbicides and the development of birth defects, including spina bifida and other health problems in veterans' offspring; and
4. that the Secretary establish a presumption of service connection for spina bifida in offspring of veterans based on exposure to an herbicide agent *if* statutory authority was enacted granting such authority (U.S. DVA, 1996).

In a May 28, 1996, news conference, President Clinton and Secretary Brown announced the Administration's intention to implement all four recommendations. DVA proposed amending its regulations regarding presumptive service connection for prostate cancer and for acute and subacute peripheral neuropathy on August 8, 1996 (61 FR 41368-71), and announced a final rule concerning these conditions on November 7, 1996 (61 FR 57586-89). Legislation regarding the authority to grant compensation for spina bifida was proposed in July 1996. Section 421 of Public Law 104-204, which was signed on September 26, 1996, changed Title 38 of the U.S. Code to provide certain benefits, including a monthly monetary allowance, to children born with spina bifida (except spina bifida occulta) who are the natural children of veterans who served in Vietnam during

the Vietnam era. DVA then proposed (62 FR 23724-31, May 1, 1997) and finalized (62 FR 51274-96, September 30, 1997) regulations implementing the law. Subsequently, Section 404 of Public Law 105-114, signed on November 21, 1997, made technical corrections to some of the definitions in the original act.

FEDERAL GOVERNMENT'S RESPONSE TO CONCERNS OVER THE MILITARY USE OF HERBICIDES IN VIETNAM

The federal government has been involved with international and domestic policy issues related to the health effects associated with the military use of herbicides, particularly Agent Orange, since the defoliation program began in Vietnam. On December 16, 1974, the U.S. Senate ratified the Geneva Protocol, which broadly sought an international commitment from all governments that they would never use chemical or biological weapons (including herbicides) in war. In April 1975, President Ford issued Executive Order 11850 renouncing future use of herbicides in war.

U.S. Congress

Congressional interest concerning Vietnam veterans' health falls primarily into three categories: (1) health care (provision of services at VA medical centers); (2) scientific research (primarily, epidemiologic research on veterans); and (3) compensation issues (for disabilities that might have resulted from exposure to herbicides). As documented in *VAO* and *Update 1996*, congressional committees have for many years held informational and oversight hearings and introduced bills on these topics, and Congress has passed several laws dealing with the human health effects of exposure to the herbicides used in Vietnam. This section focuses on congressional action since the release of *Update 1996*.

Hearings on the *Update 1996* Report

Two congressional hearings were conducted to review the finding of *Update 1996*. The first of these was held on April 16, 1996, by the Subcommittee on Hospitals and Health Care of the House Committee on Veterans' Affairs (U.S. Congress, House, 1996). The IOM committee responsible for the report was represented by committee chair Dr. David Tollerud and member Dr. Andrew Olshan. Representatives of DVA and researchers involved in the CDC and the Ranch Hands studies of Vietnam veterans also participated. On September 19, 1996, the Senate Committee on Veterans Affairs heard testimony from Dr. Tollerud, Secretary of Veterans Affairs Brown, and others regarding the committee's classification of spina bifida into the *limited/suggestive evidence of an association* category (U.S. Congress, Senate, 1996).

Legislation Regarding Herbicide Exposure and the Health of Vietnam Veterans

In 1970, Congress enacted the first public law dealing with the military use of herbicides. Congress has since promulgated legislation to appropriate funds for herbicide exposure research, to provide clarification of payments received from the Agent Orange settlement fund, and to specify the conditions under which Vietnam veterans and their families may receive disability compensation for medical conditions.

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. The effect of this legislation was to provide health care for Vietnam veterans for conditions that require treatment and may have resulted from exposure to Agent Orange. Veterans need not demonstrate any direct 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 103-452 extended the program through June 30, 1995. A further extension through December 31, 1997, was authorized in P.L. 104-110. The Veterans' Health Care Eligibility Reform Act of 1996—which became P.L. 104-262 on October 9, 1996—significantly revamped VA medical care eligibility requirements for all veterans and superseded the provisions of previous acts with regard to Vietnam veterans. As mentioned earlier, the 1997 appropriations for the Department of Veterans Affairs (P.L. 104-204) included provisions extending health care benefits to the children of Vietnam veterans who are born with spina bifida.

Epidemiologic Studies. Public Law 96-151, enacted on December 20, 1979, ordered the Veterans Administration (VA) to conduct an epidemiologic study of the possible health effects in veterans of exposure to dioxin found in the herbicides used in Vietnam. The legislation also required the Office of Technology Assessment to review and approve the protocol for the study. In 1981, Public Law 97-72 expanded the scope of the epidemiologic study to include an evaluation of the impact on the health of Vietnam veterans of other environmental factors existing in Vietnam; this study was later transferred from the VA to the CDC and is referred to as the "Vietnam Experience Study." On April 7, 1986, President Reagan signed Public Law 99-272, which included provisions directing the VA to conduct an epidemiologic study of the long-term health effects of herbicide exposure on women who served in Vietnam. The Women Veterans Health Programs Act of 1992 (P.L. 102-585) expanded the program for women veterans.

Compensation. On October 24, 1984, Congress enacted Public Law 98-542, 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 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. As discussed earlier, this law also mandated the review of the scientific literature that resulted in the *Veterans and Agent Orange* series of reports.

Department of Veterans Affairs

The Department of Veterans Affairs is responsible for providing health care, compensation, and benefits to veterans of the Vietnam era. DVA has also been involved in conducting and assessing research and in monitoring studies on the health effects of herbicide exposure in veterans.

Health Care

The DVA provides certain health care services to veterans of the Vietnam era (defined as January 9, 1962, through May 7, 1975, in P.L. 105–114) who were possibly exposed to herbicides as a result of their service in Southeast Asia. Prior to receiving health care services, veterans must provide proof of service in Vietnam. When a veteran requests DVA medical care, he or she undergoes 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 (AOR) serves as a health surveillance data base; it contains records on approximately 10 percent of the entire Vietnam veteran population (self-selected) and is reviewed routinely for changes in health outcomes and mortality patterns. Since completion of the *Update 1996* report, DVA has published studies regarding Hodgkin's disease incidence (Dalager et al., 1995), the risk of death from trauma and selected cancers among Marine veterans (Watanabe and Kang, 1995), mortality patterns among Army and Marine veterans (Watanabe and Kang, 1996), mortality among Army Chemical Corps veterans (Dalager and Kang, 1997), and lung

cancer incidence (Mahan et al., 1997). These studies are reviewed in subsequent sections of this report. EES is also conducting or managing ongoing epidemiologic studies of women Vietnam veterans and Army Chemical Corps veterans.

Compensation and Benefits

The DVA compensates veterans for certain diseases related to exposure to dioxin-containing herbicides during their service in Vietnam. Whenever the Secretary determines that there is sound medical and scientific evidence indicating a positive association between exposure to an herbicide agent and occurrence of a disease in humans, DVA issues regulations stating that a presumption of service connection is warranted for the disease.

DVA's compensation policy provides that the Secretary take into account reports from the National Academy of Sciences and all other sound medical and scientific information and analysis in making determinations. In evaluating any study, the Secretary must take into consideration whether the results are statistically significant, are capable of replication, and can 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 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 regarding compensation or denial of compensation for these diseases are published in the *Federal Register*, and DVA solicits comments from the public before final regulations are issued.

Outreach Activities

The DVA's Environmental Agents Service (EAS) is responsible for developing and implementing national medical policies and procedures regarding the exposure of military veterans to possible environmental hazards, including Agent Orange. EAS maintains the Agent Orange Registry, a computerized index of Agent Orange medical examinations. As of December 29, 1997, there were 259,554 veterans in the Registry (Rosenblum, 1998). In addition to diagnostic data, the AOR also contains a variety of self-reported demographic and military characteristics (U.S. DVA, 1992). The registry's participants (all self-selected) receive the *Agent Orange Review,* a newsletter that provides updated information about Agent Orange and related matters. EAS also compiles fact sheets, called *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.

Department of the Air Force

In 1979, the Air Force began an epidemiologic study of Operation Ranch Hand personnel who participated in the aerial spraying of herbicides in Vietnam.

The Ranch Hand study, formally known as the "Air Force Health Study," is designed to assess whether long-term adverse health effects exist and can be attributed to occupational exposure to Agent Orange and other herbicides and dioxins. It is managed by the Population Research Branch of the Air Force Armstrong Laboratory. The study population consists of approximately 1,000 Ranch Hand personnel and approximately 1,300 Air Force personnel involved in aircraft missions in Southeast Asia during the same period that the Ranch Hand unit was active. Comparison veterans were not involved with spraying herbicides. The study includes periodic analyses of postservice mortality, physical examinations, in-person interviews, medical record retrievals, and psychological testing. Examinations were administered in 1982, 1985, 1987, and 1992. The 1997 follow-up examinations began in May 1997 and were scheduled to be completed in March 1998. A final follow-up is planned for 2002.

Numerous reports and papers regarding the Ranch Hand study population have been published. Many of these are reviewed in the earlier *Veterans and Agent Orange* reports. A complete listing of research publications is available at the study's Web site: http://www.brooks.af.mil/AFRL/HED/hedb/afhs.html. The National Technical Information Service maintains copies of the reports and publicly available data files.

Environmental Protection Agency

In 1991, the Environmental Protection Agency (EPA) began a scientific reassessment of the risks of exposure to the dioxin 2,3,7,8-TCDD and chemically similar compounds. EPA undertook this project in response to newly emerging scientific knowledge about the mechanisms of action of dioxin (U.S. EPA, 1992). The reassessment is part of EPA's efforts to improve the research and scientific base of the agency and to incorporate solid research and science into its decisions. In 1994, the EPA released a draft report on the project seeking comment on its technical accuracy and policy implications (U.S. EPA, 1994). The draft stated there was the potential for adverse impacts on human metabolism, developmental biology, reproductive biology and possibly other effects in the range of current human exposures to TCDD. According to the draft, evidence also suggested that dioxin and related compounds were likely to present a cancer hazard to humans. It was noted that there were significant data gaps and that additional information was needed to reduce uncertainty in these conclusions. The EPA Science Advisory Board (SAB) submitted its evaluation of the draft in September 1995 (SAB, 1995). It faulted the draft for its reliance on the standard EPA default assumption of a linear non-threshold model for carcinogenic risk. The SAB asserted that the report's presentation of scientific findings concerning possible risks was not balanced, with a tendency to overstate the possibility for danger; and that important uncertainties associated with the conclusions were not fully identified. However, it also noted almost all the SAB reviewers concurred with EPA's draft

judgment that dioxin, under some conditions of exposure, was likely to increase human cancer incidence. The EPA report was undergoing revision at the end of 1998.

International Agency for Research on Cancer

IARC was established in 1965 by the World Health Organization to coordinate and conduct research on the causes of human cancer and to develop scientific strategies for cancer control. One of IARC's primary efforts is a program to evaluate the carcinogenic risk of chemical, radiation, and other exposures to humans. This work is conducted by international working groups of experts who review and evaluate the scientific literature. To date, more than 800 agents (including chemicals, groups of chemicals, complex mixtures, occupational exposures, and biological or physical agents) have been examined.

A working group of 25 scientists from 11 countries was convened in 1997 to review evidence for the potential carcinogenicity of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (IARC, 1997). The working group reviewed published scientific data on the occurrence of cancer in human populations known to have been exposed to high levels of dioxins, assessed the evidence for carcinogenic effects of dioxins in experimental animals, and evaluated possible biologic mechanisms of carcinogenesis for these substances.

The IARC working group concluded on the basis of its review that 2,3,7,8-TCDD is carcinogenic to humans. It cited three major categories of supporting evidence:

1. 2,3,7,8-TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah Receptor.
2. This receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals.
3. Tissue concentrations are similar both in heavily exposed human populations in which an increased overall cancer risk has been observed and in rats exposure to carcinogenic dosage regimens in bioassays.

REFERENCES

Conway F. 1993. Memorandum to the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. Washington: Department of Veterans Affairs. May 18.

Dalager NA, Kang HK. 1997. Mortality among Army Chemical Corps Vietnam veterans. American Journal of Industrial Medicine 31:719–726.

Dalager NA, Kang HK, Burt VL, Weatherbee L. 1995. Hodgkin's disease and Vietnam service. Annals of Epidemiology 5:400–406.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

International Agency for Research on Cancer (IARC). 1977. Some Fumigants, The Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 15. Lyon, France: World Health Organization (WHO), IARC.

International Agency for Research on Cancer, Working Group on the Evaluation of Carcinogenic Risk of Humans. 1997. Polychlorinated Dibenzo-*para*-dioxins and Polychlorinated Dibenzofurans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 69. Lyon, France: WHO, IARC.

Mahan CM, Bullman TA, Kang HK, Selvin S. 1997. A case-control study of lung cancer among Vietnam veterans. Journal of Occupational and Environmental Medicine 39(8):740–747.

Rosenblum DJ. 1998. January 12 fax to the staff of the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. Washington: U.S. Department of Veterans Affairs, Environmental Agents Service.

Science Advisory Board (SAB), U.S. Environmental Protection Agency. 1995. Letter from Drs. Genevieve Matanoski, Morton Lippmann, and Joan Daisey, Chairs, to the Honorable Carol M. Browner, Administrator, U.S. Environmental Protection Agency; Subject: Science Advisory Board's review of the Draft Dioxin Exposure and Health Effects Reassessment Documents. September 29. EPA-SAB-EC-95-021.

U.S. Congress, House. 1996. Committee on Veterans' Affairs, Subcommittee on Hospitals and Health Care. Hearing: Institute of Medicine Update on Veterans and Agent Orange. 104th Cong., 2nd Sess. Serial No. 104-17.

U.S. Congress, Senate. 1989. Committee on Veterans' Affairs. Report on Veterans' Agent Orange Exposure and Vietnam Service Benefits Act of 1989. 101st Cong., 2nd Sess. Report 101-82.

U.S. Congress, Senate. 1996. Committee on Veterans' Affairs. Hearing: Effect of Herbicide Exposure on Reproductive Outcomes. 104th Cong., 2nd Sess. S. Hrg. 104-723.

U.S. Department of Veterans' Affairs (DVA). 1992. Agent Orange Briefs A1-D5. Washington: DVA, Environmental Agents Service.

U.S. Department of Veterans' Affairs. 1993. Report to the Secretary of Veterans' Affairs: VA Agent Orange Task Force. Washington: DVA.

U.S. Department of Veterans' Affairs. 1994. News Release: VA Announces Rules in Place for More Agent Orange-Related Diseases. Washington: DVA, Office of Public Affairs.

U.S. Department of Veterans' Affairs. 1996. Report to the Secretary of Veterans Affairs on Veterans and Agent Orange. Washington: DVA.

U.S. Environmental Protection Agency (EPA). 1992. Workshop Review Draft of Health Assessment for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds. Washington: EPA, Office of Research and Development.

U.S. Environmental Protection Agency. 1994. Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds. Review Draft. Volumes I-III. Washington: EPA, Office of Research and Development.

Watanabe KK, Kang HK. 1995. Military service in Vietnam and the risk of death from trauma and selected cancers. Annals of Epidemiology 5:407–412.

Watanabe KK, Kang HK. 1996. Mortality patterns among Vietnam veterans: a 24-year retrospective analysis. Journal of Occupational and Environmental Medicine 38(3):272–278.

3

Toxicology

As in *Veterans and Agent Orange* (*VAO*) and *Veterans and Agent Orange: Update 1996* (*Update 1996*), this review summarizes the experimental data that serves as a scientific basis for assessment of the biologic plausibility of health outcomes reported in epidemiologic studies. Efforts to establish the biologic plausibility of effects due to herbicide exposure in the laboratory strengthen the evidence for the herbicide effects suspected to occur in humans. Differences in chemical levels, frequency of administration, single or combined exposures, preexisting health status, genetic factors, and routes of exposure significantly influence toxicity outcomes. Thus, any attempt to extrapolate from experimental studies to human exposure must carefully consider such variables before conclusions are made.

Multiple chemicals were used for various purposes in Vietnam. Four herbicides documented in military records were of particular concern and are addressed here: 2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); picloram; and cacodylic acid. In addition, the toxicologic properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin), a contaminant of 2,4,5-T, are discussed. This chapter focuses to a large extent on the toxicological effects of TCDD, because considerably more information is available on TCDD than on the herbicides.

SUMMARY

Toxicokinetics

New information on the distribution of 2,4-D and the metabolism of cacodylic acid has improved understanding of how the body handles these sub-

stances. 2,4-D enters the brain, but only to a limited extent, and its uptake by the brain appears to be an energy-dependent process. Cacodylic acid is one of the major metabolic products of ingested arsenic in mammals. Studies using skin taken from mice report that the absorption of cacodylic acid is influenced by the substance in which it is dissolved and the length of time that cacodylic acid remains in contact with the skin.

TCDD, unlike the herbicides, stays in the body for a long time. In humans about half is eliminated every 8.5 years. It is removed from the body as it is metabolized to less toxic forms that are more easily eliminated in the urine than TCDD itself. The length of time that TCDD remains in the body increases with increasing body fat.

New evidence based on animal models suggests that rats and humans tend to handle TCDD in body tissues in similar ways. However, rats tend to excrete TCDD more quickly. Rats are most likely to absorb TCDD through food and air and this fact may carry over to humans. However, the types of TCDD and other dioxins that accumulate in the body may differ markedly between humans and rodents.

Mechanisms of Toxic Action

Little is known about the way in which the herbicides produce toxic effects in animals. Recent studies have focused on the mechanisms of cellular toxicity of 2,4,5-T. For example, some studies using animal tissues suggest that 2,4,5-T may alter nerve and muscle function by interacting with chemicals that participate in nervous system function. 2,4,5-T may induce mutations at different stages of cell development. Finally, it may alter the cellular process involved in the elimination of harmful carcinogens.

To date, the consensus is that TCDD is not directly toxic to the body's genetic material. However, it may affect enzymes and hormone levels, which in turn may produce adverse effects.

Recent studies confirm earlier findings that most of the toxic effects of TCDD are caused by its binding to a protein called the aryl hydrocarbon receptor (AhR). The binding of TCDD to this protein triggers various events that result in toxic sequelae. However, some tests suggest that other events, in addition to the binding of TCDD to the AhR, are involved. Studies of the AhR and its partner protein Arnt (aryl hydrocarbon nuclear translocator protein) indicate that similar proteins exist in different species and interact with a number of other proteins to produce an effect. Researchers have recently bred mice that lack the AhR protein. It is anticipated that these mice will allow more informative studies of the way TCDD reacts with the AhR to produce a toxic effect.

Disease Outcomes

Disease outcomes associated with herbicide exposures continue to be debated. Some cellular-level effects have been identified, although it is not clear

what impact these may have on living organisms. Other studies suggest disease effects including neurotoxicity and kidney, liver, and muscle damage at certain high dose levels in particular animal species; however, translating these results to the exposures experienced by veterans and others remains problematic.

2,4-D appears to affect the membrane sheath around nerve cells. Other studies support the view that 2,4-D may disrupt cellular processes in the liver, and reports of kidney and muscle damage have been published. Results from studies indicate that high doses of 2,4-D are necessary to produce these effects. A case-control study of dogs exposed to 2,4-D in addition to other pesticides used in yard work, reported an increase in lymphomas associated with exposure.

The limited evidence published during the past two years suggest that cacodylic acid may promote cancer in rats.

Several recent studies have examined the role of TCDD in producing certain disease outcomes in animals, including acute toxicity, dermal toxicity, liver toxicity, neurotoxicity, immunotoxicity, reproductive and developmental toxicity, and cancer.

A prominent symptom of the acute toxicity of TCDD is the loss of fat tissue and body weight, a phenomenon known as wasting syndrome. Several mechanisms are under investigation including inhibition by TCDD of sugar transport activity, effects on fat cell differentiation, and effects on certain receptors and enzymes. There is some evidence to suggest that gender differences exist in the response of fat cells to TCDD.

TCDD has also been shown to affect the development of skin cells by binding to the AhR. This effect is antagonized by retinoids.

Liver enlargement has been shown to occur following high doses of TCDD. The mechanism by which TCDD affects the liver is still under investigation. Recently, it has been shown to inhibit DNA synthesis of liver cells, decrease certain receptors in liver cell membranes, and inhibit liver enzymes.

Animal and test-tube studies continue to emphasize the importance of alterations in neurological systems as underlying mechanisms of TCDD-induced behavioral dysfunction. TCDD can affect the metabolism of serotonin, a substance in the brain that can modulate food intake. This biochemical change is consistent with observations of progressive weight loss and anorexia in experimental animals exposed to TCDD. In certain brain cells, there is evidence that TCDD may increase the uptake of calcium.

It is known that TCDD exposure causes a broad range of immunologic effects in experimental animals. Recent studies support earlier data that TCDD decreases immunity and host resistance to pathogenic microorganisms. Despite considerable laboratory research, the mechanisms underlying the immunotoxic effects of TCDD are still unclear. TCDD immunotoxicity appears to be mediated primarily through the AhR, but some components of immunosuppression have been shown to act independently of this receptor.

Low doses of TCDD administered to experimental animals alter reproductive development and fertility of the offspring. When TCDD is administered to pregnant rats, malformations of the external genitalia are observed in female offspring. Functional reproductive alterations in female offspring are also observed after TCDD exposure, including decreased fertility rates and reduced fecundity.

Studies in male rats and hamsters have shown that decreased daily sperm production is one of the most sensitive effects of exposure to TCDD in the womb and through breast milk. Results also suggest that TCDD exposures selectively impair rat prostate growth and development.

TCDD has been shown to affect blood serum hormone levels. This outcome is thought to be due partially to the action of TCDD on the pituitary gland.

Several reports published during the reference period focused on the mechanism by which TCDD induces cleft palates in experimental animals. Evidence suggests that this effect involves the AhR. There have also been reports of developmental defects in the cardiovascular system of TCDD-treated animals. Evidence suggests that cells lining the blood vessels are a primary target of TCDD-induced developmental cardiovascular toxicity.

Studies continue to focus on the mechanism by which TCDD induces cancer in animals. Although there is considerable evidence that TCDD-induced cancer is mediated by the AhR, it does not appear to be solely responsible. There is also evidence that the mechanism by which TCDD induces tumor promotion may involve reactive molecules containing oxygen, which are known as oxygen radicals. It is hypothesized that a release of oxygen radicals by TCDD causes DNA damage that could lead to mutation and cancer. There is also evidence that TCDD tumor promotion may be due to its ability to interfere with intercellular communications.

Inconsistencies reported in the molecular basis of dioxin's actions reflect the degree of tissue, cell, and gene specificity that characterizes the toxic response.

Relevance to Human Health

Exposure to 2,3,7,8-TCDD, a contaminant in some of the herbicides used in Vietnam, has been associated with both cancer and noncancer end points in animals. Studies in animals indicate that TCDD effects are mediated through the AhR. Although structural differences in the AhR have been identified, it operates in a similar manner in animals and humans, and a connection between TCDD exposure and human health effects is, in general, considered biologically plausible. Evidence has also begun to accumulate for non-AhR mediated effects. Animal research indicates that TCDD can both cause cancers or tumors and enhance the incidence of certain cancers or tumors in the presence of known carcinogens. However, experimental animals greatly differ in their susceptibility to TCDD-induced effects, and the sites at which tumors are induced also varies

from species to species. Other noncancer health effects vary according to dose and to the animal exposed. Controversy exists over whether the effects of TCDD and other exposures are threshold dependent, that is, whether some exposure levels may be too low to induce any effect.

Limited information is available on the biologic plausibility of herbicide health effects not connected with TCDD. Although concerns have been raised about non-dioxin contaminants of herbicides, far too little is known about the ubiquitousness and concentration of these compounds in the formulations used in Vietnam to draw conclusions about their impact.

Considerable uncertainty remains about how to apply this information to evaluation of the potential health effects in Vietnam veterans of herbicide or dioxin exposure. Scientists disagree over the extent to which information derived from animal and cellular studies predicts human health outcomes and the extent to which health effects resulting from high-dose exposure are comparable to those resulting from low-dose exposure. Research on biological mechanisms is burgeoning, and subsequent *VAO* updates may have more and better information on which to base conclusions.

VAO AND *UPDATE 1996*—OVERVIEW

Chapter 4 of *VAO* and Chapter 3 of *Update 1996* review the results of animal and test-tube studies published until 1995 that investigated the toxicokinetics, mechanism of action, and disease outcomes of TCDD and herbicides. According to these earlier reviews, TCDD elicits a diverse spectrum of biological sex-, strain-, age-, and species-specific effects, including carcinogenicity, immunotoxicity, reproductive and developmental toxicity, hepatotoxicity, neurotoxicity, chloracne, and loss of body weight. The scientific consensus is that TCDD is not genotoxic and that its ability to influence the carcinogenic process is mediated via epigenetic events such as enzyme induction, cell proliferation, apoptosis, and intracellular communication. The toxicity of the herbicides used in Vietnam has been poorly studied. In general, the herbicides 2,4-D, 2,4,5-T, cacodylic acid, and picloram have not been identified as particularly toxic substances since high concentrations are often required to modulate cellular and biochemical processes. A comprehensive description of the toxicological literature published until 1995 can be found in *VAO* and *Update 1996*.

UPDATE OF THE SCIENTIFIC LITERATURE—OVERVIEW

Toxicokinetics

A limited number of studies have been published since *Update 1996* that examine the biologic and toxic effects of 2,4-D. Toxicokinetic studies using rabbits suggest that uptake of 2,4-D by the brain is restricted by the developing,

as well as the mature, blood–brain barrier. In hamsters, cellular 2,4-D uptake appears to be an energy-dependent process.

During the reference period since the publication of *Update 1996*, the disposition of TCDD in humans has been investigated in two studies. Based on multiple serum measurements collected over a 10-year period from 213 veterans of Operation Ranch Hand, the mean decay rate of TCDD was estimated to be 0.0812 per year, with a corresponding half-life estimate of 8.532 years. In these veterans, half-life increased significantly with increasing body fat, but not with age or relative changes in the percentage of body fat. In another human study, the impact of breastfeeding on the body burden of dioxin-like chemicals in Arctic Inuit people was investigated. Toxicokinetic modeling revealed that breast feeding strongly influences the body burden of TCDD during childhood but not after 20 years of age. In addition, liver and adipose tissue concentrations in adults greater than 20 years of age appeared to be lower than those associated with cancer and adverse reproductive effects in laboratory animals.

Using a physiologically based model that describes the distribution kinetics of dioxin-like chemicals in various mammalian species, the kinetic profile of TCDD was found to be similar in rats and humans, although the half-lives differ considerably between species. The half-life of TCDD in rats and humans is measured in weeks and years, respectively. Comparative studies of the systemic absorption of TCDD in rats following oral and inhalation exposures indicate that both exposures are significant routes of absorption—an observation that is of relevance to humans given the similarities in kinetic profiles between rats and humans. In addition, for a given body burden, the adipose tissue concentrations have been found to vary in an inversely proportional manner to the mass of adipose tissues. Despite similarities in the toxicokinetic profile of rats and humans, some data suggest that humans may bioaccumulate higher levels of certain dioxins than mice due to interspecies metabolic differences.

Results from another model of the disposition of TCDD in the rat indicate that TCDD increases the enzymatic activity of UDP-glucuronosyltransferase (UGT) and the levels of blood thyroid-stimulating hormone (TSH). Calculated increases in blood TSH levels are consistent with prolonged stimulation of the thyroid and may represent an early stage in the induction of thyroid tumors identified in previous two-year bioassays. This suggests that increases in UGT activity may be a useful biomarker for tumorigenic changes in hormone levels after TCDD exposure. However, certain noncancer end points may be more significant in assessing human health risks to TCDD than cancer end points. For instance, immune suppression and enzymatic induction have been found to occur at lower doses and under conditions more relevant to general population exposure conditions. In assessing the risk of humans to dioxins, it should also be noted that recent data suggest that toxic equivalency factors (TEFs) derived from short-term assays may not adequately predict the relative potencies of this class of compounds following chronic exposure.

Mechanisms of Toxic Action

The mechanisms of cellular toxicity of 2,4,5-T have been the focus of a number of recent studies. One study presents compelling evidence that 2,4,5-T interacts with choline to generate false cholinergic messengers that alter neuronal and muscular function. Another study found that 2,4,5-T can induce mutations at different germ cell stages. Finally, there is some evidence that 2,4,5-T modulates cellular metabolism to alter the expression of membrane pumps and drug-metabolizing enzymes involved in the disposition of chemical carcinogens.

Dimethylarsinic acid (cacodylic acid, DMA) is one of the major methylated metabolites of ingested arsenicals in mammals. During the reference period, toxicokinetic studies reported that the rates of in vitro dermal absorption of DMA can be influenced by both the vehicle of administration and the duration of exposure.

Scientific reports published during the past two years continue to focus on the mechanism by which TCDD exerts its effects. Structural and functional studies of the AhR and Arnt indicate that both proteins are highly conserved, are found in diverse vertebrate groups, and interact with a large number of proteins to influence nuclear events. In vitro studies have confirmed in vivo findings regarding the functional binding domains of mouse AhR that interact with the heat shock protein (hsp90). Other results continue to support the view that TCDD influences patterns of gene expression by modulating transcriptional and post-transcriptional events. Such responses are often mediated by the AhR but exhibit considerable tissue and cell specificity. From a toxicologic perspective, the development of AhR knockout mice has been an important advance because it has helped establish a definitive association between the AhR and TCDD-mediated toxicities. Some studies suggest that specific patterns of Arnt expression differ in certain tissues from those of the AhR and that Arnt may have roles in normal embryonic development independent of the AhR. The recent discovery that the oxygen-regulated transcription factor HIF-1α and the AhR share a common heterodimerization partner Arnt (HIF-1β) has fueled intensive investigation into the possible crosstalk between oxygen and dioxin signal transduction pathways.

Disease Outcomes

While disease outcomes associated with 2,4-D exposures continue to be debated, neurotoxic effects have been reported in rats administered high acute doses, possibly as a result of neuronal demyelination. Studies on rats continue to support the view that the hepatotoxic effects of 2,4-D may involve disruption of thiol homeostasis. Reports of kidney and muscle damage have also been published. A case-control study of dogs exposed to 2,4-D in addition to other pesticides used in yard work, reported an increase in lymphomas associated with exposure. Although 2,4-D induced significant numbers of mutations in a *Droso-*

phila cell line and increased mRNA levels of multidrug resistance (mdr) genes in mouse liver, cancer bioassays show no carcinogenic effect. Results from chronic and subchronic toxicity studies indicate that 2,4-D is relatively nontoxic.

Limited research has been conducted on the offspring of male animals exposed to herbicides. A study of male mice fed varying concentrations of simulated Agent Orange mixtures concluded there were no adverse effects in offspring. A statistically significant excess of fused sternebra in the offspring of the two most highly exposed groups was attributed to an anomalously low rate of the defect in controls. Another study reported an increase in the incidence of malformed offspring of male mice exposed to subacute levels of a mixture of 2,4-D and picloram in drinking water. However, the paternal toxicity observed in the high dosage levels used and inconsistent dose–response pattern are of concern.

Limited evidence presented during the past two years suggests that DMA acts as a promoter of urinary bladder, kidney, liver, and thyroid gland carcinogenesis in rats. DMA induces apoptosis and sensitizes DNA to oxidative injury.

TCDD has been shown to adversely affect a number of organ systems that have been or may be linked to a variety of disease outcomes. TCDD lethality has been associated with changes in brain serotonin metabolism. However, the wide interspecies differences in TCDD-induced lethality cannot be explained by changes in tryptophan metabolism or carbohydrate homeostasis.

A prominent symptom of the acute toxicity of TCDD is the loss of adipose tissue and body weight, a phenomenon known as wasting syndrome. Several mechanisms are under investigation including inhibition by TCDD of glucose transport activity and hepatic phosphoenolpyruvate carboxykinase (PEPCK, the rate-limiting enzyme of hepatic gluconeogenesis); the effects of TCDD on adipocyte differentiation; and the effects of TCDD on epidermal growth factor receptor and protein-tyrosine kinase. There is some evidence to suggest that gender differences exist in the response of cells to TCDD. Glucose uptake and lipoprotein lipase activity were significantly decreased in adipose tissue in vitro after intraperitoneal (ip) injection of TCDD in male guinea pigs. No significant effect was observed in females. In addition, radiolabeled-TCDD binding affinity studies in adipose explant tissues showed that tissues from male guinea pigs and monkeys had a higher binding capacity for TCDD than female tissues.

TCDD has been shown to induce differentiation in human keratinocytes, which may be initiated by TCDD binding to the AhR. This effect is antagonized by retinoids and may involve interactions between TCDD and retinoids in the regulation of epithelial differentiation.

The mechanism by which TCDD induces hepatotoxicity is still under investigation. TCDD has been shown to inhibit hepatocyte DNA synthesis; decrease hepatic plasma membrane epidermal growth factor receptor; inhibit hepatic pyruvate carboxylase activity as a consequence of a reduction in pyruvate carboxylase mRNA levels (this effect was ten-fold greater than in congenic Ahb/b mice, suggesting that a competent AhR is required); and induce cytochrome P4501A1

(CYP1A1) in fish and chick embryo hepatocyte cultures, resulting in porphyrin accumulation. Studies have been conducted to examine the short- and long-term effects of TCDD on rat ethoxyresorufin *o*-deethylase (EROD) activity and liver enzymes. Four days after oral dosing, EROD activity was considerably elevated. Hepatic PEPCK and glutamyl transpeptidase activities were inhibited and stimulated, respectively. Ninety days after dosing, liver EROD activity and PEPCK activity revealed considerable reversibility, whereas glutamyl transpeptidase activity remained elevated. Hepatomegaly has been shown to occur following high subchronic doses.

Using Mardin Darvey canine kidney cells, TCDD has been shown to stimulate transcription of the PGHS-2 gene. It has been suggested that PGHS-2 expression may be involved in toxic reactions that involve inappropriate cellular growth, such as tumor promotion.

Animal studies and in vitro mechanistic studies continue to emphasize the importance of alterations in neurotransmitter systems as underlying mechanisms of TCDD-induced behavioral dysfunction. Lethal doses of TCDD administered to rats affect the metabolism of serotonin, a neurotransmitter in the brain able to modulate food intake. This biochemical change is consistent with observations of progressive weight loss and anorexia in experimental animals exposed to TCDD. In primary cultures of rat hippocampal neuronal cells, there is evidence that TCDD may increase the uptake of intracellular calcium. This concentration-dependent increase in calcium is associated with a decrease in mitochondrial membrane potentiation and activation of α-protein kinase C (α-PKC).

TCDD and structurally related halogenated aromatic hydrocarbons cause a broad range of immunologic effects in experimental animals. Recent studies support earlier data that TCDD decreases innate immunity and host resistance to pathogenic microorganisms; impairs cell-mediated immune responses, such as the generation and lytic activity of cytotoxic T cells; and suppresses humoral immunity by inhibiting B-lymphocyte differentiation into antibody-producing cells. Despite considerable laboratory research, the mechanisms underlying the immunotoxic effects of TCDD are still unclear. TCDD immunotoxicity appears to be mediated primarily through AhR-dependent processes, but some components of immunosuppression have been shown to act independently of the AhR.

Low doses of TCDD administered to experimental animals alter reproductive development and fertility of the progeny. Studies in male rats and hamsters have shown that decreased daily sperm production and cauda epididymal sperm number are some of the most sensitive effects of in utero and lactational TCDD exposure. However, in utero and lactational TCDD exposure does not appear to alter radiolabeled sperm transit time through the whole epididymis. Studies have been conducted to determine whether in utero and lactational TCDD exposure decreases male rat accessory sex organ weights during postnatal development and whether this effect involved decreases in testicular androgen production or changes in peripheral androgen metabolism. Results suggest that in utero and

lactational TCDD exposure selectively impairs rat prostate growth and development without inhibiting testicular androgen production or consistently decreasing prostate dihydrotestosterone (DHT) concentration. Male mice treated with a mixture of 2,4-D, 2,4,5-T, and TCDD exhibited dose-related liver and thymus toxicity and reduced weight gain, although no significant effects were observed on sperm function, reproductive outcomes, survival of offspring, or neonatal development.

In female rats, a single dose of TCDD administered on gestational day (GD) 15 results in malformations of the external genitalia in Long Evans (LE) and Holtzman rats. There was complete to partial clefting of the phallus. Treatment on GD 8 was more effective in inducing functional reproductive alterations in female progeny (e.g., decreased fertility rate, reduced fecundity, cystic endometrial hyperplasia, increased incidence of constant estrus).

TCDD administered by gastric intubation altered serum hormone levels in immature female rats. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin levels were increased. This effect is due partially to the action of TCDD on the pituitary and is calcium dependent.

After water-borne exposure of newly fertilized eggs to TCDD, the toxicity and histopathology of TCDD in zebrafish revealed that TCDD did not increase egg mortality or affect time to hatching. However, pericardial edema and craniofacial malformations were observed in zebrafish larvae. Reports indicate that in ovo TCDD exposure of the domestic chicken, domestic pigeon, and great blue heron adversely affected the body and skeletal growth and hatchability of the domestic pigeon but had no effect on the domestic chicken or great blue heron.

Studies involving human luteinizing granulose cells have shown that glucose transporting activity can be used as a sensitive biomarker to detect the very early response to TCDD in these steroid-producing cells and that the effect of TCDD on progesterone is mediated through cyclic adenosine 5′-monophosphate (cAMP)-dependent protein kinase.

TCDD-induced cleft palate and hydronephrosis involve mechanisms that are AhR mediated. There are data to suggest that TCDD interacts with other signaling pathways in inducing cleft palate. For example, cross-regulation of the receptors is believed to be important in the synergistic interaction between TCDD and hydrocortisone. When female mice are treated with TCDD and retinoic acid simultaneously, palatal clefts can be observed in 100 percent of offspring at dose levels far lower than those required for either agent to produce clefting if given alone. This synergy suggests that the pathways controlled by these agents converge at one or more points in cells of the developing palate.

The effects of TCDD on the estrogen-signaling system during fetal and perinatal development of peripubertal female rats has been investigated. The mechanism for the reduction in female fertility that accompanies in utero and lactational exposure to TCDD remains unknown, although it could be linked to estrogenic effects such as clefting of the phallus and hypospadias.

Several reports published during the reference period describe developmental deficits in the cardiovascular system of TCDD-treated animals. Evidence suggests that the endothelial lining of blood vessels is a primary target site of TCDD-induced cardiovascular toxicity. CYP1A1 is induced in mammalian endothelial cells in culture. The vascular endothelium in lake trout is also uniquely sensitive to induction of CYP1A1 by TCDD in developing animals. CYP1A1 induction in the endothelium may be linked to early lesions that result in TCDD-induced vascular derangements leading to the yolk sac, pericardial, and meningial edema associated with lake trout sac fry mortality. CYP1A1 induction has also been observed in adult quail aortic tissue.

The cardiotoxicity induced by TCDD was examined in chick embryo. The spatial and temporal expression of AhR and Arnt suggests that the developing myocardium and cardiac septa are potential targets of TCDD-induced teratogenicity, and such targets are also consistent with cardiac hypertrophy and septal defects observed following TCDD exposure.

DNA damage and consequent cell death in the embryonic vasculature are key physiological mediators of TCDD-induced embryotoxicity in medaka (a small Japanese freshwater fish [*Oryzias latipes*]). Treatment of TCDD-exposed medaka embryos with an antioxidant provides significant protection against TCDD-induced embryotoxicity and suggests that reactive oxygen species may participate in the teratogenic effects of TCDD.

TCDD has been shown to significantly induce CYP1A1 mRNA levels and EROD activity in several human cancer cells. Experiments involving several strains of mice provide evidence that a functional Ah receptor is required for TCDD induction of CYP1A1 and liver tumor promotion. However, the AhR does not appear to be exclusively responsible. CYP1A1 induction in various mice strains was not directly related to the degree of tumor-promoting capability, which suggests that other undefined genetic factors may play an important role.

Studies comparing liver induction in TCDD-responsive (C57BL/6J) and less responsive (DBA/2J) mice indicate that induction of CYP1B1 and CYP1A1 mRNA content is more pronounced in the former. CYP1A1 was more responsive to TCDD that CYP1B1 in both strains, suggesting that CYP1B1 mRNA expression is less inducible by TCDD but that both genes are AhR regulated. Other studies indicate that the expression of CYP1A1 and CYP1B1 is highly cell specific even though each is regulated through the AhR. However, each P450 exhibits a surprising similar pattern of hormonal regulation even though expressed in different cell types.

Studies conducted to compare AhR in cultured fetal cells and adult liver tumors from TCDD-responsive (C57BL/6J) and less responsive (DBA/2J) mice indicate that the responsiveness of fetal cells is likely mediated by the AhR and is not due to a different allelic form of AhR ligand binding subunit in fetal versus adult cells.

There is evidence that the mechanism by which TCDD induces tumor promotion may involve oxygen radicals since scavengers of hydroxyl radicals or antioxidants hinder the tumor-promoting effects of TCDD in transformed mice fibroblasts. In support of this, other studies have shown that TCDD induction of CYP1A1 in hepatoma 1c1c7 cells appears to lead to a release of oxygen radicals and subsequent oxidative DNA damage that could result in mutation and cancer. This is also evidence that tumor promotion by TCDD may be due to its ability to interfere with gap junctional intercellular communications.

In human CYP1A1 genes from MCF-7 cells, there is some evidence for cell-specific autoregulation of CYP1A1 tumor promotion. Other studies indicate that topoisomerase I activity is necessary for the primary CYP1A1 induction response and that the decreased expression of CYP1A1 in high-passage rat epidermal cells may be mediated by altered negative regulatory DNA (NeRD) binding factors present in these cells. TCDD induction of CYP1A1 in rainbow trout hepatocytes does not appear to depend on protein kinase activity.

TOXICITY PROFILE UPDATES

This section updates the toxicity profiles of the five substances discussed in *VAO* and *Update 1996*: (1) 2,4-D, (2) 2,4,5-T, (3) picloram, (4) cacodylic acid, and (5) TCDD (dioxin). The chemical nature of these substances is discussed in more detail in Chapter 6 of *VAO*.

Each profile update contains a review of experimental studies published during 1995–1997. Information in this literature update is organized under the topics (1) toxicokinetics, (2) mechanisms of toxic action, (3) disease outcomes, and if applicable, (4) estimating potential health risks and factors influencing toxicity.

Toxicity Profile Update of 2,4-D

Toxicokinetics

Kim et al. (1996) constructed a physiologically based pharmacokinetic (PBPK) model describing the kinetics of 2,4-D in developing fetal rabbit brain. Pregnant rabbits were administered 2,4-D intravenously (1, 10, or 40 mg/kg). The concentrations of 2,4-D in maternal and fetal brain, maternal and fetal plasma, and amniotic fluid were examined over time. Results indicated that the uptake of 2,4-D was membrane limited by the blood–brain barrier, with saturable clearance from the cerebrospinal fluid into the venous blood observed in both fetus and mother. In related studies, Sandberg et al. (1996) measured the relationship between 2,4-D concentration in maternal and fetal tissues following intravenous administration of radiolabeled 2,4-D (1, 10, and 40 mg/kg) to pregnant New Zealand rabbits (GD 28–30). The highest levels of 2,4-D accumulated in mater-

nal kidney and uterus and the lowest in maternal and fetal brain. At levels between 10 and 40 mg/kg, the maternal plasma binding capacity of 2,4-D became saturated and fetal levels increased more than four-fold. The organic anion transporter of the brain barrier system is functional in the late-gestational phase of the fetal rabbit. But its development is probably not complete because higher brain tissue-to-plasma ratios of 2,4-D were found in the fetus than in the dam.

At the cellular level, Bergesse and Balegro (1995) have shown that 2,4-D influx into Chinese hamster ovary cells is an energy-dependent process. These cells take up 2,4-D against a concentration gradient but do not metabolize its undissociated form. The uptake process is inhibited by sodium azide and dinitrophenol, but not ouabain, indicating that (Na^+/K^+) adenosine triphosphatase (ATPase) is not involved. Although pH less than 4.5 favors the occurrence of the undissociated form of 2,4-D (pKa 2.9), a decrease in cellular uptake is observed under these conditions. Alterations at the carrier level induced by changes in electrical charge at the cell membrane are believed to favor movement of the dissociated form of 2,4-D through the membrane.

Mechanisms of Toxic Action

Studies published since *Update 1996* continue to support the view that the mechanism of toxic action of 2,4-D involves disruption of thiol homeostasis. Palmeira et al. (1995a) showed that the viability of freshly isolated rat hepatocytes decreases significantly when incubated with 10 mM 2,4-D for 60 minutes. Basal calcium ion levels increased only slightly with concentrations of 2,4-D ranging from 1 to 10 mM, suggesting that cell death in hepatocytes was not related to early increases in calcium ion concentration. In a related study, Palmeira et al. (1995b) demonstrated that the metabolism of 2,4-D rapidly depletes glutathione (GSH) and protein thiols and induces lipid peroxidation, suggesting that 2,4-D is hepatotoxic by a mechanism related to disruption of GSH homeostasis.

Kale et al. (1995) evaluated the mutagenicity of several pesticides. 2,4-D induced significant numbers of mutations in at least one of the cell types tested. Because these results differed from earlier studies, it was hypothesized that different germ cell stages and treatment regimens might account for the observed inconsistencies. Treatment with 2,4,5-T led to similar results which are reported later in this chapter.

A recent report by Miranda et al. (1997) concluded that to a small extent, 2,4-D increases mRNA levels of mdr genes in mouse liver. The study also reported effects due to 2,4,5-T exposure, which are discussed later.

Disease Outcomes

Lethality In studies by Paulino et al. (1996), rats were exposed acutely (600 mg/kg), subchronically (200 mg/kg per day for 30 days), and chronically (200

mg/kg per day for 180 days) to 2,4-D by the oral route. Macroscopic or histopathological lesions were not observed following acute, subchronic, or chronic exposures. However, acute exposure to 2,4-D was associated with decreased locomotor activity and ataxia, sedation, muscular weakness (mainly of the hind quarters), and gasping for breath; increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), and amylase activities and elevated creatine levels; decreased total protein (TP) and glucose levels; and increased hematocrit values. Subchronic herbicide exposure increased AST activity and albumin and hematocrit values. Chronic exposure increased AST, AP, and LDH activities; decreased amylase and glucose levels; but induced no change in hematocrit values. Chromatographic analysis of the serum of chronically exposed rats suggested that 2,4-D does not accumulate in the body.

In related studies, Paulino and Palermo-Neto (1995) evaluated the acute toxicity of 2,4-D (100–600 mg/kg) in cattle by monitoring levels of serum AST, ALT, AP, *O*-glutamyl transferase (*O*-GT), and creatine kinase (CK); LDH activity and urea, creatinine, glucose, total protein, and albumin levels. The lowest dose (100 mg/kg) did not affect any of the biochemical parameters studied, whereas 300 mg/kg decreased AST, *O*-GT, and CK activities and increased urea glucose levels. Increased LDH and CK activities and protein, urea, creatine, and glucose levels were observed in animals treated with 600 mg/kg. These changes were time and dose dependent as well as reversible. Collectively, the results indicate that acute 2,4-D intoxication disrupts serum levels of several enzymes and blood components that reflect kidney and muscle damage induced by this herbicide.

Charles et al. (1996c) conducted subchronic toxicity studies in dogs comparing three forms of 2,4-D: (1) the parent form, 2,4-D acid; (2) 2,4-D dimethylamine salt; and (3) 2,4-D 2-ethylhexyl ester. Groups of four dogs of each sex received in their food, on an acid equivalent basis, either 0, 0.5 (parent form only), 1.0, 3.75, or 7.5 mg/kg daily for 13 weeks. The dogs exhibited reductions in body weight gain and food consumption, and minor increases in blood urea nitrogen, creatinine, and alanine aminotransferase as a function of chemical treatment. A no-observed-adverse-effect-level (NOAEL) of 1.0 mg/kg per day was identified for all three forms indicating the comparable and generally low toxicity of different forms of 2,4-D.

Charles et al. (1996b) reported that, in Fischer rats, 2,4-D decreases red cell mass, 3,5,3′-triiodothyronine (T3) and 3,5,3′,5′-tetraiodothyronine (thyroxine, T4) levels, and ovary and testes weights, and increases liver, kidney, and thyroid weights, and cataracts and retinal degeneration (high-dose females). These data suggest that the three forms of 2,4-D acid evaluated exert comparable low toxicities and support a subchronic no-observed-effect level (NOEL) of 15 mg/kg per day for all three forms.

Neurotoxicity Using a gas–liquid chromatographic method for determination of 2,4-D levels in serum and brain tissue of rats, Oliveira and Palermo-Neto (1995)

showed that the toxic effects of 2,4-D in rats were observed within one-half hour after its oral administration and correlated with signs and symptoms of central nervous system (CNS) depression. The data were interpreted to suggest that the toxic mechanism of 2,4-D is related to an action on the central nervous system.

According to Duffard et al. (1996), 2,4-D renders the developing rat nervous system vulnerable by hindering the process of myelination in the brain. Investigators examined rat pups following 100 mg/kg administration of 2,4-D to dams during the period of rapid demyelination (day 15 to 25 postnatal). Brains of both male and female pups showed a significant diminution of myelin markers, such as monohexosylceramide, phospholipids, and free fatty acids, and an increase of cholesteryl esters.

Immunotoxicity Blakley (1997) reported that female CD-1 mice exposed to Tordon 202C (a mixture of 2,4-D and picloram) in the drinking water for 26 days at concentrations ranging from 0 to 0.42 mg/kg significantly reduced antibody production in response to inoculation with sheep red blood cells. Although the individual component responsible for this effect was not identified, it is important to note that these levels of exposure are only marginally higher than the levels encountered following recommended application of the herbicide.

Reproductive or Developmental Toxicity In 1980, Lamb et al. conducted studies to evaluate the effects of a mixture of 2,4-D (20–40 mg/kg), 2,4,5-T (20–40 mg/kg), and the herbicide contaminant TCDD (0.16–2.4 μg/kg) on the reproduction and fertility of male C57BL/6 mice. These mixtures were intended to simulate Agent Orange. Dose-related liver and thymus toxicity and reduced weight gain were observed in treated animals. No significant effects were observed on sperm function (concentration, motility, or abnormalities) or reproductive outcomes (including litter size, number of dead fetuses, and sex ratio). Survival of offspring and neonatal development were unaffected by paternal exposure to the mixture. No increase in most congenital anomalies was found. A statistically significant excess of fused sternebra in the offspring of the two most highly exposed groups was observed, but this was attributed to an anomalously low rate of the defect in the controls.

Blakley et al. (1989) exposed male CD-1 mice to Tordon 202C (a mixture of 2,4-D and picloram) in drinking water at concentrations of 0.21–0.84 mg/kg for 60 days prior to mating with untreated females. Symptoms of paternal toxicity were observed in all exposure groups. Pregnancy failure was increased. Fetal weight and crown–rump length were reduced in offspring of the highest-dosage group, and various malformations (including ablepharon, cleft palate, unilateral agenesis of the testes, extra ribs, and incomplete ossification) were observed in the offspring of mice at all three dosage levels. However, the paternal toxicity observed at the high dosage levels used and the inconsistent dose–response pattern are of concern.

Carcinogenicity Charles et al. (1996a) evaluated the carcinogenicity of 2,4-D in a two-year rodent bioassay. Groups of 50 rats per sex per dose group received varying doses (0, 5, 75, and 150 mg/kg per day) of 2,4-D for two years. Groups of 50 mice per sex per dose group were administered 0, 5, 62.5, and 125 mg/kg of 2,4-D per day for two years. Clinical chemistry, hematology, and urinalysis were performed at six month intervals in both species. No evidence of astrocytomas was observed in rats or mice, even at concentrations equaling the maximum tolerated dose. No other oncogenic effects were reported in either test species.

Hayes et al. (1991, 1995) conducted a retrospective case-control study of malignant lymphoma in dogs. Researchers gathered data from 492 dogs diagnosed with lymphomas from three veterinary medical teaching hospitals in Minnesota, Indiana, and Colorado. There were two control groups, dogs with cancers other than lymphomas and dogs without cancer. Owners of the dogs were questioned about the frequency and duration of various pesticides used in yardwork. The authors reported a statistically significant association between the risk of canine malignant lymphoma and 2,4-D exposure. However, it must be emphasized that the dogs were concomittantly exposed to other pesticides in addition to 2,4-D.

Toxicity Profile Update of 2,4,5-T

Toxicokinetics

No toxicokinetic studies were identified for the reference period.

Mechanisms of Toxic Action

Sastry et al. (1997) examined the in vitro formation of 2,4,5-T-acetylcoenzyme A by acetylcoenzyme A synthetase and 2,4,5-trichlorophenoxyacetylcholine (2,4,5-T-Ach) by human placental choline acetyltransferase. The measured reaction rates for both endogenous and exogenous substrates were comparable. Low concentrations of synthetic 2,4,5-T-Ach decreased contraction heights of the rat phrenic nerve hemidiaphragm when the nerve or muscle was electrically stimulated. Collectively, these results suggest that 2,4,5-T can enter cellular metabolic pathways involving acetylcoenzyme A, resulting in altered metabolism and cholinergic transmission.

Kale et al. (1995) evaluated the mutagenicity of several pesticides. 2,4,5-T induced significant numbers of mutations in at least one of the cell types tested. Because these results differed from earlier studies, it was hypothesized that different germ cell stages and treatment regimens may account for the observed inconsistencies. Similar results were shown for 2,4-D treatment.

Miranda et al. (1997) concluded that 2,4,5-T increases mRNA levels of mdr

genes in mouse liver. At the protein level, P-glycoprotein traffic ATPase content increased in the canalicular domain of hepatocytes treated with 2,4,5-T. This response appeared to be mediated at the transcriptional level. Similar results were shown for 2,4-D treatment.

Voskoboinik et al. (1997) reported that the formation of DNA adducts by cytochrome P450-derived metabolites of benzo[*a*]pyrene (BP) increased in hepatocytes isolated from rats treated with 2,4,5-T for 14 days. The activity of CYP1A1 was increased, whereas the activity of glutathione *S*-transferase (GST) was decreased by the herbicide, suggesting that the increased formation of DNA adducts, following carcinogen treatment of 2,4,5-T treated rats, is due to increased metabolic activation to reactive intermediates coupled with reduced detoxification. The latter effects are particularly interesting since they are strikingly similar to those elicited by dioxin.

Disease Outcomes

Results from in vitro mechanistic studies suggest that 2,4,5-T may acutely affect neuronal and muscular function by altering cellular metabolism and cholinergic transmission. Mechanistic studies also suggest that repeated exposures to 2,4,5-T may be associated with modulation of xenobiotic metabolizing enzymes that alter the disposition of chemical carcinogens, such as BP. These alterations coupled with the ability of 2,4,5-T to induce mutations under certain conditions can influence the carcinogenic process.

Toxicity Profile Update of Cacodylic Acid

Toxicokinetics

Hughes et al. (1995) evaluated the in vitro dermal absorption of radiolabeled DMA. Discs of preclipped dorsal skin were cut from adult female B6C3F1 mice, mounted in flow-through diffusion cells, and then challenged for 24 hours with DMA at doses of 10, 100, and 500 µg using solid compound and aqueous solution (20, 100, and 250 µl) and soil (23 µg/cm^2) as vehicles. Absorption of the compound into the skin and receptor fluid ranged from < 1 to 40 percent in all exposure scenarios examined. The rank order of DMA absorption into the skin was 20 µl water > 100 µl water > solid > 250 µl water > soil. No dose or pH effects were observed. There was also no pH effect on the partitioning of DMA between 1-octanol and buffer. Short-term (1-hour) exposure of DMA in water followed by wash of the skin resulted in < 1 percent of the dose being absorbed. Although the implications of this work for human risk assessment remain to be established, the studies suggest that vehicles and duration of exposure are important factors in the in vitro dermal absorption of DMA.

Mechanisms of Toxic Action

Ochi et al. (1996) investigated the ability of certain arsenic compounds, including DMA, to induce apoptosis or programmed cell death in cultured human HL-60 cells. Of the agents examined, DMA was the most potent inducer of apoptosis. Depletion of cell glutathione following inhibition of γ-glutamylcysteine synthetase by *L*-buthionine-SR-sulfoximine enhanced the cytotoxicity of arsenite, arsenate, and methylarsonic acid, but suppressed the toxicity of DMA. The implications of these findings are not clear.

According to Rin et al. (1995), paraquat, a superoxide-generating agent, enhances DMA-induced DNA single-strand breaks in cultured alveolar cells. This enhancement occurs following washing of cells treated with DMA, suggesting that DMA-induced DNA modifications are recognized by active forms of oxygen leading to single-strand breaks. This interpretation is consistent with ultraviolet (UV) irradiation and electron spin resonance studies showing that superoxide produced by paraquat in DMA-exposed cells was more efficiently consumed than in nonexposed cells. Collectively, DMA may induce DNA modifications that sensitize it to free-radical injury.

DMA treatment (50, 100, 200, or 400 mg/kg) in the drinking water of male rats initiated by sequential treatment with nitrosamines (diethylnitrosamine [100 mg/kg, i.p., single dose]; *N*-methyl-*N*-nitrosourea [20 mg/kg, i.p., four times, on days 5, 8, 11, and 14]; 1,2-dimethylhydrazine [40 mg/kg, subcutaneously, 4 times, on days 18, 22, 26, and 30]; *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine [0.05 mg/kg in drinking water, during weeks 1 and 2]; and *N*-bis(2-hydroxypropyl)-nitrosamine [BBN; 0.1 mg/kg in drinking water, during weeks 3 and 4]) showed that DMA significantly enhanced tumor induction in the urinary bladder, kidney, liver, and thyroid gland (Yamamoto et al., 1997). Induction of preneoplastic lesions as reflected by GST-positive foci in the liver and atypical tubules in the kidney, respectively, was also significantly increased in animals treated with 100 or 400 parts per million (ppm) of DMA alone. Ornithine decarboxylase activity in the kidneys of rats treated with 100 ppm DMA was significantly increased compared to control values ($p < .001$). From these studies it was concluded that DMA is a promoter of urinary bladder, kidney, liver, and thyroid gland carcinogenesis in rats.

In related studies, Wanibuchi et al. (1996) examined the promotion potential of DMA for rat urinary bladder carcinogenesis. Six-week-old male F344 rats were treated with 0.05 mg/kg BBN for four weeks and then given DMA in their drinking water (0, 2, 10, 25, 50, and 100 mg/kg) for 32 weeks. The development of preneoplastic lesions and tumors (papillomas and carcinomas) in the urinary bladder was enhanced by treatment with DMA in a dose-dependent manner. A significant increase in the multiplicity of tumors (papillomas and carcinomas) was observed even at a low concentration of DMA (10 mg/kg). On the other hand, no preneoplastic lesions and tumors were observed in rats treated with

DMA alone. Administration of DMA alone (0, 10, 25, and 100 ppm) in drinking water for eight weeks without prior initiation was associated with a significant increase in the 5-bromo-2′-deoxyuridine labeling index and alteration of the surfaces of urinary bladder epithelial cells. These results suggest that the potential of DMA to promote rat urinary bladder carcinogenesis may be related to its ability to stimulate cell proliferation in the urinary bladder epithelium.

Disease Outcomes

The limited evidence presented during the past two years suggests that DMA acts as a multiorgan promoter of carcinogenesis.

Toxicity Profile Update of Picloram

No studies published since *Update 1996* have reported on the toxicity of picloram alone. As described earlier, Blakley (1997) reported that female CD-1 mice exposed to Tordon 202C (a mixture of 2,4-D and picloram) in the drinking water for 26 days at concentrations ranging from 0 to 0.42 mg/kg significantly reduced antibody production in response to sheep red blood cell inoculation. Although the individual component responsible for this effect was not identified, it is important to note that these levels of exposure are only marginally above the levels encountered following recommended application of the herbicide.

Toxicity Profile Update of TCDD

Toxicokinetics

Michalek and coworkers (1996a, 1997) examined TCDD disposition in veterans of Operation Ranch Hand, the Air Force unit responsible for aerial spraying of Agent Orange in Vietnam. The half-life of TCDD in 213 veterans was estimated based on multiple serum measurements collected over a 10-year period. Researchers took into account the potential influence of age, percentage of body fat, and changes in the percentage of body fat. In agreement with previous estimates, the mean decay rate of TCDD for these veterans is 0.0812 per year; with a corresponding half-life estimate of 8.532 years. Half-life increased significantly with increasing body fat, but not with age or relative changes in percentage of body fat. Investigators warned that serum dioxin measurements should not be used when the first measurement exceeds 50 mg/kg due to low reliability (Michalek et al., 1996b).

Ayotte et al. (1996) estimated the impact of breastfeeding on the body burden of dioxin-like chemicals in Arctic Inuit people from birth to age 75. Toxicokinetic modeling in the Inuit revealed that breastfeeding strongly influences body burden during childhood but not after age 20. Liver and adipose tissue

concentrations appeared to be lower than those associated with cancer and adverse reproductive effects in laboratory rats. On the other hand, a substantial proportion of Inuit women may have adipose tissue concentrations close to or higher than those associated with increased incidence of endometriosis in rhesus monkey.

Recent studies have also addressed the toxicokinetics of TCDD in experimental animals. For example, Diliberto et al. (1996) compared the absorption, tissue distribution, and elimination of TCDD in laboratory rats. Researchers exposed male rats to radiolabeled-TCDD (0.32 mg/kg) through inhalation or oral treatment. They also injected rats directly with TCDD because this exposure route serves as a standard for evaluating the others. Injection eliminates problems with absorption. Three days after exposure, fecal excretion accounted for 22, 26, and 32 percent of the injected, inhaled, and orally administered doses, respectively. Urinary excretion accounted for only 2.2, 1.3, and 1.4 percent, respectively. Thus, 95 percent of the administered dose was absorbed by inhalation, whereas 88 percent was absorbed after oral administration. After absorption, the liver and fat serve as major TCDD depots. Following injection, 37 percent and 21 percent of the absorbed dose were distributed to the liver and fat, respectively. After inhalation, the numbers were 35 percent and 16 percent. After oral treatment, 28–30 percent of the absorbed dose was distributed to both liver and fat. These results indicate that both the oral and the inhalation routes are important for the systemic absorption of dioxins. Carrier et al. (1995 a,b) proposed a physiologically based model that describes the distribution kinetics of polychlorinated dibenzodioxins or polychlorinated dibenzofurans (PCDD/Fs) in various mammalian species. Simulations were in agreement with published data on the distribution kinetics of PCDD/Fs in rodents, monkeys, and humans. The model takes into account intercellular diffusion, PCDD/F–receptor and PCDD/F–protein binding, and PCDD/F-dependent enzyme induction in the liver. Investigators formulated nonlinear differential equations, with anatomically and biochemically relevant parameters to predict functional dependencies between the fraction of total PCDD/F body burden contained in liver and adipose tissues and the overall body concentration at any time. The liver fraction of the total body burden decreases as a function of the overall body concentration. Since elimination of these chemicals occurs principally via the liver, this results in slower elimination rates and longer half-lives. The kinetic profiles were also found to be similar for rats and humans. However, the half-lives differed considerably, with rats calculated in weeks but humans in years. For a given body burden, adipose tissue concentrations vary in inverse proportion to the mass of adipose tissues, an observation that is relevant to humans.

Aozasa et al. (1995) evaluated the EROD activity and liver accumulation of dioxin-like chemicals (i.e., 2,3,7,8-chlorine substituted PCDD/F) in two species of mice (C57BL/6 and DBA/2) administered these chemicals orally for 28 days. The C57BL/6 mice with high EROD induction or DBA/2 mice with low EROD

induction did not accumulate high levels of the chemicals. However, C57BL/6 mice accumulated larger amounts in their liver than DBA/2 mice. A remarkable difference in congener levels was observed when comparing the ratios in human tissue and in food. The tissue–food ratio of octachlorodibenzo-*p*-dioxin (OCDD) level was 20 times higher than that of octachlorodibenzofuran (OCDF), despite the same number of chlorine substituents in each compound. Taken collectively, the finding that in humans, OCDD accumulates more readily than OCDF is potentially significant because it suggests the possibility that the high bioaccumulation of OCDD in humans may be caused by metabolic pathways different from those in mice.

During the reference period, Kohn et al. (1996) extended a model of the physiological disposition of TCDD in the rat (Kohn et al., 1993) to include the distribution of blood among major vessels and tissue capillary beds; resorption of TCDD released from the liver into the gut lumen as a consequence of cell lysis; and compartments for thyroid and thyroxine-sensitive tissues (e.g., pituitary, kidney, brown fat) that take into consideration secretion and tissue uptake of thyroid hormones; the binding of T3 and T4 to proteins in blood and tissues; the deiodination of iodothyronines; and TCDD induction of T4 glucuronidation by hepatic UGT. The extended model fit the observed dose–response of P450 isozymes and Ah and estrogen receptors reported in the previous model after repeated oral doses. The extended model provided a better fit with respect to liver and fat TCDD levels after single and repeated oral and subcutaneous doses. Predicted liver TCDD concentrations at very low doses were verified experimentally. In addition, the model reproduced the responses observed for blood T3, T4, and TSH after 31 weeks of biweekly oral dosing of rats with TCDD. The model also predicted the responses of UGT mRNA and UGT enzymatic activity observed in experiments with TCDD-treated rats. Based on the model, TCDD increases UGT enzymatic activity and blood TSH levels. Calculated increases in blood TSH levels are consistent with prolonged stimulation of the thyroid and may represent an early stage in the induction of thyroid tumors identified in previous two-year bioassays. This suggests that increases in UGT activity may be a useful biomarker of tumorigenic changes in hormone levels following TCDD exposure.

Analysis of linear, sigmoid-E_{max}, and power law functions by McGrath et al. (1995) suggests that the use of a wide dose range may bias the interpretation of low-dose phenomena. This interpretation was based on the change in slope observed from low- to high-dose subsets for thymic atrophy, immune suppression, BP hydroxylase activity, and EROD activity. These investigators suggest that the power law function can provide a more accurate and biologically relevant assessment of risk and that noncancer end points may be more significant than cancer end points in assessing human health risks from TCDD. For example, immune suppression and enzyme induction occur at lower doses and under conditions more relevant to general population exposure conditions.

DeVito and Birbaum (1995) studied the pharmacokinetic factors that can

influence the relative potency of TCDD and tetrachorinated dibenzofuran (TCDF), in vivo. TCDD and TCDF were administered to female B6C3F1 mice for 4 and 13 weeks and EROD activity, a useful marker of CYP1A1 gene expression, was measured in liver and skin. Mice received either 150 ng TCDD/kg or 1,500 ng TCDF/kg daily, 5 days per week, for either 4 or 13 weeks, a regimen designed to provide equipotent doses. At four weeks, hepatic EROD was induced eleven- and seven-fold by TCDD and TCDF, respectively, suggesting that published TEFs accurately estimate the relative potency of TCDD and TCDF after four weeks of treatment. In marked contrast, the induction of hepatic EROD after 13 weeks was elevated 41- and 5-fold for TCDD and TCDF, respectively. Investigators suggested that the inability of TEFs to predict the relative potency of these compounds after 13 weeks of treatment may be due in part to differences in the pharmacokinetic properties of each congener since the half-lives of TCDF and TCDD are approximately 2 and 15 days, respectively. As such, steady-state levels of TCDD were not attained by 4 weeks, which is reflected in the increase in hepatic EROD between 4 and 13 weeks, whereas steady-state levels of TCDF were reached within 4 weeks. Similar results were observed for skin EROD activity. These findings are potentially significant since they suggest that TEFs derived from short-term assays may not adequately predict the relative potencies of this class of compounds following chronic exposure.

Mechanisms of Toxic Action

The biochemical and toxic effects of TCDD are in large measure mediated by the AhR, a cytoplasmic protein that binds several classes of xenobiotics including polycyclic aromatic hydrocarbons (PAHs), benzoflavones, heterocyclic amines, and halogenated aromatic hydrocarbons. The AhR is ubiquitously expressed in almost every organ and cell in the body. The receptor is normally found in association with two heat shock proteins (hsp90), which are thought to aid in stabilization of the receptor protein. Ligand binding (e.g., with TCDD) transforms the receptor to a form that exhibits increased DNA binding affinity leading to translocation to the nucleus where it dimerizes with a partner protein, Arnt. The AhR–Arnt complex binds to specific sequences in the regulatory region of target genes called aryl hydrocarbon-responsive elements (AhREs) to influence patterns of gene expression. Based on extensive studies of the CYP1A1 gene, binding of the AhR to the promoter region alters chromatin structure, leading to increases in the rate of gene transcription. Although several other genes are known to be influenced via a comparable mechanism, the net influence of AhR binding to DNA is dictated by the nature of the molecular interactions governing the regulation of the particular gene. Scientific reports published during the past two years have identified several genes that are involved in the regulation of development, growth, and differentiation of mammalian tissues as targets of TCDD action. As such, the ability of TCDD to influence the expression

of these "target genes" may mediate at least in part the health outcomes associated with herbicide exposures. Other recent studies summarized below discuss the structural and functional aspects of the AhR and Arnt, DNA binding and transcriptional events, biological consequences associated with activation, significant and insignificant interactions and interspecies, and interindividual differences in sensitivity. The section ends with a discussion of methods used to estimate the potential health risk associated with exposure to TCDD and related compounds.

Abbott et al. (1996) examined the disposition of TCDD in pregnant C57BL/6N mice 24 hours after oral administration on GD 12. TCDD was detected in maternal blood, liver, and fat and in the placenta, embryonic liver, and palate within 30 minutes of dosing on GD 12. The levels peaked in blood and placenta at 3 hours and in other tissues at 8 hours. The early peak level found in maternal blood is in agreement with findings for distribution of this class of compounds in adult rats and mice. In general, dioxins and furans are rapidly absorbed from the gastrointestinal tract, and tissue distribution in the first hour parallels blood levels and reflects blood flow and tissue size. During this period the embryo grows rapidly (e.g., liver weight almost doubles from GD 12 to 13). This suggests that although the embryo continues to accumulate TCDD, increasing mass results in a relatively constant tissue concentration.

Structural and Functional Aspects of AhR and Arnt Both the AhR and Arnt are members of the basic helix–loop–helix (bHLH) superfamily of gene regulatory proteins. A conserved domain of 200–350 amino acids, designated PAS (Per, Arnt, AhR, Sim), defines this superfamily. The AhR is a highly conserved protein found in diverse vertebrate groups. For example, in the PAS domain, the N-terminus of human AhR shows 87 percent and 86 percent amino acid identity with mouse and rat AhR, respectively. Recently, Hahn and Karchner (1995) have shown that the amino acid sequence of the PAS domain of a teleost AhR is 62–64 percent identical to the PAS domains of mammalian AhR, and Brown et al. (1995) detected the presence of two proteins (28 and 39 kDa) in the cytosols of the hard-shell clam species, *Mercenaria Mercenaria*, that bind a chemical analogue of TCDD. The latter studies reported that these proteins are homologous to the AhR.

Arnt, like the AhR, also shows a high degree of conservation between vertebrate groups. Two cDNAs encoding bHLH–PAS proteins with similarity to Arnt protein have been isolated from RTG-2 rainbow trout gonad cells (Pollenz et al., 1996). The deduced proteins, termed rtARNTa and rtARNTb, are identical over the first 533 amino acids and contain a bHLH domain that is 100 percent identical to human Arnt.

Both the AhR and Arnt have been shown to interact with a large number of proteins to influence receptor function. Of particular significance from a biochemical and toxicological perspective has been the recent demonstration that

Arnt interacts in vivo with HIF-1α to form the active HIF-1 transcription factor complex. This complex appears responsible for mediating the cellular response to hypoxia by transcriptional regulation of genes such as erythropoietin and other mediators of cellular oxygenation. Five new members of the PAS superfamily that interact with the AhR or Arnt were identified by Hogenesch et al. (1997). Two of these proteins (MOP1 and MOP2) dimerize with Arnt and form complexes that are transcriptionally active, an interaction suggesting that cellular pathways mediated by MOP1 and MOP2 may influence or respond to the dioxin signaling pathway.

In general, the AhR and Arnt proteins appear to be coexpressed; however, pronounced differences in relative expression levels exist between the two dimeric partners in some tissues, suggesting that additional dimerization partners and signaling pathways may be present. Arnt is able to homodimerize, as well as heterodimerize, with Sim and Per. Identification of a human Sim homologue that may play a causal role in Down's syndrome (trisomy 21) has recently been demonstrated. This human Sim protein is found in embryonic CNS and facial tissues, where it may interact with Arnt or an Arnt-like protein to regulate important developmental processes.

Abbott and Probst (1995) characterized the expression of Arnt in mouse embryos during GDs 10–16. On GD 10–11, embryos showed the highest levels of Arnt in neuroepithelial cells of the neural tube, visceral arches, otic and optic placodes, and preganglionic complexes. The heart also had significant expression of Arnt with strong nuclear localization. After GD 11, expression in heart and brain declined. On GD 12–13, embryonic expression was highest in the liver, where expression increased from GD 12 to 16. On GD 15–16, the highest levels of Arnt occurred in adrenal gland and liver, although it was also detected in submandibular gland, ectoderm, tongue, bone, and muscle. In all of these tissues, Arnt was cytoplasmic as well as nuclear, except in some of the cortical adrenal cells in which it was strongly cytoplasmic with little or no nuclear localization. These specific patterns of Arnt expression differ in certain tissues from those of the AhR, suggesting that Arnt may have roles in normal embryonic development that are independent of the AhR. In Arnt-deficient cells, the AhR can still translocate to the nucleus in vivo, a process therefore independent of Arnt.

The PAS domain of the AhR also harbors the contact region for association with hsp90. Interestingly, this region colocalizes with a domain previously identified as a repression domain on AhR signaling (Dolwick et al., 1993). It has been shown that in a purified system the AhR–hsp90 complex is not dissociated by the addition of ligand (McGuire et al., 1994) and that addition of a cellular fraction from Hepa cells, but not Arnt-deficient Hepa mutants, can promote hsp90 dissociation. These results suggest that the Arnt protein plays an active role in this process.

Studies by Coumailleau et al. (1995) defined a minimal ligand binding domain of the AhR within the central PAS region that interacts with hsp90 in vitro. The minimal ligand binding domain maintains the quantitative and qualitative

aspects of ligand binding exhibited by the full-length receptor. These studies are consistent with in vitro findings by Whitelaw et al. (1995).

In contrast to the results of McGuire et al. (1994), Fukunaga et al. (1995) found that ligand binding was localized to a region encompassing the PAS B repeat. One hsp90 molecule appears to bind within the PAS region, whereas the other appears to require interaction over the bHLH region. In addition, ligand-mediated dissociation of AhR from hsp90 did not require Arnt. These results also suggested that equivalent regions of the AhR and Arnt associate with each other since both the first and the second helices of the bHLH motif and the PAS region are required for dimerization. Finally, Fukunaga et al. (1995) suggested that the carboxyl-terminal half of the AhR plays a more prominent role in transcriptional activation of the CYP1A1 gene than the corresponding region of Arnt. Deletion of the carboxyl-terminal half of AhR did not affect dimerization or AhRE binding, but did eliminate biological activity as assessed by an in vivo transcriptional activation assay. Deletion of the carboxyl-terminal half of Arnt did not affect biological activity in the same assay system.

In contrast to the findings of Fukunaga et al. (1995), Li et al. (1994) have reported that the activation of an AhRE-driven reporter plasmid by AhR–Arnt is dependent on the transactivation domain (TAD) of Arnt. In vivo, the TADs of the AhR and Arnt may synergize, because removal of the Q-rich region of Arnt did not impair AhR–Arnt dimerization but diminished transactivation of a AhRE-driven CAT reporter gene in Arnt-defective Hepa cells.

The C-terminal 34 amino acids of Arnt harbor a TAD that functions independently of other sequences in the AhR complex (Corton et al., 1996). The strength of the Arnt TAD is cell-type specific since Arnt and herpes simplex virus VP16 TAD were equally strong in COS-1 cells, but the Arnt TAD had weak activity in an Arnt-deficient mouse hepatoma cell line and was not needed for restoration of CYP1A1 activation. These results imply that for CYP1A1 activation the AhR provides the dominant activation function for the heterodimer in hepatoma cells. This may not be the case for other genes or for different transcription factor complexes.

Analyses of AhR cDNA deletion mutants from C57BL/6 mouse liver indicate that the carboxyl half of AhR contains several TADs that function independently of the domains that mediate TCDD recognition, DNA binding, and heterodimerization with Arnt (Ma and Whitlock, 1996). The transactivation domains function independently of each other, display different levels of activity, and act synergistically when linked.

In addition to dimerizing with bHLH–PAS proteins, the AhR interacts with other cellular proteins involved in the regulation of rabbit liver cell function. Dunn et al. (1996) identified two active protein fractions in ranging in molecular weight from 12 to 14 kDa that bind to the AhR. Of particular significance was the identification of the protein histone H4, which is known to interact with transcription factors in a variety of systems.

In other studies, a yeast two-hybrid system was used to identify proteins that interact with the AhR (Li and Dougherty, 1997). These investigators cloned a mouse cDNA that encodes a novel 37 kDa protein that binds to the AhR. The amino acid sequence of this protein exhibits homology with proteins required for cell cycle control and RNA synthesis and is related to steroid receptor binding immunophilins. The 37 kDa protein is thought to be cytoplasmic and to associate with unliganded AhR and with hsp90. TCDD treatment disrupts the protein–protein interaction, whereas overexpression of the AhR interacting protein augments the response of the CYP1A1 gene to TCDD.

The regulation of AhR gene expression has not been studied in as much detail as other aspects of receptor function. To study tissue-specific regulation of the mouse AhR gene, Fitzgerald et al. (1996) transfected chimeric deletion constructs containing the AhR 5′-flanking region and the firefly luciferase reporter gene into five established mouse cell lines: Hepa 1c1c7 (derived from hepatoma), JB6-C1 41-5a (epidermis), MLE-12 (lung epithelium), F9 (embryonal carcinoma), and NIH/3T3 (fibroblasts). In all cell lines except F9 cells, maximal constitutive expression occurred with constructs containing 78 base pairs of AhR promoter sequences. This region includes several putative binding sites for the transcription factor Sp1. It is interesting that in F9 cells, other transcription factors appear to be important in AhR gene expression since up to 174 promoter sequences were required for induction. Results suggests that regulation of the AhR gene occurs in a tissue- and cell-type specific manner.

Although the ability of TCDD to modulate gene expression often involves transcriptional mechanisms, posttranscriptional events may also be important in the regulation of gene expression (Gaido and Maness, 1995). Treatment of SCC-12F cells with 10 nM TCDD increases urokinase-plasminogen activator (u-PA) mRNA. Transcription of u-PA was not altered by TCDD; instead, induction of u-PA occurred as a result of a stabilization of the u-PA mRNA. Tissue-plasminogen activator and plasminogen activator inhibition (PAI-1) expression were not altered by TCDD. Thus, TCDD acts through different mechanisms in SCC-12F cells to induce both a plasminogen activator and a specific inhibitor of plasminogen activation.

Of perhaps greatest significance during the past two years has been the development of AhR-deficient mice ($AhR^{-/-}$). Using gene targeting, Gonzalez and coworkers (1995) developed AhR-deficient mice by inactivation of the first exon of the AhR. In a separate study, Schmidt et al. (1996) used gene targeting to delete exon 2, which encodes the bHLH DNA binding and dimerization domain. These knockout mice do not express receptor protein, and as expected, transcriptional activation of AhR target genes by TCDD is abolished. The results from knockout experiments present compelling evidence that the AhR plays a fundamental role in cell and organ physiology and homeostasis. If ligand activation is indeed required for receptor function, an endogenous ligand must be present in most cells. From a toxicologic perspective, AhR knockout mice have been impor-

tant since they helped establish a definitive association of the AhR with TCDD-mediated toxicity.

Peters and Wiley (1995b) have shown that culturing embryos in medium with an AhR antisense oligodeoxynucleotide reduces the incidence of blastocyst formation as well as mean embryo cell number, suggesting that AhR may function in embryonic cell differentiation and proliferation independent of its known function in mediating TCDD toxicity.

$AhR^{-/-}$ mice are relatively unaffected by doses of TCDD (2,000 mg/kg) tenfold higher than those found to induce severe toxic and pathologic effects in littermates expressing a functional AhR. The resistance of $AhR^{-/-}$ mice to TCDD-induced thymic atrophy and cortical lymphocyte depletion appears restricted to processes involving AhR since the corticosteroid dexamethasone, which induces thymic atrophy by a pathway unrelated to the AhR, rapidly and efficiently induces cortical depletion in both $AhR^{-/-}$ and normal littermate control mice. It is important to note, however, that at high doses of TCDD, $AhR^{-/-}$ mice display limited vasculitis and scattered single-cell necrosis in lung and liver, respectively. The mechanism(s) responsible for these apparently receptor-independent processes remain unclear but may involve novel, alternative pathways for TCDD-induced toxicity.

Evidence also continues to accumulate that ligand-independent events can mediate activation of the receptor. Benzimidazole derivatives are potent CYP1A1 inducers in rabbit and human liver cells, but do not bind the AhR, suggesting that ligand-independent mechanisms may activate the AhR. Lesca et al. (1995) showed that benzimidazoles bind early and transiently to an unknown protein in rabbit liver cells, causing a depletion of AhR in a time- and dose-dependent manner. In contrast, benzimidazoles are unable to induce CYP1A1 mRNA in specific mouse liver cells and to deplete the high-affinity AhR form from these cells. A signal transduction pathway similar to that involved in the ligand-independent activation of steroid receptors may activate the low-affinity forms of AhR present in rabbit and human cells, but not in mouse cells.

DNA Binding and Transcriptional Interference As described previously, modulation of gene expression by TCDD is dependent on its ability to bind DNA to influence transcriptional events. Recent studies have focused on characterizing the nature of TCDD-induced ligand binding (e.g., transactivation domains) and transcriptional events (e.g., conformational characteristics and protein–DNA complexes).

Weiss et al. (1996) evaluated transient and stable AhR expression in AhR-deficient clones. The AhR that was transiently expressed into receptor-deficient variants exhibited high basal transactivation activity on promoters containing AhR binding sites compared to wild-type cells. Hybrid receptors also showed high basal activity in the absence of exogenous TCDD in AhR-deficient variant cells, indicating that endogenous AhR activating signal acts directly on the recep-

tor. Stable expression of AhR in variant cell clones fully reconstituted TCDD responsiveness, including target gene induction and delay of cell cycle progression. These AhR-reconstituted cells, like wild-type cells that contain AhR, showed low basal activity of the transiently expressed AhR hybrid. The increased basal activity in AhR-deficient cells suggests a negative feedback control of AhR activity compatible with the existence of an endogenous AhR ligand.

Bacsi et al. (1995) completed studies to evaluate TCDD-induced binding of AhR–Arnt to the asymmetric AhRE in nuclear extracts of Hepa 1c1c7 cells. Covalent cross-linking analysis and immunoprecipitation with antibodies specific for AhR or Arnt demonstrated that Arnt directly contacts the 3′-most thymine position, the AhR directly contacts the second thymine position, and neither protein contacts the 5′-most thymine position. The thymine position contacted by Arnt lies within a three-nucleotide sequence (5′-GTG-3′) identical to a half-site of an E-box element (5′-CACGTG-3′) recognized by other bHLH transcription factors, whereas AhR binds to a portion of the AhRE that does not resemble an E-box. In this study, evidence was also presented that neither protein loops over to contact residues located beyond the other's binding site.

In studies to characterize the binding of transformed guinea pig hepatic AhR to DNA, Bank et al. (1995) identified two distinct TCDD-inducible protein–AhRE complexes, but only a single high-affinity binding site. The formation of both DNA–binding complexes exhibited nucleotide specificity for the AhR complex.

Santostefano and Safe (1996) investigated ligand-dependent differences in molecular properties of the transformed cytosolic and nuclear AhR. For several different AhR ligands including TCDD, TCDF, 1,2,7,8-TCDF, and α-naphthoflavone, the pattern of proteolytic protein–DNA products using transformed cytosolic or nuclear AhR complexes was comparable. In contrast, significant differences were observed in the pattern of degraded protein–DNA products using nuclear AhR complexes derived from mouse Hepa 1c1c7 cells treated with TCDD or 6-methyl-1,3,8-trichlorodibenzofuran (MCDF). Such differences may be related to conformational characteristics induced by TCDD, a potent AhR agonist, relative to MCDF, a partial AhR agonist and antagonist.

Yamaguchi and Kuo (1995) have demonstrated that ligand-free AhR has no transactivating properties in yeast. However, the C-terminal portion (amino acid residues 580–797) of the AhR, including the Q-rich domain, confers transactivation activity in the same system, suggesting that the N-terminal portion of the AhR contains transcription repression properties. In contrast, the 75 C-terminal amino acids of Arnt, including the Q-rich domain, exhibited full transactivation function in yeast and mammalian cells, suggesting that structural organizations of the transactivation properties differ between AhR and Arnt, although both contain transactivation domains at the C-termini.

Lindebro et al. (1995) have shown that Arnt interacts with the AhR via the PAS domain. The PAS domain of Per could dimerize with both AhR and Arnt in

vitro and disrupt the ability of these subunits to form a DNA binding heterodimer. Ectopic expression of Per blocked dioxin signaling in mammalian cells. Thus, the PAS domains of the dioxin receptor and Arnt are novel dimerizing regions critical in the formation of a functional AhR–Arnt complex, whereas the PAS domain in Per is a potential negative regulator of bHLH–PAS function. Evidence was also presented that hsp90 may modulate dioxin receptor function by directing correct folding of the ligand binding domain, interference with Arnt heterodimerization, and folding of a DNA binding conformation of the bHLH domain.

Swanson et al. (1995) examined the DNA recognition and pairing of several bHLH–PAS families of proteins. The AhR–Arnt complex exhibits a preference for the sequence commonly found in dioxin-responsive enhancers in vivo (GCGTG). As discussed previously, the Arnt protein is capable of forming a homodimer with a binding preference for the palindromic E-box sequence (CACGTG). These studies revealed that Arnt has a broader range of interactions than other members of the bHLH–PAS proteins.

Dioxin-induced binding of the AhR–Arnt heterodimer to enhancer chromatin is associated with a localized alteration in chromatin structure is manifest by increased accessibility of the DNA (Okino and Whitlock, 1995). These changes likely reflect disruption of a nucleosome by AhR–Arnt. However, in the CYP1A1 promoter, such changes must occur by a different, more indirect mechanism because they are induced from a distance and do not reflect a local effect of AhR–Arnt binding. Dose–response experiments indicate that changes in chromatin structure at the enhancer and promoter are graded and correlated with the graded induction of CYP1A1 transcription by dioxin. Thus, TCDD induces a shift in equilibrium between nucleosomal and nonnucleosomal chromatin configurations.

Certain ellipticine derivatives have been reported to bind the AhR and inhibit the ability of TCDD to transform it to a form that recognizes an AhRE upstream of the CYP1A1 gene. Gasiewicz et al. (1996) examined more than 30 ellipticine derivatives and structurally related compounds for their ability to bind the AhR, activate it to a dioxin response element (DRE) binding form, induce the luciferase gene under the control of a DRE containing enhancer, and block activation of the AhR by TCDD. The ability of ellipticine derivatives to inhibit TCDD-elicited DRE binding and TCDD-induced luciferase activity was inversely related to their ability to stimulate these responses. Their antagonistic activity was related to their binding affinity for the AhR as predicted by their van der Waals dimensions and the presence of an electron-rich ring nitrogen at or near a relatively unsubstituted *x*-axis terminal position.

The dioxin-inducible transcriptional control mechanism for the mouse CYP1A1 gene in its native chromosomal context was recently evaluated by Ko et al. (1996). The C-terminal segment of the AhR was shown to contain latent transactivation capability and to communicate the induction signal from enhancer to promoter. Heterodimerization activates the latent transactivation function of the AhR and silences that of Arnt since removal of Arnt's transactivation domain

does not affect dioxin-induced CYP1A1 transcription in vivo. Dioxin-induced changes in chromatin structure at the CYP1A1 enhancer and promoter may occur by unique mechanisms.

Rowlands et al. (1996) used a series of fusion proteins with a heterologous DNA binding domain to study the trans activating function of the human AhR and Arnt proteins in yeast. The results of these studies confirmed that human AhR and Arnt both contain carboxyl-terminal TADs. The AhR has a complex TAD composed of multiple segments that function independently, exhibit varying levels of activation, and cooperate to induce synergistic activation of transcription. In the absence of a DNA binding domain, the AhR and Arnt TAD probably inhibit activated and basal transcription because of selective binding of basal transcription factors, the TATA-binding protein and TFIIF. Thus, activation of target gene expression by AhR–Arnt may involve direct interactions with basal transcription factors. However, Henry et al. (1997a) published data suggesting that a purified TCDD–AhR complex retains both specific DNA binding and transcriptional activities in the absence of other factors. Purified and partially purified receptors gave a similar footprint of interaction with G-residues within the AhRE consensus sequence and were able to stimulate transcription from a AhRE-containing template in a cell-free system in the presence of HeLa cell nuclear extract.

Biological Consequences of Activation The ability of TCDD to influence patterns of gene expression involves modulation of transcriptional and posttranscriptional events. Such responses are often mediated by the AhR and thus exhibit considerable tissue and cell specificity. However, evidence continues to accumulate to suggest that some actions of TCDD may be independent of the AhR. Hoffer et al. (1996) examined mouse hepatoma Hepa-1 cells to analyze the mechanism of *fos*/*jun* activation by TCDD. Their results suggested that TCDD induces expression of the immediate early-response genes *fos* and *jun* by activation of three separate signal transduction pathways, at least one of which does not require a functional AhR complex. The serum response elements (SRE) mediate the response of c-*fos* to TCDD in serum in a dose-dependent manner independent of the AhR. The SRE also mediates c-*fos* induction by growth factors, cytokines, UV irradiation, oxidants, and other stimuli that activate mitogen-activated protein kinases (MAPKs). It was proposed that activation of the SRE ternary complex by TCDD may be initiated by a signal triggered at the cell surface that precedes binding of TCDD to the cytosolic AhR.

Selmin et al. (1996) used the differential display technique to identify genes regulated by TCDD. Sequencing of one of the differentially expressed clones showed 71 percent homology with the transmembrane domain of the precursor for the interleukin-6 receptor and a conserved consensus sequence found in the cytokine growth factor–prolactin receptor superfamily, respectively. TCDD appears to modulate cytokine expression and function in multiple systems and with

some degree of selectivity. The effect of TCDD on a series of TGF-β_2 (transforming growth factor β_2) gene promoter deletions ranging from 1,391 to 64 base pairs upstream of the transcription start site was investigated by Lee et al. (1996) to identify the region necessary for down-regulation by TCDD. The effect appears to be localized to the TATA box and is dose dependent, with saturation kinetics maximal by 10 nM and complete by 24 hours. TCDD can modulate gene transcription by acting on the transcription initiation complex via a tyrosine kinase-dependent pathway. These data support an alternative mechanism by which TCDD can alter gene expression, an indirect route mediated by the AhR complex through a tyrosine kinase-dependent pathway affecting the transcription initiation complex. Signaling interactions between AhR and other growth pathways are described below.

Ma and Whitlock (1996) reported that AhR-deficient Hepa 1c1c7 cells exhibited a different morphology, decreased albumin synthesis, and a prolonged doubling time relative to wild-type counterparts. Introduction of AhR cDNA into deficient cells by stable transfection induced acquisition of the wild-type phenotype. Conversely, introduction of antisense AhR cDNA into wild-type cells induced the AhR-deficient phenotype. Flow cytometric and biochemical analyses suggest that the slow growth rate of AhR-deficient cells reflects prolongation of G_1, suggesting a link between AhR and the G_1 phase of the Hepa 1c1c7 cell cycle. Also of significance was the fact that the effects of AhR occurred in the absence of TCDD and thus may represent responses to an endogenous AhR ligand.

AhR Signaling Interactions Evidence has continued to accumulate during the past two years that AhR interactions with other signal transduction pathways are complex and often redundant in nature. The complexity of AhR signaling interactions in evident from the large number of proteins that interact with the AhR to influence growth and differentiation, redox signaling, modulation of kinase activities, and estrogen receptor signaling. New data with respect to these areas are discussed below.

Growth and Differentiation Signaling During the reference period, evidence continued to lend support to the fact that the AhR signal transduction pathway is involved in the regulation of development and growth. Enan and Matsamura (1996) described the ability of TCDD to increase protein-tyrosine kinase activity in the cytosol of male guinea pig adipose tissue, an effect believed to be AhR dependent. The protooncogene c-Src, involved in growth control, was reported to be associated with the AhR–protein complex in cytosol from adipose tissue as well as in liver of guinea pig and C57BL/6J mice and NIH 3T3 mouse fibroblasts. The c-Src protein is functionally attached to the AhR and specifically activated on ligand binding.

Wanner et al. (1996) have shown that the transcript levels of the AhR and Arnt increase as a function of differentiation in a human keratinocyte cell line. In

situ hybridization studies established that in normal human skin, AhR expression is absent in proliferating basal cells and increases in the upper cell layers as differentiation progresses. In agreement with these correlations, AhR expression in differentiation-deficient hyperproliferative psoriatic skin is markedly decreased. When keratinocytes were continuously treated with retinoic acid (RA), the upregulation of AhR and Arnt mRNA levels was inhibited as was keratin 4 expression, a marker of keratinocyte differentiation. In contrast, treatment of already differentiated cells with RA did not down-regulate transcript levels. The mRNA levels of retinoic acid receptors in keratinocytes, RAR gamma and RXR α, were not influenced by the process of differentiation or by the addition of RA. These data suggest that the regulation of AhR, Arnt, and keratin 4 expression by RA is mediated via an indirect mechanism. To examine interactions between retinoid and AhR signaling, Fiorella et al. (1995) evaluated RA metabolism in microsomes from four retinoid-responsive tissues in male Sprague-Dawley rats three days after a single exposure to TCDD (80 μg/kg, i.p.). Microsomes from all four tissues catalyzed increased rates of RA metabolism, with a rank order of induction of liver > lung = kidney = testis. These data were interpreted to suggest that one aspect of TCDD toxicity involves alterations in the metabolism of RA.

Significant interactions between TCDD and TGF-β_1 have been described by several investigators. Dohr et al. (1997) reported that basal mRNA expression of CYP1A1, CYP1B1, and AhR, as well as inducible CYP1A1 expression, is down-regulated by TGF-β_1 in cells treated with TCDD. In contrast, mRNA expression of the AhR partner protein Arnt was not influenced. Treatment of cells with cycloheximide led to superinduction of TCDD-induced CYP1A1 and CYP1B1 mRNA expression and abolished the inhibitory effect of TGF-β_1 on basal as well as TCDD-induced CYP1 and AhR mRNA expression. These results suggest that TGF-β_1 induces rapid transcription and translation of an as-yet-unknown negative regulatory factor or factors that may directly regulate expression of the AhR and genes of the Ah gene battery at the transcriptional level.

TCDD and related chemicals also interact with insulin, a hormone involved in the growth regulation of several cells. Lu et al. (1996b) reported that TCDD and TCDF inhibit insulin-induced cell proliferation and DNA synthesis in MCF-7 cells, a response blocked by α-naphthoflavone, a partial AhR antagonist. TCDD alone did not affect K_d and B_{max} values for binding of insulin to the receptor. However, the insulin-induced K_d value for insulin receptor ligand binding was decreased and the B_{max} value was increased by TCDD cotreatment. TCDD elevated insulin receptor mRNA levels and inhibited several other insulin-induced responses including c-*fos* protooncogene expression, phosphorylation of the insulin receptor, and a 185 kDa protein in MCF-7 cells.

Redox Signaling The discovery that the oxygen-regulated transcription factor HIF-1α and the AhR share a common heterodimerization partner Arnt (HIF-1β) has fueled intensive investigation into the possible cross-talk between

oxygen and dioxin signal transduction pathways. HIF-1α is activated following changes in O_2 concentration and translocates to the nucleus to form a heterodimer with Arnt. This complex activates gene expression by binding to a hypoxia-responsive element. All of these transcription factors (HIF-1α AhR, Arnt–HIF-1β) share an N-terminal bHLH region followed by a PAS domain, as described previously for the AhR and Arnt. Gradin et al. (1996) demonstrated that HIF-1α required Arnt for DNA binding in vitro and functional activity in vivo. Both the bHLH and the PAS motifs of Arnt were critical for dimerization with HIF-1α. Strikingly, HIF-1α exhibited very high affinity for Arnt in coimmunoprecipitation assays in vitro, resulting in competition with the ligand-activated AhR for recruitment of Arnt. These findings have implications for risk assessment in that TCDD itself may induce alterations in the redox status of cells.

Consistent with these observations, activation of HIF-1α function in vivo or overexpression of HIF-1α inhibited ligand-dependent induction of DNA binding activity and AhR function on minimal reporter gene constructs. However, HIF-1α- and AhR-mediated signaling pathways were not mutually exclusive since activation of AhR did not impair HIF-1α-dependent induction of target gene expression. Both HIF-1α and Arnt mRNAs are expressed constitutively in a large number of human tissues and cell lines, and steady-state expression levels is not affected by exposure to hypoxia. HIF-1α is associated with hsp90. Given the critical role of hsp90 for ligand binding activity and activation of the AhR, it is therefore possible that HIF-1α is regulated by a similar mechanism, possibly by binding an as-yet-unknown class of ligands.

Gassmann et al. (1997) showed that Arnt is indispensable for hypoxia-inducible HIF-1 DNA binding as well as for oxygen-regulated reporter gene activity medicated by the hypoxia response element present in the 3′ enhancer of the erythropoietin gene (EPO 3′). Hypoxic induction of the vascular endothelial growth factor (VEGF) gene, however, was only partially abrogated in Hepa 1C4 cells, suggesting that an HIF-1-independent oxygen signaling pathway is present. De novo translation, phosphorylation, and redox processes seem to be involved in hypoxic HIF-1α and probably also Arnt activation.

Yao et al. (1995) studied the effect of TCDD and BP on the transient expression of a chloramphenicol acetyltransferase (CAT) reporter gene linked to the promoter sequences in the long terminal repeat (LTR) of human immunodeficiency virus type 1 (HIV-1). Induction of a functional CYP1A1 monooxygenase by TCDD stimulates a pathway that generates thiol-sensitive reactive oxygen intermediates, which in turn are responsible for the TCDD-dependent activation of genes linked to the LTR. NFκB and an adjacent AhRE are required for TCDD-dependent CAT expression. In addition, mutation of the NFAT/AP-1 binding sites in the negative regulatory region of the promoter increases the magnitude of the TCDD effect. In related studies, the inducibility of three phase II genes in the mouse dioxin-inducible Ah battery: Nmo1 [encoding reduced nicotinamide–adenine dinucleotide [NAD(P)H]: menadione oxidoreductase], Ahd4 (encoding

the cytosolic aldehyde dehydrogenase ALDH3c), and Ugt1*06 (encoding UGT) were evaluated by Vasiliou et al. (1996). Increases in Ah phase II gene expression in the 14CoS/14CoS mouse correlated with the electrophile response element (EpRE) found in the 5′-flanking regulatory regions of these genes. Gel mobility shift assays with a synthetic oligonucleotide probe corresponding to the EpRE showed that EpRE binding proteins are more than twice as abundant in 14CoS/14CoS than in the wild-type ch/ch nuclear extracts. Competition studies of EpRE-specific binding with an excess of EpRE, mutated EpRE, AP-1, AhRE3, mutated AhRE3, and C/EBP α-oligonucleotides suggest that several common transcriptional factors bind to the EpRE and AhRE3 motifs. Two monospecific antibodies to the AhR protein block formation of an EpRE-specific complex on gel mobility electrophoresis, suggesting that AhR or an AhR-related protein might be an integral part of the EpRE binding transcriptional complex associated with the oxidative stress response.

Protein Kinases Reports linking TCDD to the modulation of kinase activities continued to appear during the reference period. These interactions are reciprocal since evidence was also published that various kinases directly modulate AhR function. Enan and Matsamura (1995b) reported that TCDD stimulates nuclear protein phosphorylation in explant tissue cultures within 10 minutes, a response that is followed by a substantial decrease in the level of total protein phosphorylation activity. Manganese-stimulated protein kinase was found to be the predominant type of nuclear protein phosphorylating activity affected by TCDD, with 60 percent of the total activity due to heparin-sensitive casein kinase II (CK II). TCDD was also found to increase protein-kinase C and microtubule-associated protein 2 kinase activities as early as 15 minutes after treatment in isolated adipose tissue in culture. Changes in kinase activities are of biological significance since DNA binding activity of the transcriptional factor AP-1 increases, while c-Myc DNA binding activity decreases. Genistein, a specific protein-tyrosine kinase inhibitor, abolished the stimulatory effect of TCDD on the AP-1 binding activity, but not the DNA binding activity of c-Myc. Although TPA (12-O-tetradecanoylphorbol 13-acetate), a phorbol ester, increased the binding activity of AP-1 and c-Myc, TCDD in combination with TPA caused a slight reduction in the binding activity of both transcriptional factors. In the presence of forskolin, the stimulatory effect of TCDD on AP-1 binding and the inhibitory effect on c-Myc were still apparent. Okadaic acid almost abolished the binding activity of c-Myc, whereas in combination with TCDD a stimulatory effect was found. Thus, TCDD regulates the DNA binding activity of AP-1 and c-Myc by modulating their phosphorylation status through alterations in protein kinase and phosphatase activities. Incubation of a nuclear-free subcellular homogenate of guinea pig adipose tissue with TCDD results in a significant elevation of protein kinase activity within 1–10 minutes (Enan and Matsamura, 1995b). The kinetics of this response is not consistent with the classic transcriptional mechanism of

action for TCDD, but interestingly, the actions of TCDD were blocked by AhR blockers. TCDD-induced increases in protein phosphorylation occurred mainly in cytosolic preparations devoid of nucleus, microsomes, and plasma membranes and were still observed in the presence of inhibitors of protein phosphatases. Furthermore, TCDD caused a rise in protein-tyrosine kinase activity in a purified AhR preparation, as well as in an isolated hsp complex preparation containing the AhR. This activation is unrelated to de novo protein synthesis. Evidence was also presented that this action of TCDD triggers the protein kinase-mediated growth factor signal transduction pathway (e.g., stimulation of mitogen-activated protein kinase 2 and tyrosine kinase activity). These results support the view that the TCDD-induced activation of protein kinases operates via a mechanism different from its conventional pathway involving changes in gene transcription in the nucleus. Finally, the association of c-Src protein kinase with AhR was described earlier (Enan and Matsamura, 1996).

Several earlier studies suggested that AhR phosphorylation may be critical in activation of the AhR to a DNA binding state. To further evaluate the functional role of AhR phosphorylation, Mahon and Gasiewicz (1995) investigated whether TCDD binding altered total AhR phosphorylation and identified phosphorylated regions in the receptor based on chemical cleavage patterns. The total level of AhR phosphorylation was not affected by ligand binding. The shortest regions of overlap determined by the chemical cleavage patterns localized phosphorylation sites to two regions in the C-terminal half of the AhR. One region was centrally located between amino acids 368 and 605 and within or adjacent to a DNA binding repressor domain. The other region was located at the glutamine-rich carboxyl terminus between amino acids 636 and 759. These data suggest that total AhR phosphorylation is not altered by ligand-induced transformation of the receptor, but that phosphorylation nevertheless plays an important role in the ability of an active AhR–Arnt complex to associate with cis-acting regulatory elements. This interpretation is consistent with studies showing that the activity of the AhR–Arnt dimer can be decreased by treatments that cause the down-regulation of protein kinase C and decrease nuclear accumulation of the receptor.

A reporter plasmid containing two xenobiotic responsive elements (XREs) was used to investigate the effects of phosphatase inhibitors on TCDD-dependent transcription by the Hepa-1 mouse liver cell line (Li and Dougherty, 1997). The inhibitors calyculin A and okadaic acid caused two- to threefold increases in TCDD-dependent transcription at concentrations capable of selectively inhibiting protein phosphatase 1 and protein phosphatase 2A. The inhibitor cyclosporin A doubled TCDD-dependent transcription at a concentration capable of selectively inhibiting protein phosphatase 2B. All three of the phosphatase inhibitors increased TCDD-dependent transcription without affecting transcription in the absence of TCDD. Nuclear extracts were prepared from cells treated with concentrations of okadaic acid or cyclosporin A that substantially stimulated TCDD-dependent transcription. Neither of the inhibitors significantly increased the level

of TCDD-dependent XRE binding in extracts. GAL4–Arnt fusion proteins were used to further investigate whether the phosphatase inhibitors affected a step other than DNA binding. Okadaic acid treatment specifically increased the ability of a GAL4 fusion protein containing the Arnt–PAS and transactivation domains to stimulate transcription. These results suggest that serine- or threonine-specific protein phosphatases can act at a level subsequent to XRE binding to inhibit the ability of the AhR–Arnt dimer to stimulate transcription.

Chen and Tukey (1996) examined the effects of phorbol 12-myristate 13-acetate (PMA), a potent activator of protein kinase C, on the ligand-induced transcriptional activation of the CYP1A1 gene and cellular function of the AhR in human HepG2 101L cells. Pretreatment of cells with PMA enhanced ligand-induced CYP1A1 gene expression two- to threefold. Inhibition of PKC activity blocked the transcriptional activation and transactivation of the CYP1A1 gene, indicating a role for PKC in the AhR-mediated transcriptional activation process. However, DNA binding activities of the in vitro activated and the induced nuclear AhR were not affected when CYP1A1 transcription was inhibited, indicating that the action of PKC is a nuclear event that works in concert with or precedes AhR binding to the gene. The effects of TCDD on growth factor-coupled activation of nuclear protein kinase C (nPKC) and on the subcellular distribution of PKC activity in rat splenocytes were investigated by Zorn et al. (1995). Seven days after a single injection of TCDD (50 fg/kg body weight), cytosolic and particulate PKC activities were elevated in splenocytes from TCDD-treated rats or pair-fed control rats compared to ad libitum-fed animals. Growth factor-stimulated nPKC activation was attenuated in splenic nuclei from TCDD-treated rats compared to vehicle-treated controls. Thus, TCDD may uncouple growth factor receptors linked to PKC activation at the level of the nucleus.

Estrogen Receptor Signaling Considerable research activity during the past two years has focused on reciprocal interactions between estrogen receptor signal transduction pathways and the AhR. These studies were fueled in part by the early observation that TCDD is a more potent hepatocarcinogen in female than in male or ovariectomized rats. Measurement of 8-oxodeoxyguanosine (8-oxo-dG), a marker for oxidative DNA damage, in livers of intact and ovariectomized Sprague-Dawley rats chronically treated with TCDD (125 ng/kg per day) with or without diethylnitrosamine as an initiator showed elevated levels of 8-oxo-dG in intact compared to ovariectomized TCDD-treated rats (Tritscher et al., 1996). Expression of CYP1B1 mRNA, a newly identified cytochrome P450 with estrogen hydroxylase activity, was highly induced in these animals by TCDD, suggesting that the increased metabolism of endogenous estrogens to catechols caused by TCDD-induced enzymes may lead to increased oxidative DNA damage and hepatocarcinogenicity in female rats.

TCDD and related hydrocarbons have also been identified as potent anti-estrogens, and this effect involves reciprocal interactions between the estrogen

and the AhR signal transduction pathway. For instance, Kharat and Saatcioglu (1996) have shown that the TCDD-mediated decrease in estradiol-inducible gene products, such as the cathepsin D gene (cat D), is due to a decline in mRNA accumulation despite changes in estrogen receptor (ER) mRNA levels. The decline in cat D mRNA levels likely involves decreased transcription rates since TCDD blocks the ability of ER to bind DNA and transactivate from an estrogen response element. Interestingly, TCDD does not function as an antiestrogen in mutant cells lacking a functional AhR. Likewise, estradiol treatment blocked TCDD-induced accumulation of CYP1A1 mRNA and AhR-mediated activation of the CYP1A1 promoter, due to the ability of liganded ER to interfere with the binding of AhR to the xenobiotic response element. Liu and Safe (1996) and Lu et al. (1996b) have shown that treatment of MCF-7 human breast cancer cells with TCDD decreases prolactin receptor (PRLR) mRNA levels within 12 hours after treatment, and for up to 48 hours, while PRLR binding is not affected. The effects of TCDD on PRLR mRNA levels were inhibited by the AhR antagonist α-naphthoflavone and were not observed in Ah-nonresponsive MCF-7 cells. TCDD antagonizes 17β-estradiol-induced increases in PRLR mRNA levels. Using MCF-7 or mouse Hepa 1c1c7 cells transiently transfected with E2-responsive Vit A2 gene 5′-promoter constructs, Nodland et al. (1997) showed that there was a correlation between the antiestrogenic activity of AhR ligands in MCF-7 cells and their rank order binding affinity for the AhR. A role for the AhR is suggested by the finding that α-naphthoflavone inhibited the antiestrogenic activity of TCDD in MCF-7 cells and TCDD inhibited E2-induced CAT activity in Ah-responsive wild-type, but not in Ah-nonresponsive, class 2 mutant Hepa 1c1c7 cells. The antiestrogenic activity of TCDD was also observed in cells that transiently overexpressed human ER, suggesting that the mechanism does not involve down-regulation of the ER by TCDD. In other studies, Krishnan et al. (1995) presented compelling evidence that AhR-mediated inhibition of estrogen-induced cat D gene expression is affected by disruption of the ER–Sp1 complex by targeted interaction with an overlapping XRE.

The interaction between TCDD and estrogen was evaluated by White et al. (1995) in weanling females Sprague-Dawley rats. Estrogen (10 μg/kg per day at days 21 and 22) increased relative uterine weight and induced keratinization of the vaginal epithelium. Estrogen reduced uterine ER protein levels and serum FSH levels. None of these parameters were affected by pretreatment with 20, 40, or 80 μg/kg TCDD on day 19. Given that other signs of TCDD toxicity were reproducibly observed in these rats, it was concluded that weanling female Sprague-Dawley rats are not sensitive to the antiestrogenic effects of TCDD at doses that cause overt toxicity. Collectively, these data suggest that the antiestrogenic effects of TCDD are species, strain, and age dependent.

Dohr et al. (1995) reported that TCDD inhibits cell growth and induces CYP1A1-associated EROD activity in MCF-7 cells that express the ER, but not in ER-negative MDA-MB 231 cells. Transcripts of CYP1B1 were detected in

both cell lines, and mRNA content was enhanced eight- and thirtyfold in MCF-7 and MDA-MB 231 cells treated with TCDD, respectively. In the gel mobility shift assay, a stronger signal of DNA binding AhR was observed in MDA-MB 231 than in MCF-7 cells treated with TCDD. A fortyfold higher AhR mRNA content was observed in untreated MDA-MB 231 than in MCF-7 cells, while the mRNA of the AhR nuclear translocator was expressed in a similar range of magnitude. Treatment of cells with TCDD did not change mRNA expression of either gene. Analysis of NADPH:quinone oxidoreductase (NMO-1) and PAI-2 mRNA expression revealed a dose-dependent induction of both genes in MDA-MB 231 cells after TCDD treatment. These studies strengthened the view that AhR-mediated transactivation is not impaired in ER-negative cells, but expression of ER is important for regulation of CYP1A1 induction after TCDD treatment in these human breast cancer cell lines. It was interesting to note, however, that ER does not appear to have a function in TCDD-induced mRNA expression of CYP1B1, NMO-1, and PAI-2 in MDA-MB 231 cells.

In summary, evidence published during the past two years indicates that the mechanisms of interaction between the TCDD- and E2-induced signaling pathways are highly complex. Some of the inhibitory effects of TCDD may involve 5′-flanking inhibitory AhREs present in target genes.

Significant Interactions Recent data suggest that TCDD and related hydrocarbons are not the only ubiquitous AhR agonists encountered by human populations. Several AhR agonists of dietary origin have been identified. For example, Kleman and Gustafsson (1996) described the formation of procarcinogenic heterocyclic aromatic amines, following cooking of protein-rich foods, capable of activating the AhR to a form that interacts with AhRE in vitro. Another group of putative dioxin receptor ligands of dietary origin involves the indolocarbazoles, produced in vivo from precursor molecules in cruciferous plants. Indolocarbazoles are potent regulators of the expression of a reporter gene driven by a minimal AhRE in both mouse and human hepatoma cells. The indolocarbazole-induced human receptor appeared to form more stable complexes with AhRE in vitro relative to those generated by the dioxin-activated receptor. Indolo-3-carbinol (I3C), a major component of *Brassica* vegetables, and its metabolite diindolylmethane (DIM) have been identified as partial AhR antagonists (Chen et al., 1996). Both compounds competitively bind to the AhR with low affinity. In Ah-responsive T47D human breast cancer cells, I3C and DIM do not induce EROD activity or CYP1A1 mRNA levels. However, cotreatment with TCDD plus different concentrations of I3C or DIM reduced the TCDD-induced response at high concentrations. In T47D cells cotreated with TCDD alone or in combination with I3C or DIM, there was a marked reduction in the formation of the nuclear AhR.

The induction kinetics and CYP1A1 mRNA half-life by the diet-derived indole derivative, indolo[3,2-*b*]carbazole (ICZ) are concentration dependent and transient due to rapid clearance of ICZ (Chen et al., 1995). TCDD and ICZ

displayed equal efficacies in the activation of a TCDD-responsive CAT (chloramphenicol acetyltransferase) reporter construct in Hepa 1 cells. ICZ is also a potent and selective noncompetitive inhibitor of EROD activity.

Significant interactions between TCDD-related AhR signaling and other environmental chemicals have long been described. In a recent study, the mechanism by which an ambient level of aged and diluted sidestream cigarette smoke (ADSS) induces CYP1A1 was investigated in C57BL/6N and DBA/2N mice, strains that exhibit high- and low-affinity forms of the AhR, respectively (Gebremichael et al., 1996). Induction of CYP1A1-associated EROD activity was observed in the lungs of C57BL/6N mice, whereas no induction occurred in DBA/2N mice. ADSS also induced EROD in wild-type mouse hepatoma (Hepa 1c1c7) cells (Hepa 1), but not in variant Hepa 1 cells defective in the Arnt protein. ADSS exposure of recombinant Hepa 1 cells stably transfected with a reporter plasmid containing the luciferase gene under the control of several dioxin-responsive enhancers resulted in a time- and exposure-dependent induction of luciferase activity. ADSS-mediated induction of luciferase activity was inhibited by α-naphthoflavone, an AhR antagonist. Exposure to ADSS induced transformation and DNA binding of the AhR complex. Collectively, these results indicate a role for the AhR in mediating the induction of CYP1A1 by ADSS and suggest that environmentally relevant levels of ADSS contain AhR ligands at sufficient concentrations to activate gene expression in an AhR-dependent manner.

Using 1- and 2-aminonaphthalene as model substrates, the influence of a second amino group on mutagenicity, binding to the cytosolic AhR, and CYP1A inducibility relative to the effects of 3,3′-diaminobenzidine and 1-naphthylethylenediamine were examined by Cheung et al. (1997). 1,5- and 1,8-Diaminonaphthalene were effective inducers of CYP1A activity and more potent than 1-aminonaphthalene. 2,3-Diaminonaphthalene was also an inducer of CYP1A, but the effect was similar to that elicited by 2-aminonaphthalene. In contrast, 3,3′-diaminobenzidine and 1-naphthylethylenediamine did not induce CYP1A activity. All aminonaphthalenes displaced radiolabeled-TCDD from the AhR, whereas 3,3′-diaminobenzidine and 1-naphthylethylenediamine failed to do so. The latter two compounds did not elicit a mutagenic response in the Ames test. Introduction of a second amino group at the 3-position of 2-aminonaphthalene did not modulate its mutagenicity. In the case of the nonmutagenic 1-aminonaphthalene, introduction of a second amino group at position 5 had no effect, but when it was incorporated at position 8, mutagenic potential was conferred on the molecule. The ability of substituted flavones to modulate AhR signal transduction in MCF-7 human breast cancer cells was evaluated by Lu et al. (1996b). The 4′-methoxy-3′-nitro- and 3′-amino-4′-methoxyflavones were characterized as AhR agonists and inducers of CYP1A1 gene expression, whereas the 3-methoxy-substituted flavones (3′-methoxy-4′-nitro- and 4′-amino-3′-methoxy-) were inactive. All four compounds inhibited induction of EROD activity by TCDD. These results were interpreted to suggest that two forms of the nuclear AhR complex exist in MCF-

7 cells and that 3-methoxy-substituted flavones inhibit nuclear uptake of the transcriptionally active form.

Disease Outcomes

Lethality TCDD lethality has been associated with changes in brain serotonin (5-HT) metabolism (Unkila et al., 1995b). This response was recently examined in the most TCDD-susceptible and TCDD-resistant species, guinea pigs and hamsters, respectively. Body weight gain of guinea pigs exposed to TCDD (0.2–2.7 μg/kg) diminished dose dependently, while the effect was marginal in hamsters (900–4,600 μg/kg). 5-Hydroxyindoleacetic acid (the primary metabolite of 5-HT), brain tryptophan (the precursor amino acid of 5-HT), and plasma-free and total tryptophan were not affected at any dose in guinea pigs. In contrast, four days after exposure, the levels of plasma-free and total tryptophan were increased in hamsters and, along with brain tryptophan, remained elevated ten days after exposure. TCDD did not affect plasma glucose level in either species. Liver glycogen was decreased in a dose-dependent manner in TCDD-treated guinea pigs, as well as in their pair-fed controls, on day 10. There was no change in liver glycogen in hamsters. The activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase was depressed only in hamsters by all doses of TCDD. Changes in tryptophan metabolism or in carbohydrate homeostasis cannot explain the wide interspecies differences in susceptibility to the acute lethality of TCDD, although they may correlate with some aspects of its toxicity in certain species.

Cells harvested six to eight days after TCDD intubation from pair-fed rats contained significantly more fat and higher levels of glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity. The mRNA for lipoprotein lipase (LPL) and GPDH genes was also higher for cells from pair-fed rats, suggesting that TCDD inhibits the differentiation of fat cells (Brodie et al., 1996). TCDD treatment in vivo inhibits the increase of mRNA for the PPAR (2, aP2 and C/EBP) during differentiation of isolated preadipocytes. C/EBPβ and CHOP mRNAs were unaffected. 3T3-L1 cells appear to provide a good model to study adipogenesis and the inhibition of this process by TCDD (Brodie et al., 1997).

Fan and Rozman (1995) completed studies to examine short- and long-term effects of TCDD in female LE rats. Female rats were dosed orally with either 5.3, 12, 18, and 60 μg TCDD/kg and sacrificed four days after dosing or 27, 40, and 60 μg TCDD/kg and sacrificed 90 days after dosing. Four days after dosing, EROD activity was fully induced at all doses studied, hepatic PEPCK and γ-glutamyl transpeptidase activities were reduced dose dependently, and hepatic tryptophan 2,3-dioxygenase (TdO) activity was stimulated at low doses but decreased at high doses. Serum total T4 (TT4) levels were dose dependently decreased, whereas serum total T3 (TT3) and tryptophan levels were unaffected. The short-term effects of TCDD examined indicate only small differences in the

response to TCDD of female LE rats compared to males. Ninety days after dosing, liver EROD activity revealed considerable reversibility although it was still elevated compared to controls. Hepatic PEPCK activity at this time was no different from controls. In contrast to four days after dosing, serum TT3, TT4, and hepatic glutamyl transpeptidase activity were elevated dose dependently at 90 days.

Male Sprague-Dawley rats were administered orally a total dose of 0, 0.2, 2.3, 11.5, 35, 70, or 115 μg/kg of TCDD over 10 weeks at 4 ml/kg of vehicle. Results show that dose–responses for the induction of EROD activity and the reduction of serum TT4 occurred at much lower doses than those for decreased TdO and PEPCK activities or elevated tryptophan levels and mortality. After a six-week recovery period, PEPCK and TdO activities in liver, as well as tryptophan in serum, returned to near-control values, whereas EROD activity and serum TT4 still displayed a dose-dependent induction and reduction, respectively, albeit both shifted to the right in accordance with toxicokinetics. These data support the interpretation that subchronic dose–responses of TCDD are similar to acute dose–responses when corrected for toxicokinetics and that at least some TCDD-induced effects are reversible (Li and Rozman, 1995).

In male mice treated with TCDD, body weights and feed intake were not much affected until day 8 after exposure (Weber et al., 1995). Hepatomegaly developed at doses greater than 3 and 97.5 μg/kg in C57 and DBA mice, respectively. EROD activity was induced in liver with a median effective dose (ED_{50}) of 1.1 and 16 μg/kg, and in kidney with an ED_{50} of 65 and 380 μg/kg in C57 and DBA mice, respectively. The PEPCK in livers of both mouse strains was reduced over the entire dose range, displaying a plateau in the dose response at the onset of acute toxicity of TCDD. This enzyme activity was decreased by as much as 80 percent at the respective lethal doses. PEPCK activity in kidney was not affected. Glucose-6-phosphatase activity (G-6-Pase) in liver was altered only in the lethal dose range, with a maximum reduction of about 50 percent. Serum glucose concentration was reduced over the entire dose range, but the reduction was significant only at doses in which G-6-Pase activity was affected, reaching levels as low as 3 mmol per liter in DBA mice. Tryptophan-2,3,-dioxygenase activity was not lowered at any dose of TCDD in either mouse strain, and no increase in serum tryptophan levels was observed. Serum levels of T4 and T3 were decreased dose dependently over most of the dose range administered, with T3 levels exactly paralleling T4 levels in both mouse strains. These data were interpreted to suggest that TCDD causes acute toxicity in male C57 and DBA mice by a severe reduction of gluconeogenesis but, in contrast to rats, does not affect tryptophan homeostasis. After administration of TCDD, serum T3 levels in the mouse appear to correlate with T4 levels, whereas in the rat they are independent of each other.

Li et al. (1997) examined the effect of single doses (0.03–30 μg/kg) of TCDD administered orally by gastric intubation on serum hormone levels in female rats (22 days old). Two distinct peaks for LH and FSH were detected, the

first of which was seen at 1 hour and appeared to be a nonspecific response to the vehicle, and a second at 24 hours that appeared to be induced by TCDD. Gonadotropin levels in these animals were dose dependently elevated. In cultured pituitary halves and primary pituitary cell cultures exposed to gonadotropin-releasing hormone (GnRH) and/or TCDD, TCDD caused a dose-dependent release of LH from pituitary halves with an ED_{50} of about 0.1 nM. This effect was abolished in calcium-free medium but was not attenuated by a GnRH antagonist. In primary pituitary cell cultures, although the cells responded to GnRH, no effect of up to 100 nM TCDD on the release of gonadotropins was detected. These results suggest that TCDD dose dependently induces a brief release of gonadotropins in immature female rats. This effect is at least partially due to an effect of TCDD on the pituitary. Increased release of gonadotropins as a result of TCDD treatment depends on the action of calcium but does not occur via activation of GnRH receptors. However, cells in a primary pituitary culture do not respond to TCDD with increased release of gonadotropins, suggesting that the effect of TCDD in the pituitary is mediated by a factor present in pituitary halves but not in primary cell culture.

Dermal Toxicity Wanner et al. (1995) reported on the relative levels of AhR mRNA and TCDD-induced CYP1A1 mRNA and their modulation by retinoic acid in the human keratinocyte cell line HaCaT. AhR mRNA was present already in proliferating keratinocytes and increased eightfold in the course of differentiation. Addition of 10 nM TCDD did not alter the level of AhR transcripts. In contrast, addition of 1 μM retinoic acid (RA) maintained the amount of AhR mRNA at the basal level only in proliferating keratinocytes. The transcription of CYP1A1 was dependent on TCDD-treatment and increased fivefold in more differentiated cells compared to proliferating cells. Simultaneous addition of RA revealed only a twofold increase. These results indicate that expression of the AhR depends on the state of differentiation of keratinocytes and seems to be affected by retinoic acid.

As discussed previously, TCDD strongly induces a switch from proliferation to terminal differentiation in keratinocytes, which can be antagonized effectively by retinoic acid and retinol. As parameters for differentiation, the [^{35}S]methionine incorporation into cross-linked envelopes (revealing the total CLE) was quantified by. TCDD is a potent inducer of both CLE biomass and number with an EC_{50} of 1.4 nM. Both effects were dependent on Ca^{2+} and increased with elevated cell density, being optimal in postconfluent cultures. Retinoic acid dose dependently decreased the effect of 10^{-8} M TCDD, 10^{-6}M having a nearly complete antagonistic action. Retinyl palmitate and etretinate were not effective as TCDD antagonists. Supplementation of hydrocortisone suppressed TCDD-induced keratinocyte differentiation (REF). Reduced increase in cell number and diminished cell biomass are the result of early withdrawal of proliferating cells from the cell cycle due to premature and accelerated induction of differentiation. TCDD-induced

differentiation in human keratinocytes may be initiated by TCDD binding to the AhR. The exact nature of the interaction between retinoids and TCDD is not clear, but may involve interactions in the regulation of epithelial differentiation.

Cardiovascular Toxicity Several reports appeared during the reference period describing developmental deficits in the cardiovascular system of TCDD-treated animals. Endothelium is a single-cell layer lining that could be a primary site of chemical effects in the cardiovasculature and systemically.

CYP1A expression and activity in cultured porcine aortic endothelial cells (PAEC) exposed to the AhR agonists TCDD, 3,3′,4,4′-tetrachlorobiphenyl (TCB), BP, or β-naphthoflavone (BNF) were evaluated by Stegeman et al. (1995). CYP1A1 was induced in cultures exposed to TCDD, TCB, BP, or BNF. Gene induction was observed at intermediate concentrations (0.1 or 1.0 μM) of TCB, BP, or BNF but inhibited at higher concentrations. The suppression response was not due to generalized cytotoxicity. Immunohistochemical analysis showed that CYP1A1 induction in PAEC was not present in all cells. PAEC exhibited a typical complement of microsomal electron transport components. NADPH–cytochrome P450 reductase showed comparable rates in induced and control cultures, and the addition of purified rat reductase to PAEC microsomes increased EROD rates threefold. EROD rates in intact cells maximally induced by BP, TCB, or TCDD ranged from 15 to 30 pmol/mg per minute of whole-cell protein. Methoxyresorufin *O*-demethylase activity induced by TCDD was 2 pmol/mg per minute (i.e., <10 percent of EROD activity). In cultures in which CYP1A1 was strongly induced, CYP1A2 was not detectably expressed. The CYP1A2 inducer acenaphthylene did not induce EROD or methoxyresorufin *O*-demethylase in intact cells. Results show that CYP1A1 but not CYP1A2 is strongly induced in mammalian endothelial cells in culture and that CYP1A1 is active in intact cells, although the catalytic rates are low.

A recent study by Guiney et al. (1997) examined CYP1A induction in endothelium and its possible association with mortality due to the edema and vascular effects of TCDD in lake trout early life stages. Lake trout eggs were injected at 24–50 hours postfertilization with 0.2 μl of 50 mM phosphatidylcholine liposomes or liposomes containing TCDD to give seven doses ranging from 11 to 176 pg TCDD/g egg. Doses of TCDD greater than 44 pg/g egg elicited hemorrhages; yolk sac, pericardial, and meningial edema; craniofacial malformations; regional ischemia; growth retardation; and mortality at the sac fry stage of development. Expression of CYP1A was assessed at four developmental stages, by immunohistochemical analysis of serial sections of individual fish with monoclonal antibody 1-12-3 to teleost CYP1A. CYP1A staining occurred in endothelial cells of many organs of TCDD-exposed but not vehicle-exposed embryos at one week prehatch and sac fry at two weeks posthatch. Earlier developmental stages examined were negative for CYP1A expression at any dose of TCDD. The strongest response occurred in sac fry at TCDD doses greater than 88 pg TCDD/g egg but

was detected at doses as low as 22 pg TCDD/g egg. CYP1A staining in endothelium appeared at lower doses and was stronger than in other cell types in both prehatch embryos and posthatch sac fry. Thus, the vascular system is a major initial site affected by TCDD in lake trout early life stages, and the vascular endothelium is a cell type uniquely sensitive to induction of CYP1A in these developing animals. Based on an index of immunohistochemical staining of CYP1A, endothelial CYP1A induction in sac fry by TCDD occurred with an ED_{50} of 64–69 pg TCDD/g egg, similar to the dose–response for mortality occurring during the sac fry stage of development (LD_{50} [median lethal dose] = 47 pg TCDD/g egg). These correlations suggest that CYP1A or AhR in the endothelium may be linked to early lesions that result in TCDD-induced vascular derangements leading to yolk sac, pericardial, and meningial edema associated with lake trout sac fry mortality, but the precise mechanism remains to be determined.

Cantrell et al. (1996) characterized embryotoxicity in medaka (*Oryzias latipes*) by TCDD. DNA degradation in cells of the embryonic vasculature and loss of functional integrity of the medial yolk vein were demonstrated in TCDD-exposed embryos. Piperonyl butoxide inhibited TCDD-induced DNA degradation, restored the functional integrity of the medial yolk vein, and protected against the embryotoxicity of TCDD. Treatment of TCDD-exposed embryos with the antioxidant *N*-acetylcysteine also provided significant protection against the embryotoxicity of TCDD. These results demonstrate that DNA damage and consequent cell death in the embryonic vasculature are key physiological mediators of TCDD-induced embryotoxicity and implicate oxidative mechanisms in this response.

Celander and colleagues (1997) examined CYP1A1 induction in cultures of porcine aortic endothelial cells and human aortic endothelial cells exposed to TCDD with or without the glucocorticoid receptor (GR) agonist cortisol or dexamethasone. In porcine cells exposed to 0.1 nM TCDD and 10 μM cortisol, the level of CYP1A1 protein and the degree of EROD activity induction were two- to threefold higher than with 0.1 nM TCDD alone. A similar enhancement of EROD induction was obtained when 0.1 or 1 nM TCDD was added together with 0.1, 1, or 10 M dexamethasone in the media. Human cell counterparts also showed potentiated EROD induction when 1 nM TCDD was coadministered with 10 μM dexamethasone. This potentiation caused by dexamethasone was abolished by the addition of 10 μM of the GR antagonist RU-38486, suggesting that potentiation of CYP1A1 induction in endothelial cells proceeds by a GR-dependent mechanism. The implications of these data have not been fully defined but demonstrate that vascular cells are responsive to TCDD.

Renal Toxicity Kraemer and colleagues (1996) examined the molecular mechanisms for TCDD-stimulated prostaglandin synthesis in Mardin Darvey canine kidney (MDCK) cells. TCDD stimulated prostaglandin synthesis in these cells, at least in part by elevation of prostaglandin endoperoxide H_2 synthase-2 (PGHS-2)

levels. TCDD-stimulated transcription of the PGHS-2 gene was maximal (six-fold) within two hours and resulted in a hundredfold increase in PGHS-2 mRNA and a twenty-five-fold increase in PGHS-2 protein levels by four hours. Transient transfection experiments using luciferase-reporter plasmids demonstrated that the control element(s) responsible for TCDD activation of the murine PGHS-2 promoter in MDCK cells are located in the first 965 nucleotides upstream from the PGHS-2 transcriptional initiation site. A canonical xenobiotic response element, similar to those that control transcription of other well-known TCDD-sensitive genes, is present at position −157, but does not appear to be sufficient for halogenated aromatic hydrocarbon activation of the PGHS-2 promoter. TCDD failed to stimulate transcription from the PGHS-2 promoter when reporter plasmids were transfected into Hepa 1c1c7 cells, a line that contains the functional AhR. It seems likely that inappropriate expression of PGHS-2 may contribute to the toxic effects of TCDD and other halogenated aromatic hydrocarbons (HAHs). In particular, PGHS-2 expression may affect those toxic reactions that involve inappropriate cellular growth, such as dermal hyperplasia and tumor formation. It is also likely that elevated synthesis of prostaglandins, which are potent regulators of immune function, could play a role in the immunotoxicity associated with HAH exposure.

Hepatotoxicity Schuetz et al. (1995) conducted studies to determine if aromatic hydrocarbons induce the mdr gene product P-glycoprotein and whether this induction involves the AhR. Induction of mdr mRNA was compared to the induction of CYP1A1 mRNA in Ah-treated cultures of primary human hepatocytes. Hepatocytes from all 15 individuals tested responded to 3-methylcholanthrene (MC) or TCDD with induction of CYP1A1 mRNA. However, only 62 percent and 55 percent of the preparations responded to treatment with MC and TCDD, respectively, with induction of mdr mRNA. Indeed, in some individuals, mdr mRNA was suppressed by MC and TCDD despite robust CYP1A1 induction. These studies suggest not only that individual variations exist in mdr induction by AH but that aryl hydrocarbons regulate mdr in humans by a novel mechanism distinguishable from the classical AhR pathway.

In a 13-week feeding study in female Sprague-Dawley rats by Van Birgelen et al. (1995), diets were supplemented with 0, 0.2, 0.4, 0.7, 5, or 20 fg TCDD/kg diet. The estimated daily intakes were calculated to be 0, 14, 26, 47, 320, or 1,024 ng TCDD/kg body weight per day. The lowest estimated daily intake associated with increased liver weights was 320 ng TCDD/kg, while a daily intake of 47 ng TCDD/kg resulted in decreased plasma thyroid hormone concentrations and decreased body weight gain. Decreases in relative thymus weights, loss of hepatic retinoids, and induction of CYP1A1 and CYP1A2 activities were found at 14 ng/kg, the lowest dose used. For increases in CYP1A1 and CYP1A2 activities, the right critical values for the 95 percent confidence intervals for the no effect levels (CNELs) ranged from 0.7 to 4 ng TCDD/kg per day. Based on hepatic TCDD

residue levels, these right critical values for CNELs ranged from 0.06 to 0.4 ng TCDD/g liver (wet weight). The CNELs in this study are consistent with the NOAELs reported before in chronic and reproductive studies with rats and TCDD (i.e., 1 ng/kg per day).

The basis for the regional specificity of TCDD in the modulation of hepatocyte proliferation that results in enhanced proliferation in the periportal region, but reduced proliferation in the remainder of the hepatic lobule, is not known. Hushka and Greenlee (1995) have shown that TCDD caused a dose-dependent inhibition of DNA synthesis in primary hepatocytes isolated from either male or female Sprague-Dawley rats in the presence or absence of known hepatocyte mitogens (epidermal growth factor [EGF], hepatocyte growth factor, and TGF-α). No change in DNA synthesis was observed at TCDD concentrations less than 1 pM. Initial characterization of the EGF response system in these cells revealed that TCDD did not alter the specific binding of EGF, or the levels of EGF receptor protein measured in intact cells or cell lysates. TCDD-dependent inhibition of DNA synthesis occurred independently of the suppression observed with TGF-β_1. Estradiol did not alter DNA synthesis in the presence or absence of TCDD. Taken together, these findings indicate that TCDD suppresses DNA synthesis via a novel pathway that is nonresponsive to estradiol, independent of TGF-β, and does not involve a decreased ability of hepatocytes to recognize (bind) EGF, a prototype mitogen.

Zhao and Ramos (1995) arrived at the same conclusions using primary cultured rat hepatocytes. Scheduled DNA synthesis in control cultures peaked at 64 hours and was negligible by 72 hours after initial seeding of freshly isolated hepatocytes. A concentration-dependent inhibition of DNA synthesis was observed in one-day-old hepatocyte cultures treated with BP (0.3–30 μM) for up to 28 hours, and comparable inhibitory responses were observed in cultures treated for 24 hours with TCDD (0.01 nM) or TCDF (0.01–1 nM) but not in cultures treated with perylene (0.01–100 nM) or benzo[*e*]pyrene (1–1,000 nM). EROD activity was highly inducible in hepatocytes challenged for 24 hours with BP (0.3–3 μM) or TCDD (0.1–100 nM), with peak induction at 12 or 36 hours after chemical challenge, respectively. To assess the role of the AhR in this response, the interactions of α-naphthoflavone (α-NF) and ellipticine (ET) with BP and TCDD in this cell system were evaluated. Pretreatment with α-NF (10 nM) for 24 hours prevented the inhibitory effects of both BP (3 μM) and TCDD (1 nM), whereas ET (0.01 nM) pretreatment selectively antagonized the effects of BP (3 μM). Pretreatment of hepatocytes with TCDD or TCDF (1 nM) for 24 hours before the onset of DNA synthesis, followed by challenge with BP (3 μM), partially antagonized the inhibitory response to BP. These data implicate AhR-related signal transduction in the inhibition of hepatocyte DNA synthesis by BP and related agents, and suggest that in the case of BP, metabolism by cytochrome P450 to toxic intermediates contributes to the inhibitory response.

Administration of TCDD to rats results in a dose-dependent decrease in

hepatic plasma membrane epidermal growth factor receptor (EGFR). Sewall et al. (1995a) monitored alterations in hepatic EGFR levels in female Sprague-Dawley rats seven days after a single oral gavage dose of TCDD (0, 1, 5, 25, and 50 fg/kg). The level of hepatic EGFR was significantly decreased at a dose of TCDD as low as 1 fg/kg. Thus, TCDD decreased total EGFR protein and maximum binding capacity without altering ligand binding affinity (K_d). The results demonstrated that ligand-induced autophosphorylation capacity and basal phosphotyrosine residues of plasma membrane EGFR both decreased parallel with the decrease in EGFR protein, suggesting no TCDD-related alteration in the inherent functional ability of the receptor to undergo activation. Furthermore, it was found that the dose–response curve for EGFR protein level determined by Western blot analysis was similar for both male and female Sprague-Dawley rats.

In other studies, Ilian et al. (1996) observed that day 8 post-TCDD treatment is associated with a dose-dependent reduction of hepatic pyruvate carboxylase (PC) mRNA levels in Ahb/b mice. This response was tenfold greater than in congenic Ahb/b mice, suggesting that previously reported reduction in PC activity by TCDD treatment of mice is a consequence of a reduction in PC mRNA levels and that the effect requires a competent AhR. TCDD inhibits normal accumulation of vitamin A in hepatic stellate cells, the main storage site for vitamin A. TCDD-induced inhibition of hepatic vitamin A accumulation does not seem to involve a reduction in the number of stellate cells or cell transformation (Hanberg et al., 1996). TCDD acts as a promoter of lesions initiated either spontaneously or by vinyl carbamate. TCDD overrides the intrinsic resistance of both male and female C57BL/6 mice to liver tumor formation (Watson et al., 1995).

Xiao et al. (1995) tested the effects of glucocorticoids on the expression of a number of genes under the control of the AhR in cultured primary rat hepatocytes. Treatment of cultured hepatocytes with 1.0 μM dexamethasone potentiated the induction of CYP1A1, glutathione *S*-transferase Ya subunit (GSTYa), and UDP-glucuronosyltransferase gene expression by polycyclic aromatic hydrocarbons, whereas the glucocorticoid agonist suppressed PAH induction of NAD(P)H: quinone oxidoreductase (QOR) subunit and aldehyde dehydrogenase 3C gene expression. Two of these rat genes, GSTYa and QOR are also induced by electrophilic agents, such as *tert*-butylhydroquinone. In the presence of *tert*-butyl hydroquinone, dexamethasone caused a similar level of potentiation of GSTYa subunit expression and suppression of QOR subunit expression as observed with the PAH 1,2-benzanthracene. Studies using the glucocorticoid receptor antagonist RU-38486 demonstrated that the modulation of PAH induction by glucocorticoids of CYP1A1 and QOR activity apparently depends on the action of the glucocorticoid receptor. These results suggest that the positive and negative changes observed are the result of specific alterations in the rates of transcription of these genes because of the action of the glucocorticoid receptor, thereby affecting regulation of GSTYa and QOR by both AhR-dependent and AhR-independent mechanisms.

Although hepatic uroporphyria is induced by HAHs in mammalian and avian systems, attempts to produce uroporphyria in vertebrate (mammalian) hepatoma lines have been unsuccessful. Dose-dependent accumulation of porphyrins was observed in cells treated for 48 hours with TCDD or 3,3′,4,4′-tetrachlorobiphenyl when the heme precursor δ-aminolevulinic acid (ALA) was present during the last 5 hours of treatment (Hahn and Chandran, 1996). Uroporphyria did not occur in cells treated with TCDD or TCB in the absence of added ALA. ALA-dependent porphyrin accumulation was also seen following treatment of PLHC-1 cells with TCDF or with the non-ortho-substituted chlorobiphenyls 3,4,4′,5-tetrachlorobiphenyl and 3,3′,4,4′,5-pentachlorobiphenyl. The EC_{50} values for porphyrin accumulation were similar to, or slightly higher than, the concentrations at which peak EROD activities were obtained, suggesting a relationship between the decline in EROD activity and enhanced porphyrin accumulation. α-Naphthoflavone inhibited TCDD-induced EROD activity and porphyrin accumulation, providing further evidence for the involvement of a fish CYP1A in the mechanism of this porphyria. Addition of TCB to TCDD-treated cells also inhibited EROD activity, but enhanced porphyrin accumulation, suggesting that an interaction between the halogenated inducer and the induced CYP1A is necessary for the porphyrogenic response.

Lorenzen et al. (1997) observed concentration-dependent induction of CYP1A and intracellular porphyrin accumulation following treatment of chicken embryo hepatocyte cultures with TCDD and related chemicals. Maximal CYP1A activity (measured as EROD activity) and immunodetectable protein were observed at concentrations coincident with those at which porphyrin accumulation became evident. These results are consistent with a role of CYP1A induction and/or AhR activation in porphyrin accumulation mediated by HAHs with a planar configuration.

Münzel et al. (1996) studied the modulation of DNA synthesis by TCDD in primary cultures of hepatocytes and rat liver epithelial cells (WB-F344). In hepatocytes, TCDD either positively or negatively modulated EGF-stimulated DNA synthesis. In the presence of ethinylestradiol, 10^{-12} M TCDD moderately increased EGF-stimulated DNA synthesis (approximately 30 percent). In contrast, in the absence of ethinylestradiols 10^{-9} M TCDD decreased DNA synthesis (approximately 30 percent). The response of "early genes" of the *jun/fos* family and the corresponding proteins was also studied under these two conditions. In agreement with DNA synthesis data, the level of c-Jun was increased or decreased in nuclear extracts. Furthermore, DNA binding of Jun/Fos proteins, including c-Jun and Fra-1, was decreased under conditions of mitoinhibition, while the level of Fra-1 in nuclear extracts was increased. In WB-F344 cells, TCDD treatment for 44 hours increased DNA synthesis two- to threefold compared to controls, based on measuring radiolabeled thymidine incorporation into DNA or on determining the nuclear labeling index with bromodeoxyuridine. This effect is probably due to the inhibition of high-density growth arrest by TCDD.

Wasting Syndrome A prominent symptom of the acute toxicity of TCDD is the loss of adipose tissue and body weight, a phenomenon known as the wasting syndrome. The effect of TCDD on glucose transport in mice was examined by Liu and Matsamura (1995). A single i.p. dose of TCDD (116 μg/kg) resulted in a time-dependent decrease in transport activity in adipose tissue and brains of C57BL/6 mice. Reduction of transport occurred within 24 hours in both tissues. In adipose tissue, a slight recovery was observed by 30 days, but in the brains of treated animals, glucose transport was significantly decreased even at the latest time. A comparison of dose–response relationships for several tissues between C57BL/6 (TCDD responsive) and DBA/2J (TCDD-nonresponsive) mice resulted in parallel curves, with C57BL/6 animals showing a ten- to twentyfold greater sensitivity. The estimated ED_{50} values for reduction of transport in adipose tissue were 50 and 800 μg/kg for the C57BL/6 and DBA/2J strains, respectively. Immunoblotting for the adipose-type (type 4) glucose transporter (GLUT) showed a 40 percent decrease in the membrane fraction of adipose tissue from C57BL/6 mice treated with 116 μg/kg TCDD for 40 hours. A similar decrease in brain-type GLUT1 was observed in the plasma membrane fraction of brain tissues isolated from the same animals. Analysis of RNA for the corresponding GLUT4 and GLUT1 genes showed a dramatic decrease in GLUT4 mRNA as early as 24 hours after treatment. In contrast, the level of GLUT1 mRNA increased slightly in the brains of treated mice. Based on these data it was concluded that regulation by TCDD of glucose transport activity in mice is an AhR-dependent process and that adipose-type GLUT4 appears to be regulated at the mRNA level, whereas brain-type GLUT1 is affected mainly at the protein level.

Enan and colleagues (1996) investigated the involvement of the EGFR and protein-tyrosine kinase (PTK) in TCDD-induced toxicity. Up-regulation in radiolabeled EGF binding to EGFR was measured after 24 hours of TCDD treatment, whereas down-regulation in EGFR binding was measured after 72 hours of TCDD treatment. Up-regulation of EGFR binding was associated with a significant decrease in postnuclear (7,000 × *g* supernatant) PTK activity, but this activity was stimulated after 72 hours of TCDD treatment. TCDD altered the level of tyrosine phosphorylation in proteins with molecular weights of 34, 40, 43, 45, 60, and > 205 kDa. TCDD caused a significant increase in postnuclear cAMP-dependent protein kinase A (PKA) after 24 hours of treatment. The action of TCDD on protein kinases was partially blocked by the protein synthesis inhibitor cycloheximide. TCDD increased nuclear PTK and decreased nuclear PKA activity. Estradiol (E_2) inhibited the postnuclear and nuclear activity of both PTK and PKA in control samples, but did not affect the action of TCDD on either postnuclear or nuclear PTK activity. However, E_2 abolished the stimulatory effect of TCDD on PKA activity in postnuclear protein. In the presence of insulin, TCDD did not induce any additional changes in postnuclear or nuclear PTK. Forskolin alone inhibited postnuclear PTK activity and stimulated its nuclear activity. Addition of TCDD 20 minutes after forskolin

resulted in an increase in postnuclear PTK, but there was little change in nuclear PTK compared to the effect of forskolin alone. The stimulatory effect of TCDD on postnuclear PKA activity was enhanced by insulin, and TCDD reversed the negative effect of forskolin, but there was no effect of either insulin or forskolin on the inhibition by TCDD of nuclear PKA activity. TCDD decreased the activity of MAP2 kinase and reduced the binding activity of AP-1 DNA when given alone; it also blocked E_2 stimulation of MAP2K. These findings suggest that TCDD may interrupt the endocrine function of human luteinized granulosa cells through the blockage of the mitotic signal directly or indirectly through the interaction of PTK-MAP2K and PKA signaling.

It had been reported that TCDD dose dependently reduces the activity of PEPCK, the rate-limiting enzyme of hepatic gluconeogenesis. To further investigate the mechanism by which TCDD decreases PEPCK activity, Stahl (1995) investigated the effect of TCDD on PEPCK activity in primary cultured rat hepatocytes. Cells were pretreated with dexamethasone (100 nM) 8 hours before PEPCK induction was initiated by the addition of glucagon (10 nM) and concurrent withdrawal of insulin. This hormonal treatment induced twofold elevation of PEPCK activity in control cells within 8 hours. Using this induction regimen, experiments were conducted in which rats were pretreated with TCDD (125 μg/kg in corn oil by gastric intubation) four days prior to isolation of primary rat hepatocytes (PRH). This resulted in a complete block of the glucagon-dependent induction of PEPCK in PRH from TCDD-pretreated animals. In another experiment, TCDD (100 nM) was added directly to the PRH either 24 or 48 hours prior to the induction regimen. Incubation of PRG with TCDD 24 hours prior to initiation of the induction regimen resulted in a slight decrease in the degree of PEPCK induction compared to controls. However, treatment of PRH with TCDD 48 hours prior to initiation of the induction regimen almost completely blocked PEPCK induction. It is, therefore, suggested that the effect of TCDD on liver PEPCK activity is due to a direct effect on liver cells and is not mediated by factors outside the liver.

Viluksela et al. (1995) analyzed the toxicological significance of reduced gluconeogenesis by studying dose–responses and time courses of effects of TCDD on the activity of PEPCK in liver and two other tissues with high specific activity, kidney and brown adipose tissue. Liver PEPCK activity was significantly decreased from 1 to 32 days after oral dosing (60 μg/kg). A clear dose–response was present 8 days after dosing, beginning at a dose of 1 μg/kg. In contrast to liver, TCDD treatment increased PEPCK activity in kidney and brown adipose tissue, but only at the two highest doses administered (30 and 60 μg/kg). PEPCK activity in kidney began to increase slowly, reaching a maximum on day 16 and declining thereafter, whereas in brown adipose tissue the activity was significantly increased on day 1 and maximally day 4 after dosing. A likely explanation for these tissue-specific effects is related partly to toxicokinetics and partly to homeostatic responses of the organism to the toxic insult of TCDD. High concen-

trations of TCDD in liver and brown adipose tissue combined with early responses (one day after dosing) suggest a direct effect in these organ or tissues, whereas very low concentrations and delayed responses in kidney indicate an indirect effect. This interesting enzymatic constellation suggests that the reduction in gluconeogenesis due to decreased PEPCK activity in liver is partially counterbalanced by increased gluconeogenesis in kidney as a result of induction of PEPCK in this organ. Induction of PEPCK in brown adipose tissue (BAT), where it is a glyceroneogenic enzyme, provides for the first time a plausible explanation for the initial accumulation of fat in brown adipose tissue of TCDD-treated rats.

Tuomisto et al. (1995) performed portocaval anastomosis and vagotomy in LE and Han/Wistar (HW) rats to elucidate the mechanism of anorexia induced by TCDD. TCDD-sensitive LE rats were given a sublethal (5 μg/kg) or lethal (20 μg/kg) dose by gavage five to eight weeks after portacaval anastomosis. TCDD-resistant HW rats were given a nonlethal dose (500 or 7,200 μg/kg). The shunt operation did not reduce the lethality of TCDD. The effect on wasting of the marginally toxic dose of 5 μg/kg in LE rats was potentiated by the portacaval operation, and the lethal dose was effective in both shunted and sham-operated LE rats. TCDD failed to decrease food intake and body weight in shunted HW rats at either dose level although it did so in sham-operated controls. The absence of an effect may be due to the already reduced weight of shunted rats at the time of TCDD dosing. TCDD anorexia was not explained by changes in histamine or serotonin turnover in the brain. Vagotomy did not influence the lethality of TCDD, although reduction in food intake was somewhat blunted in HW rats. The results were interpreted to suggest that the anorectic effect of TCDD is modified when portal blood bypasses the liver. The results are not consistent with the suggestion that the liver plays a role as the major initiator of TCDD anorexia. Little evidence was found to support a crucial role of vagal afferent input.

The fact that TCDD toxicity in adipose tissue causes severe wasting suggests that TCDD could have effects on adipocyte differentiation. Using 3T3-L1, cells Phillips et al. (1995) demonstrated that when cells were treated with 10 nM TCDD before differentiation or during the first two days of induction in the presence of dexamethasone and isobutylmethylxanthine (IBMX), the number of fat cell colonies measured seven to ten days later decreased. Researchers observed an accompanying reduction in the amounts of mRNA encoding several adipocyte markers. In contrast, when TCDD was added after differentiation, it had no effect on maintenance of the adipose phenotype. Dose–response and structure–activity relationships were consistent with a process mediated by the interaction of TCDD with the AhR. TCDD did not interfere with glucocorticoid-inducible transcription. Treatment of cells with TCDD augmented the increase in protein kinase A activity elicited by either IBMX or forskolin, suggesting that if TCDD disrupts the cAMP signaling pathway, interference occurs after activation of PKA.

Enan et al. (1996c) presented evidence that gender differences in the response of nonreproductive cells to TCDD exist and that some of these differences involve differential effects in the cytoplasmic and nuclear compartments of the cell. Glucose uptake by adipose tissue in vitro was decreased significantly in male guinea pigs within 1 day of i.p. injection of TCDD, but there was no significant effect in females, even 28 days after treatment. A similar difference between male and female guinea pigs was detected in the effect of TCDD on lipoprotein lipase (LPL) activity, except that a significant decrease in LPL activity was observed 28 days after treatment. Experiments with adipose tissue explants from untreated guinea pigs and macaques revealed similar gender differences in the effect of TCDD in vitro on glucose uptake and LPL activity. Both time course and dose–response studies with TCDD in vitro confirmed the greater sensitivity of male tissues to TCDD toxicity. TCDD induced lipid peroxidation in the adipose tissues of male guinea pigs, but had no effect on females. Radiolabeled-TCDD binding affinity studies in adipose explant tissues showed that tissues from male guinea pigs and monkeys had a higher binding capacity for TCDD than female tissues. TCDD induced a significant reduction in nuclear protein phosphorylation and an increase in cytosolic protein phosphorylation in adipose tissue from male guinea pigs; the effects in female tissues were the opposite: nuclear protein phosphorylation increased and cytosolic protein phosphorylation decreased. In a cell-free system in the absence of the nucleus, adipose tissues from male guinea pigs and monkeys responded to TCDD with a rapid stimulation of tyrosine kinase activity, but female tissues from both species had a significantly lower and slower response. TCDD induced the DNA binding of AP-1 in adipose tissues of male guinea pigs, but in female tissues, TCDD reduced the DNA binding of AP-1.

Endocrine Effects Sewall et al. (1995b) reported follicular hyperplasia and hypertrophy of the thyroid gland in rats administered 0.1–125 ng/kg TCDD per day via oral gavage biweekly for 30 weeks. TCDD induction of UGT1 resulted in increased excretion of T3 glucuronide. The observed hyperplasia and hypertrophy are consistent with elevated TSH levels. Results suggest that TCDD induces alterations in thyroid hormone function, probably as a result of chronic perturbations of the liver–pituitary–thyroid axis.

Enan and colleagues (1996a) examined the effects of TCDD on cellular glucose uptake, cAMP-dependent PKA, and progesterone production in human luteinizing granulosa cells (LGCs) in culture. Treatment of human LGCs with TCDD produced a time- and dose-dependent decrease in cellular uptake of glucose. The V_{max} and K_m of glucose transport were decreased by TCDD treatment. Furthermore, cytochalasin B, a specific inhibitor of facilitative glucose transporter proteins, totally abolished the portion of glucose transport activity that is sensitive to TCDD. Pretreatment of cells with the AhR blockers 4,7-phenanthroline and α-naphthoflavone antagonized the effect of TCDD on [^{3}H]Me-glucose

uptake. Structure–activity relationship studies with TCDD and three dioxin congeners revealed a rank order that is consistent with their previously determined biological activity. Treatment of cells for 48 hours with 10 nM TCDD substantially reduced PKA and progesterone production. The inhibitory effect of TCDD on progesterone production was more pronounced in the presence of insulin (10 μg/ml) and *D*-glucose (13.3 μM). However, cytochalasin B abolished the effect of TCDD on progesterone production. Forskolin (an adenylate cyclase activator) abolished the effect of TCDD on glucose uptake and progesterone production but did not alter its effect on PKA activity. A relationship between glucose transporting activity and progesterone production in human LGCs treated with TCDD is suggested by the finding that cytochalasin B down-regulated glucose transporting activity and progesterone production, insulin plus *D*-glucose down-regulated glucose uptake and amplified the negative effect of TCDD on progesterone production, and forskolin abolished the negative effect of TCDD on glucose transporting activity and on progesterone production. From these data it was concluded that glucose transporting activity can be used as a sensitive biomarker to detect the very early response to TCDD in human steroid-producing cells and that the effect of TCDD on steroid production is mediated through the cAMP-dependent protein kinase.

Neurotoxicity The behavioral signs exhibited by animals exposed to TCDD (progressive anorexia and loss of body weight) suggest a role for the CNS in TCDD toxicity. At lethal doses, TCDD affects the metabolism of serotonin, a neurotransmitter that can modulate food intake in the brain, and this effect is associated with elevated concentrations of free tryptophan in the plasma (Unkila et al., 1995a). No major changes in catecholaminergic neurotransmitter systems were observed in TCDD-treated rats. Cytochrome P450-related enzyme activities are induced by TCDD in the brain. As in the liver, this induction does not correlate with susceptibility to TCDD lethality in rats.

Hanneman et al. (1996) examined the effects of TCDD and related compounds on the uptake of intracellular calcium in primary cultures of rat hippocampal neuronal cells. Treatment of cell cultures with 2,3,7,8-TCDD (10–100 nM) resulted in a rapid, concentration-dependent increase in calcium associated with a decrease in mitochondrial membrane potential and activation of α-protein kinase C. In contrast, 1,2,3,4-TCDD, a weak AhR agonist, had no effect on calcium at concentrations as high as 10 μM, and similar results were observed with TCB. Maximal calcium concentrations were observed within 30 seconds after addition of 2,3,7,8-TCDD and remained elevated above resting levels for the duration of the experiment. This rapid increase in calcium was blocked by addition of ethylenediamine tetraacetic acid (EDTA) (2 μM) to the external medium or by pretreatment of cells with the calcium channel antagonist nifedipine (10 μM). However, the pretreatment of cells with 100 μM cycloheximide failed to block calcium uptake in neuronal cells. These data indicate that rat hippocam-

pal neuronal cells are responsive to 2,3,7,8-TCDD; however, the mechanism is not associated with altered gene transcription and may involve cellular targets. Animal studies and in vitro mechanistic studies continue to emphasize the importance of alterations in neurotransmitter systems and thyroid function as underlying mechanisms of behavioral dysfunction caused by TCDD and related chemicals (Golub and Jacobson, 1995).

Immunotoxicity TCDD and structurally related halogenated aromatic hydrocarbons have a broad range of immunologic effects in experimental animals, including effects on host resistance and innate, cell-mediated, and humoral immune responses (Kerkvliet, 1995). As discussed in *VAO* and *Update 1996*, thymic atrophy is the most consistent biological effect found in laboratory animals treated with TCDD and is believed to be mediated primarily through the T-cell arm of the immune system. TCDD prevents the maturation of thymocytes to mature T cells by inducing differentiation of thymic epithelial cells. Suppression of humoral immunity by TCDD results in an inhibition of B-lymphocyte differentiation into antibody-producing cells. Summarized below are recent studies that support and expand on these findings.

The thymus plays an important role in generating the ability of the immune system to distinguish self from nonself, thereby avoiding autoimmune responses. The potential of TCDD to disrupt self–nonself discrimination was evaluated using the popliteal lymph node (PLN) assay (Fan et al. 1995). Male Sprague-Dawley rats were injected subcutaneously with either 10 μg/kg TCDD or 5 mg per 50 μl chlorpromazine (CPZ), a structural analogue of TCDD (dissolved in dimethylsulfoxide), into the right hind footpad. Vehicle was injected into the contralateral footpad of treated animals, as well as into both hind footpads of control rats. When the animals were sacrificed on day 7, the weight ratio of right PLN over left PLN was significantly higher in both CPZ- and TCDD-treated rats than in controls. Mild follicular hyperplasia of the PLN with no evidence of an acute inflammatory response was found in both groups. These results indicate that TCDD has the potential to induce or exacerbate autoimmune-like reactions.

Fan et al. (1996) studied the effect of TCDD on delayed-type hypersensitivity reaction as a measure of cell-mediated immunity in Sprague-Dawley rats. A time-course evaluation demonstrated that the greatest effect on cell-mediated immunity occurred when TCDD treatments were administered five days before immunization with the antigen keyhole limpet hemocyanin (KLH). A dose–response experiment of the effect of 1, 3, 10, 20, 30, 40, and 90 μg/kg TCDD on delayed-type hypersensitivity to KLH showed an inverted U-shaped dose–response curve, indicating that low doses enhanced and high doses suppressed this immune function.

The effects of TCDD on another measure of cell-mediated immunity, cytotoxic T-lymphocyte (CTL) activity, has also been evaluated (De Krey and Kerkvliet, 1995). When mice were administered single oral doses of 2.5–40 μg/kg

TCDD, a dose-dependent suppression of CTL activity was observed. In contrast, plasma corticosterone (CS) levels were not significantly altered at doses lower than 40 μg/kg TCDD, suggesting that TCDD-induced CTL suppression is not dependent on CS elevation. The direct effect of TCDD on CTL generation was tested in vitro by adding 10^{-13} to 10^{-9} M TCDD to cultures of mixed lymphocyte–tumor cells. No alteration of CTL activity was observed after five days of culture at any of the doses tested. In contrast, CS alone significantly suppressed CTL activity. CS-induced CTL suppression in vitro was neither enhanced nor inhibited by the presence of TCDD, which suggests that TCDD causes CTL suppression in vivo by a mechanism that does not involve CS.

In a study with nonhuman primates, Neubert et al. (1995) vaccinated marmosets with tetanus toxoid and administered a second booster in conjunction with 100 ng/kg TCDD. The proliferative response of lymphocytes to recall antigen was measured in vitro in blood samples. No reduction in lymphocyte response was observed, but when the ratio of the responses between the first and second booster was compared, a slight but statistically significant increase in this ratio was observed in the lymphocytes of TCDD-treated marmosets compared to controls.

Based on the observed suppressive effects of TCDD on T-cell activity, reports of increased susceptibility of laboratory animals to pathogenic microorganisms that interact primarily with cell-mediated immunity are not surprising. Recently, Burleson et al. (1996) found that a single oral dose of 0.01, 0.05, or 0.10 μg/kg TCDD increased mortality in mice when they were subsequently infected with influenza A/Hong Kong/8/68 (H3N2) virus. There was no effect on the virus-enhanced increase in lung weight-to-body ratio or the virus-induced decrease in thymus weight. Thus, TCDD-augmented mortality did not appear to be due to additive or synergistic effects of TCDD and virus on pulmonary edema or thymic atrophy. However, another study reported minimal effects of TCDD on *Trichinella spiralis* infection in rats (Luebke et al., 1995), which was markedly different from the increased persistence of infection observed in an earlier study with mice (Luebke et al., 1994). Researchers suggested that this difference was a clear indication of differential species sensitivity and underscored the need to determine which species more closely reflects the potential outcome of human exposure to TCDD (Luebke et al., 1995).

The toxic action of TCDD on the thymus of rats and humans has been compared by treating Wistar rats and SCID-ra and SCID-hu mice (engrafted with fetal rat or human thymus, respectively) with 1, 5, or 25 μg/kg TCDD (de Heer et al., 1995a). Four days after exposure, the thymuses were removed, weighed, and examined histopathologically. There was a dose-dependent decrease in the relative size of the cortex of both normal rat thymus and grafted human thymus; the decrease was significant in the highest-dose group. Only limited data were obtained from grafted rat thymus because of a cutaneous graft-versus-host reaction, but they were consistent with those in normal rat and grafted human thymus.

TCDD tissue concentrations in normal rat thymus and grafted human thymus were similar. Thus, it appears that the human thymus and the Wistar rat thymus display a comparable sensitivity to the toxic action of TCDD.

To determine if TCDD interferes with intrathymic negative selection processes, de Heer et al. (1995b) exposed M1s-1[a] DBA/2 mice to a single thymotoxic dose of 75 or 225 μg/kg TCDD and evaluated the emergence of Vβ6+ cells in thymus, spleen, and mesenteric lymph nodes during the subsequent recovery of TCDD-induced thymic atrophy. In addition, the extrathymic differentiation of T lymphocytes in the liver was studied. TCDD exposure resulted in severe thymic atrophy and an increase in hepatic mononuclear cells. However, no evidence of potentially autoreactive Vβ6+ cells, differentiated either intrathymically or extrathymically, in TCDD-exposed DBA/2 mice was observed.

Fan et al. (1996) examined the effects of TCDD primary antibody response to sheep red blood cells (SRBCs), as an end point of the effects of TCDD on humoral immunity, in studies with male rats. At doses of 10, 20, and 40 μg/kg TCDD, enzyme-linked immunosorbent assay (ELISA) revealed that serum immunoglobulin M (IgM) levels measured seven or fourteen days after immunization were not affected by TCDD compared to controls. In contrast, serum IgG levels were elevated in a dose-dependent manner at both times. In a related study, the involvement of cytokines (interleukin 1 [IL-1] and tumor necrosis factor [TNF]) in mediating the enhanced IgG response and delayed-type hypersensitivity reaction to 1, 2, 10, 30, and 90 μg/kg TCDD was investigated (Fan et al., 1997). Levels of mRNA for IL-1β were elevated in all dose groups, with a fivefold increase above controls in the 90 μg/kg TCDD group. The mRNA levels of TNF were also significantly elevated, beginning at 30 μg/kg TCDD. These results suggest that at low doses of TCDD, increased levels of IL-1β may account for immune function stimulation, whereas at high doses, greatly elevated TNF and IL-1β levels might exacerbate or mediate acute toxicity such as immune suppression and related biochemical effects. A time course study using 60 μg/kg TCDD without immunization indicated that mRNA levels of TNF in the liver were significantly elevated starting at 24 hours, and reached a maximum at 48 hours. This change was accompanied by a transient increase in mRNA levels of IL-1β at day 4. Thus, TCDD alone and without immunization can cause transient increases in mRNA levels of TNF and IL-1β in liver.

Smialowicz et al. (1996) studied the effect of TCDD on the antibody plaque-forming cell (PFC) response to the T-cell-independent antigen trinitrophenyl-lipopolysaccharide (TNP-LPS) in female B6C3F1 mice and F344 rats. The animals were injected i.p. with a single dose of 1–30 μg/kg TCDD seven days prior to immunization with TNP-LPS. In mice, thymus weights were decreased at 10 and 30 μg/kg TCDD, whereas spleen weights were decreased and liver weights increased at 3, 10, and 30 μg/kg. Mice treated with 10 and 30 μg/kg TCDD also had suppressed PFC responses and serum hemagglutination titers. In rats, thymus

weights were decreased and liver weights increased at 3, 10, and 30 μg/kg TCDD; however, the PFC response and serum hemagglutination titers to TNP-LPS were suppressed only at 30 μg/kg. No effects on splenic lymphocyte subsets were observed. Collectively, these data suggest that TCDD suppresses the T-cell-independent antibody response to TNP-LPS in both B6C3F1 mice and F344 rats and that mice are more sensitive to immune suppression by TCDD than rats.

In addition to immune suppression, TCDD has been shown to promote inflammatory responses. This effect could be a result of upregulation of the production of inflammatory cytokines, such as TNF and IL-1. Recently, Moos and Kerkvliet (1995) examined the effects of exogenous TNF and the effects of blocking TNF activity with a soluble TNF receptor (rhuTNFR:Fc) on antibody production to SRBCs in control and TCDD-exposed C57BL/6 mice. Their results indicated that increased TNF can suppress antibody production to SRBC, but that TNF itself does not appear to mediate TCDD-induced antibody suppression.

Investigation into the effect of TCDD on cytokine production was conducted using a novel in vitro model based on injection of hamster monoclonal antibody to the CD3 epsilon portion of the mouse T-cell receptor (Prell et al., 1995). T-cell activation resulted in the release of several cytokines, including TNF, interferon (IFN), IL-2, IL-3, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF). In an in vivo study, administration of 15 μg/kg TCDD to mice followed by an injection of anti-CD3 two days later significantly reduced plasma levels of IFN and elevated plasma levels of IL-6 and GM-CSF, suggesting that increased IL-6 and GM-CSF contributed to the toxic effects of TCDD.

Kerkvliet et al. (1996) characterized changes in CTL, alloantibody, and cytokine responses to the P815 tumor allograft in mice treated with 15 μg/kg TCDD. TCDD suppressed CTL activity as well as cytotoxic antibody responses, and suppression correlated with a reduced percentage of CD8+ T cells. The cytokine profile of TCDD-treated rats was markedly different from that of control animals, which showed increases in IL-1 and TNF on day 5 and IL-2 on day 6, followed by peak induction of IL-6, IL-7, IL-2, IFN, TNF, and IL-1 on subsequent days. In contrast, cytokine production in TCDD-treated mice showed early increases in IFN, IL-2, and TNF up to day 5 but failed to increase normally thereafter; the production of IL-1, IL-4, or IL-6 was unaffected by TCDD. This differential effect of TCDD on cytokine production was reflected in the degree of suppression of cytotoxic antibody isotypes. TCDD abrogated the production of IgG2a (generally associated with IFN production) but had much less effect on the level of IgG (associated with IL-4).

The effects of TCDD on the production of cytokines IL-1 and IL-2 were evaluated by Badesha et al. (1995). Young adult male Leeds rats fed a total dose of 3 μg/kg TCDD showed a duration-dependent reduction of in vitro lipopolysaccharide-induced production of IL-1 by splenic macrophages within 30 days of exposure. A prolonged, 180-day exposure was required before a significant suppression in the generation of IL-2 by activated splenic T cells was measured.

Thymic atrophy has been shown to be influenced by prostanoid metabolites of arachidonic acid. Olnes et al. (1996) reported that TCDD inhibits prostaglandin G/H synthase, one of the important enzymes in the cyclooxygenase pathway from arachidonic acid to prostaglandin H_2. It was reported that incubation of thymocytes with TCDD resulted in inhibition of synthase gene expression in a concentration-dependent manner.

Kraemer et al. (1996) studied TCDD-stimulated prostaglandin synthesis in canine kidney cell cultures. TCDD stimulated prostaglandin synthesis in these cells, at least in part, by elevating PGHS-2 levels. This enzyme is believed to be responsible for producing inflammatory prostaglandins and, indirectly, to modulate cytokines such as TGF and IL-1β. Results suggest that inappropriate expression of synthase may contribute to the diverse immunotoxic effects of TCDD.

Despite extensive laboratory research, the mechanism of TCDD-mediated immunotoxicity remains uncertain. This is due, in part, to unsuccessful attempts to demonstrate a direct effect of TCDD on immune function in vitro. As discussed in earlier reports, the immunotoxic effects of TCDD and related substances appear to be mediated predominantly through binding to the Ah receptor. However, AhR-independent mechanisms also appear to be involved.

Recently, Fernandez-Salguero et al. (1995) demonstrated that AhR-deficient mice are relatively unaffected by 2,000 μg/kg TCDD, a dose that is tenfold higher than that found to cause severe pathologic effects on the thymus of littermates expressing the functional receptor. These results suggest that thymic involution by TCDD is mediated entirely by the AhR. However, a number of other pathological effects, such as vasculitis and scattered single cell necrosis of the lung and liver, were present in receptor-deficient mice, suggesting that some effects of TCDD may be AhR-independent.

Thymic atrophy is a prominent effect of TCDD exposure. In the presence of TCDD, the distribution of CD4/CD8 thymocyte subsets is strongly skewed toward CD4-CD8+ single positives. The primary target of TCDD action appears to be stroma cells, which have an important role in thymocyte maturation and in the selection of thymocytes bearing T-cell receptors specific for foreign antigen in the context of self. Using staphylococcus enterotoxin B as a superantigen, Kremer et al. (1995) investigated whether the effects of TCDD on thymocyte differentiation and maturation had further consequences for the selection process by analyzing the repertoire of Vβ genes as a measure of negative selection and the expression of CD69 and bcl-2 by thymocytes as a measure of positive selection. TCDD had no effect on negative selection but did increase the parameters for positive selection. Researchers suggested that these effects on thymocyte maturation are mediated through the action of TCDD on the thymus stroma via the AhR. This hypothesis is strengthened by the results of Germolec et al. (1996), who reported an increased expression of CYP1A1 in thymus cells from rats exposed to TCDD. This pattern of induction was related to the expression of the AhR on macrophages or other stromal cells of the thymus.

The role of the AhR in the immunosuppressive effect of TCDD on B lymphocytes has also been investigated. In order to identify the genes potentially regulated by TCDD in B lymphocytes, Masten and Shiverick (1995) searched the published data on genes important in B-cell function for DNA sequences that have homology to the consensus AhR binding site. This approach identified a subset of DNA binding sites for the transcription factor B-cell lineage-specific activator protein (BSAP), which resembles the consensus binding site of the receptor. BSAP expression is essential for B-cell development, and DNA binding sites for BSAP occur in regulatory regions of the immunoglobulin heavy-chain gene locus. The BSAP binding sites were localized in the promoter region of the CD19 gene. CD19 is a cell surface signal-transducing protein expressed exclusively on B lymphocytes at early stages of development. This evidence, therefore, suggests a role for BSAP in the regulation of CD19 gene expression and further suggests that binding of TCDD to the AhR could interfere with transcription by competing with BSAP for binding to this site.

Masten and Shiverick (1996) also compared TCDD responsiveness and AhR complex formation in a cultured human hepatoma cell line and two human B-cell lines. The B lymphocytes were found to express the AhR as well as the Arnt, and gel mobility shift analysis demonstrated that the AhR complex in B cells was functional with respect to TCDD activity. Furthermore, TCDD treatment induced CYP1A1 activity in one of the two B-cell lines. The lack of response in the other B-cell line was probably due to a relatively low level of AhR expression.

AhR-independent responses have been reported in certain strains of mice immunized with SRBCs and exposed to TCDD or polychlorinated biphenyls. These results, however, may have been due to the particular method of cultivating B cells in vitro using fetal bovine serum. Using a standard in vitro culture method for spleen cells of B6C3F1 female mice, Harper et al. (1995) reported that B lymphocytes cultured with mouse serum showed a dose-dependent suppression of plaque-forming activity when exposed to TCDD. There was, furthermore, excellent correlation between the immunosuppressive activity of a number of halogenated aromatic hydrocarbons and their binding affinity for the AhR. These results support the role of the AhR in mediating TCDD-induced humoral immunosuppression.

Macrophages are important cellular components of the innate immune response. Although TCDD exerts profound immunosuppressive effects on B and T lymphocytes, it appears to have much less activity against macrophages. TCDD does not alter macrophage-mediated antigen presentation, phagocytosis, or tumor cytolysis and cytostasis. However, there is some evidence suggesting that TCDD treatment may stimulate macrophage-generated inflammatory cytokines and reactive oxygen species. The toxic effects of TCDD and related compounds may require activation to toxic metabolites by drug-metabolizing enzymes, such as CYP1A1 and alcohol dehydrogenase (ALDH). Germolec et al. (1995) compared the induction of these enzymes in various macrophage populations following

treatment of F344 rats with TCDD. Kupfer cells, alveolar macrophages, and splenic macrophages from TCDD-treated animals expressed elevated levels of inducible CYP1A1 compared to other macrophage subpopulations or to cells from control rats. In contrast, CYP1A1 induction was not detectable in resident peritoneal macrophages or peripheral blood monocytes. Examination of AhR levels in macrophage populations indicated that the ability of TCDD to induce metabolic enzymes in specific cell types correlated well with AhR expression. In a related study, Germolec et al. (1996) followed the induction of CYP1A1 and ALDH in various lymphoid tissues from F344 rats exposed to TCDD. They found that macrophages of the spleen and liver were the primary sites for generation of the metabolic enzymes. Other cells, such as thymocytes, showed enzyme induction only if previously stimulated by mitogens. These effects correlated with increased expression of the AhR and indicate that TCDD-induced increases in these enzymes are related to the level of expression of the AhR in different populations of immune cells.

Evidence for AhR-independent immunologic effects is supported by a recent study in which the distribution and behavior of the AhR in isolated spleen T lymphocytes and T-cell clones derived from Ah-responsive mouse strains were evaluated (Lawrence et al., 1996). Western immunoblot were used to determine the presence of the AhR in whole-cell extracts of resting and activated splenic lymphocytes and T-cell clones. Increased EROD activity was observed in T-cell clones and spleen cells, and the level of induction was about a hundredfold less than in Hepa cells. The AhR was detected in all cell types examined, but the it translocated to the nucleus only in activated, TCDD-treated T cells. Whereas AhR derived from TCDD-treated wild-type Hepa cells bound specifically to a dioxin response element, no binding was detected when an identical amount of AhR obtained from activated T cells was used. The inability to detect binding of the T-cell nuclear AhR complex to a consensus response element, combined with difficulties of reproducing in vivo immunotoxic effects of TCDD in vitro suggests that T cells may lack one or more factors required for AhR binding to a DRE or may contain a suppressor factor that inhibits AhR binding to DNA. Based on these data, it was suggested that TCDD affects T-cell function via an indirect mechanism.

Rhile et al. (1996) studied the role of Fas (CD95), an important molecule involved in the induction of apoptosis, and major histocompatibility complex (MHC) genes in TCDD-mediated immunotoxicity. When TCDD was orally administered to different strains of C57B1 mice at doses of 0.1, 1.0, or 5.0 μg/kg for 11 days, it was less toxic to thymocytes from C57BL/6 lpr/lpr mice (Ah responsive, Fas-) than to those from C57BL/6+/+ mice (Ah responsive, Fas+). Similar results were obtained when peripheral T-cell responsiveness to antigenic challenge with conalbumin was studied in these mice. When mice that differ only at the MHC were compared for immunotoxic effects of TCDD, it was noted that B10.D2 mice (Ah responsive H-2^d) were more sensitive to TCDD-mediated ef-

fects than B10.A mice (Ah responsive H-2^b). In all TCDD-sensitive strains tested, thymic atrophy was accompanied by a uniform depletion in all four subsets of T cells (CD4+, CD4+CD8+, CD4-CD8-, and CD8+) and the proportion of the subsets was not altered. In these strains, TCDD suppressed the antigen-specific peripheral T-cell responsiveness, but not the responsiveness of naive resting T cells to mitogens. It was also demonstrated that TCDD directly affected T cells responding to conalbumin, but not antigen-presenting cells. Thus, although the Ah locus has the primary role in determining the toxicity of TCDD to T cells, secondary factors such as the expression of Fas or the MHC phenotype might also play an important role in TCDD-mediated immunotoxicity.

The effects of TCDD on the expression of costimulatory molecules B7-1 and B7-2 in P815 allograft immunity were evaluated in C57BL/6 mice (Prell and Kerkvliet, 1997). Expression of B7-2, but not B7-1, was up-regulated in splenic B220+ and Mac-1+ cells in P815-challenged mice. Exposure to TCDD significantly decreased the expression of B7-2 on B220+ and Mac-1+ cells in P815-challenged mice. Providing exogenous B7-mediated costimulation, in the form of B7-transfected P815 tumor cells, induced CTL activity in TCDD-treated mice by a mechanism that was independent of CD4+ T cells. In contrast, B7-transfected P815 cells did not restore the cytotoxic alloantibody response in TCDD-treated mice. Based on these results, it was suggested that MHC class II B7-transfected P815 tumor cells can directly activate CD8+ CTL precursors, but cannot directly stimulate CD4+ T-helper cells required for B-cell activation. In addition, these results demonstrated that CTL precursors in TCDD-treated mice are functional and able to differentiate into effector CTL provided they receive adequate costimulation via B7. Thus, defective costimulation, through reduced B7-2 expression, may play a role in TCDD-induced immunotoxicity. In support of this hypothesis, evidence was presented that blocking B7-2/CD28 interactions, and to a lesser degree B7-1/CD28 interactions, suppressed the alloimmune responses to P815 tumor cells.

To determine the basis for TCDD-induced suppression of the humoral immune response, Karras et al. (1996) examined the effects of TCDD using in vitro models of T-independent (antibody directed against surface IgM) and T-dependent (activated T-helper cells bearing CD40 ligand) B-cell maturation. TCDD suppressed murine B-cell IgM secretion induced by anti-IgM, but did not affect IgM secretion stimulated by activated T cells through the CD40 pathway. Because mobilization of calcium has been shown to be an integral event in the stimulation of proliferation via the antigen receptor in B cells, the effect of TCDD exposure on B-cell intracellular calcium concentration and mobilization was examined. TCDD suppressed calcium mobilization in B cells, whereas stimulation by activated T cells was unaffected. The results support a role for the disruption of calcium homeostasis as another AhR-independent mechanism for TCDD toxicity.

Reproductive or Developmental Toxicity The effects of TCDD on reproductive development and fertility of the progeny have been investigated in a number

of studies. Results are presented below for male and female mammals (specifically rodents) and for other nonmammalian species (i.e., fish and birds).

Low doses of TCDD in pregnant rats alter the reproductive development and fertility of the progeny. In comparative reproductive studies, Gray et al. (1995) administered TCDD to LE hooded rats and pregnant Syrian hamsters, a species relatively insensitive to the lethal effects of TCDD. When the rats and hamsters were dosed on GDs 15 and 11, respectively, puberty was delayed by about three days, ejaculated sperm counts were reduced by at least 58 percent and epididymal sperm storage was reduced by 38 percent. Testicular sperm production was less affected. The accessory sex glands were also reduced in size in rat offspring treated on GD 15 despite the fact that serum testosterone (T), T production by the testis in vitro, and androgen receptor (AR) levels were not reduced. Some reproductive measures, such as anogenital distance and male sex behavior, were altered by TCDD treatment in rat but not hamster offspring. Since T and AR levels appeared normal in the accessory sex glands and the epididymis following perinatal TCDD exposure, alterations in these tissues are not likely to have resulted from an alteration of the androgenic status of the male offspring.

Roman et al. (1995) recently completed studies to determine whether in utero and lactational TCDD exposure decreases male rat accessory sex organ weights during postnatal development and whether this effect involves decreases in testicular androgen production or changes in peripheral androgen metabolism. Pregnant rats were administered a single dose of TCDD on GD 15, and offspring were exposed via placental and subsequent lactational transfer until weaning on postnatal day (PND) 21. No significant differences were observed between PNDs 21 and 63 in circulating androgen concentrations and intratesticular androgen content. In vitro human chorionic gonadotropin-stimulated testosterone production from TCDD-exposed animals did not differ from control, although 5-androstane-3,17α-diol production was decreased on PNDs 32 and 49 and increased on PND 63. Thus, in utero and lactational TCDD exposure can cause subtle decreases in testicular androgen production. These observed reductions, however, do not correlate temporally with one another or with decreases in androgen-dependent male accessory sex organ weights. Of the male accessory sex organs, the ventral prostate (VP) and dorsolateral prostate (DLP) were the most severely affected. Between PNDs 21 and 63, relative VP and DLP weights were decreased to 65–84 percent and 57–80 percent of control, respectively, and the magnitude of observed decreases was greatest at early times. In contrast, relative weights of the seminal vesicle and coagulating gland ranged from 80 to 104 percent of control, and the magnitude of observed decreases was greatest at later times. The sensitivity of the prostate to TCDD could not be explained by tissue-specific decreases in dihydrotestosterone concentrations. Although VP DHT concentration was decreased to 63 percent of control on PND 21, DHT concentration was not decreased in the VP between PNDs 32 and 63 or in the DLP at any time. These results suggest that in utero and lactational TCDD exposure selectively

impairs rat prostate growth and development without inhibiting testicular androgen production or consistently decreasing prostate DHT concentration.

Decreased daily sperm production (DSP) and cauda epididymal sperm number are some of the most sensitive effects of in utero and lactational TCDD exposure. To determine if TCDD exposure increases the rate of sperm transit through the excurrent duct system, thereby decreasing the number of sperm in the system at any given time, pregnant Holtzman rats were administered a single dose of TCDD (1.0 fg/kg, p.o.) on GD 15 and offspring were weaned on PND 21 (Sommer et al., 1996). On PND 50, testicular sperm were labeled with radiolabeled thymidine in five males per litter from control and TCDD-exposed litters. On PNDs 92–93, TCDD exposure significantly decreased DSP and testis, corpus and cauda epididymis, vas deferens, and ejaculated sperm numbers by 28, 30, 36, 39, and 46 percent, respectively. Decreases in sperm number in the distal excurrent duct system were greater than the decrease in DSP, consistent with the hypothesis that TCDD exposure has an effect other than decreased DSP that reduces epididymal and ejaculated sperm numbers. However, in utero and lactational TCDD exposure did not alter radiolabeled sperm transit time through the whole epididymis (15 days). With TCDD exposure causing no obvious alteration in sperm transit rate, a plausible explanation for sperm loss is an increase in sperm phagocytosis in the excurrent duct system.

The reproductive alterations in female progeny after gestational administration of TCDD were evaluated by Gray et al. (1995). In these experiments, LE hooded rats were given a single dose of 1 fg TCDD/kg by gavage on GD 8 (i.e., a period that includes major organogenesis) or GD 15 (i.e., a period prior to sex differentiation and a dosing regime that alters sex differentiation of the male LE rat). In a second experiment, Holtzman rats were dosed with TCDD at 1 fg/kg on GD 15 to determine if the progeny of this strain displayed malformations of the external genitalia and vaginal orifice as did LE rats. TCDD-treated female LE offspring displayed a number of unusual reproductive alterations. In the GD 15 group, puberty was delayed, more than 65 percent of the female offspring displayed complete to partial clefting of the phallus, and 80 percent displayed a permanent thread of tissue across the opening of the vagina. In the GD 8 treatment group, 25 percent displayed partially cleft phallus and 14 percent had a vaginal thread. GD 15 TCDD administration also induced a high incidence of malformations in Holtzman female progeny (100 percent clefting and 83 percent with a vaginal thread). At necropsy (>550 days old), ovarian weight was significantly reduced by 23 percent in both rat strains. In the LE rat, vaginal and behavioral estrous cyclicity, estrous cycle-mediated running wheel activity, and female sexual behavior at proestrus (darting and lordosis to mount ratios) were not affected by GD 15 TCDD treatment. However, untreated stud males had difficulty attaining intromission and took longer to ejaculate, and vaginal bleeding was displayed during mating by GD 15 TCDD-exposed female offspring. GD 8 TCDD-treated female offspring displayed enhanced incidences of constant

estrus (CE) (47 percent CE versus 16 percent CE in the control and GD 15 groups at middle age) and cystic endometrial hyperplasia. In addition, in the GD 8 group fertility rate declined significantly faster than in controls and fecundity was reduced by 38 percent. These data suggest that administration of a single dose of 1 fg TCDD/kg on GD 15 results in malformations of the external genitalia in female LE and Holtzman rats. Although treatment on GD 15 is generally more toxic to the offspring than treatment on GD 8 with respect to growth, viability, male reproductive effects, and malformations of the external genitalia in female progeny, treatment on GD 8 is more effective in inducing functional reproductive alterations in female progeny.

Twenty-one days prior to induction of surgery to produce endometriosis, female Sprague-Dawley rats and B6C3F1 mice were pretreated with at 0, 3, or 10 fg TCDD/kg. Animals were treated again at the time of surgery and at three, six, and nine weeks following surgery. TCDD produced a dose-dependent increase in endometriotic site diameter when all time points were pooled within each dose in rats and a dramatic increase in site diameter in mice at 9 and 12 weeks (Cummings et al., 1996). In rats but not mice, ovarian weight was decreased at 9 and 12 weeks. The occurrence of persistent vaginal estrus was increased at these times, and histological evaluation of the ovaries revealed ovulatory arrest at 12 weeks. In both species, thymic atrophy and hepatomegaly were also observed. Histological evaluations of endometriotic sites revealed fibrosis in control rats, necrotic and inflammatory changes in sites from TCDD-treated rats, and predominantly fibrotic changes in sites from TCDD-treated mice. Differences observed between rat and mouse with respect to the magnitude of changes in endometrial site diameter (rat < mice), ovarian function (rat > mice) and immune response suggest that the mechanisms mediating the promotion of endometriosis by TCDD are complex and may differ in rats and mice. Endometriosis in the rhesus monkey, which bears a close parallel to the human disease, is exacerbated by TCDD (Rier et al., 1993).

Treatment of pregnant female Sprague-Dawley rats on GD 15 with a single oral dose of TCDD (0.5, 1.0, or 2.0 μg /kg) or indole-3-carbinol (I3C; 1.0 or 100 μg/kg), an AhR agonist found in cruciferous vegetables, resulted in reproductive abnormalities in male offspring (Wilker et al., 1996). Anogenital distance and crown-to-rump length were altered by both compounds; however, the timing of the effects (day 1 or 5) was variable and the responses were not necessarily dose dependent. In 62-day-old offspring, seminal vesicle, prostate, testicular parenchymal, and epididymal weight were decreased by one or more doses of TCDD. The total number of sperm in the epididymis was significantly decreased in rats perinatally exposed to TCDD due to a decreased number of sperm in the tail of the epididymis. Perinatal exposure to I3C did not affect any of these parameters. TCDD did not affect the transit time of sperm through the complete epididymis at any doses (0.5–2.0 fg/kg). However, at the two highest doses (1.0 and 2.0 fg/kg), TCDD increased the transit rate of sperm through the tail of the epididymis. In

contrast, primarily due to the decreased transit rate of sperm through the head plus body of the epididymis, I3C (1 mg/kg) significantly increased total epididymal transit time by 31 percent. The authors concluded that perinatal exposure of pregnant rats to I3C causes reproductive abnormalities in male offspring but that, relative to TCDD, both common and different responses are present.

After water-borne exposure of newly fertilized eggs to TCDD (35–2,100 ng per liter), Henry et al. (1997b) characterized the toxicity and histopathology of TCDD in zebrafish during early life stages from 12 to 240 hours postfertilization (hpf). TCDD did not increase egg mortality (0–48 hpf), nor did it affect time to hatching (48–96 hpf). Eggs exposed to 1.5 ng or more of radiolabeled TCDD per gram of egg elicited toxic responses in zebrafish larvae. Pericardial edema and craniofacial malformations were first observed at 72 hpf, followed by the onset of yolk sac edema (96 hpf) and mortality (132 hpf). The LD_{50}, determined at 240 hpf, was 2.5 ng TCDD/g egg. Severe hemodynamic changes, observed as slowed blood flow in vascular beds of the trunk, head, and gills and decreased heart rate, occurred in TCDD-treated zebrafish prior to or coincident with the onset of gross signs of toxicity. Histological examination of TCDD-treated zebrafish revealed a variety of epithelial tissue lesions including arrested gill development and ballooning degeneration and/or necrosis of the renal tubules, hepatocytes, pancreas, and all major brain regions. Mesenchymal tissue lesions included subcutaneous edema in the head, trunk, and yolk sac; edema of the pericardium and skeletal muscle; and underdevelopment of the swim bladder.

Using a TCDD photoaffinity analogue, Brown et al. (1995) detected the presence of two proteins (28 and 39 kDa) in the cytosol of the hard-shell clam, *Mercenaria Mercenaria* that bind to this chemical. Expression of these proteins is tissue dependent, with the highest concentrations observed in gill and gonadal tissue. Gonadal tissue also exhibited gender-specific expression, with female clams exhibiting higher levels of the 39 kDa protein. The varying concentrations in different tissues suggest that these proteins are not proteolytic fragments of a larger precursor, although they could be homologous to the AhR.

Janz and Bellward (1996a) evaluated the effects of in ovo TCDD exposure on perinatal plasma thyroid hormone concentrations (total T3, total T4) and body and skeletal growth in the domestic chicken, domestic pigeon, and great blue heron. EROD activity in the liver was employed as an enzymatic marker of CYP1A1 induction by TCDD. Although the EROD activity was induced 13 to 43 times above control values in chickens treated with TCDD, there was no effect on hatchability, body growth, subcutaneous edema, or plasma thyroid hormone levels. In pigeons exposed to TCDD, EROD was induced significantly, hatchability was decreased, liver-to-body weight ratio was elevated, and body and skeletal growth decreased ($p < .01$); however, there was no effect on plasma thyroid hormone levels. In heron, EROD activity was induced two- to threefold above control birds; however, no effect was observed on plasma thyroid hormone levels or body growth. Thus, in ovo TCDD exposure adversely affected the body and

skeletal growth, and hatchability of the domestic pigeon, but had no effect on the domestic chicken or great blue heron. Collectively, these results suggest that perinatal plasma thyroid hormone levels cannot be used as a relatively noninvasive biomarker of TCDD exposure during embryonic development in these species.

In another set of experiments, Janz and Bellward (1996b) reported no effect of in ovo TCDD exposure on liver ER levels or plasma estradiol concentrations in female chickens and pigeons exposed early in incubation. In female pigeons exposed during the latter third part of incubation to a TCDD dose that would cause high embryo lethality if injected early in incubation, hepatic ER concentrations were elevated ($p < .001$) and plasma estradiol concentrations were decreased ($p < .01$) at hatch. There was no effect of TCDD exposure on plasma estradiol levels in male pigeons. In herons, TCDD exposure had no effect on hepatic ER levels or plasma estradiol and testosterone concentrations at either time. Based on these results it was concluded that in chicken, pigeon, and great blue heron hatchlings exposed early in incubation to low doses of TCDD, hepatic ER levels and plasma estradiol concentrations are not altered.

Several studies have been published during the reference period on the developmental effects of TCDD in mice, rats, chicks, and medaka. These are discussed below. Effects discussed include cleft palate, hydronephrosis, cardiotoxicity, and angiogenesis. Advances in the understanding of the mechanisms underlying these effects are also discussed.

Developing mice seem to be sensitive to TCDD, which acts to alter the proliferation and differentiation of epithelial, as well as mesenchymal cells. A mouse line deficient in TGF-β_3 exhibits cleft palate remarkably similar to that seen with TCDD, suggesting that the AhR may be involved, directly or indirectly, in the regulation of TGF-β_3 in developing palate. TCDD exposure would increase the formation AhR–Arnt dimers, decreasing the amount of Arnt available for other interactions and resulting in decreased TGF-β_3 expression. Decreased concentrations of free Arnt owing to recruitment by liganded AhR may shift the balance of this general dimeric partner away from HIF-1α or other partners.

Structural defects following dioxin exposure have been reported in the mouse at doses that do not cause either maternal or fetal toxicity, the best described of which is cleft palate. There is a critical window for the induction of this defect, with peak incidence following exposure on day 11 or 12 of gestation (Couture et al., 1990a). In contrast, the induction of hydronephrosis does not appear to have a peak window of sensitivity during organogenesis and can even be induced lactationally (Couture et al., 1990b). Interestingly, hydronephrosis is a more sensitive response to TCDD than cleft palate.

When EGF, TGF-α, EGFR, and the TGF-βs are considered as a combinatorial, interacting set of regulators, TCDD and the synthetic glococorticoid hydrocortisone (HC) each produce a unique pattern of increased and/or decreased expression of these genes (Abbott, 1995). HC in combination with TCDD pro-

duced increased expression of both receptors; this pattern would produce HC-like clefts since the GR-mediated responses would result in small palatal shelves. The observed cross-regulation of the receptors is believed to be important in the synergistic interaction between TCDD and HC for the induction of cleft palate.

Peters and Wiley (1995a) investigated the developmental expression of Arnt mRNA with the goal of identifying the mechanisms by which AhR functions during preimplantation embryo development. Blastocyst-stage preimplantation mouse embryos were collected after 72 hours of in vitro culture. Hepa 1c1c7 cells served as a positive control. Arnt was detected in blastocyst-stage embryos as well as in positive controls. Southern analysis with a human Arnt cDNA probe confirmed that the detected reverse transcription (RT) polymerase chain reaction (PCR) product in blastocysts was similar in sequence to human Arnt mRNA. These data suggest that the AhR–Arnt pathway may function during embryonic development. When mice are treated with TCDD and RA simultaneously, palatal clefts can be observed in 100 percent of offspring of mothers at dose levels far lower than those required for either agent to produce clefting if given alone (Weston et al., 1995). This synergy suggests that the pathways controlled by these agents converge at one or more points in cells of the developing palate. The effects of TCDD on induction of the type II cellular RA binding protein and the RA receptor β by RA in murine embryonic palate mesenchymal cells were also examined. Although TCDD alone had no effect on basal levels of expression of either gene, the induction of both genes by RA was strongly inhibited by TCDD. These results represent the first evidence for a direct molecular interaction between the RA and TCDD-mediated signaling pathways.

Recent reports have investigated TCDD-induced cardiotoxicity. Walker et al. (1997) injected chicken eggs with TCDD (1.0 pmol/g) prior to incubation and collected them after cardiac development was complete. Relative to controls, TCDD increased heart wet weight (27.2 ± 0.5 mg versus 36.6 ± 1.3 mg, $p < .001$) and dry weight (2.7 ± 0.1 mg versus 3.1 ± 0.1 mg, $p < .01$) and tended to increase heart myosin content (3.5 ± 0.6 μg versus 6.3 ± 2.5 μg, $p < .07$), suggesting an increase in cardiac muscle mass and edema. Histologic and morphometric analyses revealed that TCDD-exposed hearts exhibited enlarged right and left ventricles, thickened ventricular septum, and a thinner left ventricular wall with increased trabeculation, and some exhibited ventricular septal defects compared to controls. The AhR was expressed ubiquitously in cardiac myocytes, whereas Arnt expression was restricted to myocytes overlying developing septa: the atrioventricular canal, outflow tract, and atrial and ventricular septa. Both proteins were absent from endocardium and endocardial-derived mesenchyme. In addition, cardiac expression of an AhR–Arnt target CYP1A1 was restricted to myocardium coexpressing AhR and Arnt. Thus, the spatial and temporal expression of AhR and Arnt suggests that the developing myocardium and cardiac septa are potential targets of TCDD-induced teratogenicity, and such targets are also consistent with cardiac hypertrophy and septal defects observed after TCDD exposure.

Hassoun et al. (1995) exposed pregnant mice to TCDD (30 μg/kg) on the twelfth day of gestation and observed 1.8- and 2.3-fold increases in DNA single-strand breaks in fetal and placental nuclei, respectively. They also observed increases in lipid peroxidation in placental and fetal tissues. TCDD administration produced increases in amniotic fluid levels of the lipid metabolites malondialdehyde, formaldehyde, acetaldehyde, and acetone. Altogether, reactive oxygen species may participate in the teratogenic effects of TCDD.

TCDD produces dose-dependent decreases in fetal weight, fetal thymic weight, and placental weight, and dose-dependent increases in fetolethality, cleft palate formation, and hydronephrosis at doses of 10–30 and 30–60 μg/kg body weight in C57BL/6J and DBA/2J mice, respectively (Hassoun and Stohs, 1996). Based on these response patterns it has been suggested that TCDD-induced cleft plate and hydronephrosis involve mechanisms that are AhR mediated. However, the fetotoxic effects appear to involve mechanisms not related to the AhR since endrin and lindane exhibited comparable responses. Finally, it has been shown that high levels of PCBs, PCDDs, and PCDFs in breast milk were related to reduced neonatal neurological optimality. These results are consistent with the suggestion of the neurotoxic effects of these compounds on the developing brain of newborn infants (Huisman et al., 1995a).

Chaffin et al. (1996) investigated the effects of TCDD exposure during fetal and perinatal development on the estrogen-signaling system in peripubertal female rats. Pregnant rats were given 1 μg/kg TCDD on GD 15. Body weights were reduced, although not significantly, on postnatal day 21. Estrogen receptor mRNA increased in the hypothalamus, uterus, and ovary and decreased in the pituitary. The results of DNA binding assays paralleled the mRNA profile of the uterus, whereas DNA binding activity was decreased in the hypothalamus and unchanged in ovarian protein extracts. Circulating concentrations of estrogen were significantly lower in TCDD-exposed rats, suggesting that the decrease in serum estrogen may be a cause of the alterations in ER mRNA. However, changes in ER DNA binding activity are suggestive of alterations in translation or posttranslational events. The mechanism of the reduction in female fertility that accompanies in utero and lactational exposure to TCDD remains unknown, although it could be linked to the estrogenic effects observed by Gray and Ostby (1995), such as clefting of the phallus and hypospadias.

In 1988, Henshel et al. (1995) started to cull wild heron eggs from contaminated areas of British Columbia and hatch them in the laboratory. Hatchling brains exhibited a high frequency of intercerebral asymmetry, which decreased in subsequent years as TCDD levels decreased. This frequency correlated with the level of TCDD and TCDD toxicity equivalence factors (TEQs) in eggs taken from the same nest. The yolk-free body weight correlated negatively and the brain somatic index correlated positively with TCDD levels in such pair-matched eggs. These results indicate that gross brain morphology, and specifically interce-

rebral asymmetry, may be useful as a biomarker for the developmental neurotoxic effects of PCDDs and related chemicals.

Carcinogenicity During the reference period, studies were conducted to examine the role of the AhR in TCDD enzyme induction and tumor promotion. The mechanism by which TCDD induces tumor promotion was also under investigation.

Wang et al. (1995) determined the Ah responsiveness of numerous human cancer cell lines (T-47D, Hep G2, LS180, MCF-7, A431, C-4II, and MDA-MB-231) on induction of CYP1A1 mRNA levels and EROD activity. With the exception of the MDA-MB-231 breast cancer cell line, TCDD significantly induced CYP1A1 mRNA levels and EROD activity in the remaining six cell lines. However, EC_{50} values for EROD induction in all cell lines were not consistent for the nuclear AhR complex. The nuclear AhR complex varied from 175 kDa (for the MDA-MB-231 cells) to 221 kDa. Altogether, the molecular properties and levels of the nuclear AhR complex from seven different human cancer cell lines do not predict Ah responsiveness.

Males of the C57BL/6, DBA/2, or F1 strain were initiated with a single i.p. dose of N-nitrosodiethylamine (14, 21, and 21 percent respectively) (Beebe et al., 1995). Although TCDD did not induce CYP1A or promote liver tumors in DBA/2 mice, in all other strains results indicate that a functional Ah receptor is required for liver tumor promotion. However, CYP1A1 induction was not directly related to the degree of tumor-promoting capability, suggesting that other genetic factors must play a role in mediating the final tumor outcome.

Abel et al. (1996) studied the dose–response relationship of cytochrome P4501B1 (CYP1B1) and CYP1A1 induction in livers of two strains of TCDD-treated female mice (C57BL/6J and DBA/2J). For both strains, CYP1B1 and CYP1A1 mRNA content increased after TCDD exposure (24 hours) in a dose-dependent manner (0.001–50 μg/kg). These effects were more pronounced in TCDD-responsive C57BL/6J mice than in the less responsive DBA/2J mice. CYP1A1 was more responsive to TCDD than CYP1B1 in both strains, suggesting that CYP1B1 mRNA expression is less inducible by TCDD than CYP1A1 but that both genes are highly AhR regulated.

Huang et al. (1995) conducted studies to compare AhR in cultured fetal cells and adult livers from TCDD-responsive (C57BL/6J) and nonresponsive (DBA/2J) mice. In each strain, the molecular mass of the AhR from fetal cells is identical to that from adult liver. The AhR in DBA/2J fetal cells was able to activate a transfected chloramphenicol acetyltransferase linked to a dioxin-responsive element nucleotide sequence. These data suggest that the responsiveness of fetal cells from "nonresponsive" mice is likely mediated by the AhR but is not due to expression of a different allelic form of AhR ligand binding subunit in fetal cells versus adult liver.

Hakkola and colleagues (1997) studied the expression of the AhR-regulated CYP1B1 gene in human adult and fetal tissues and cell cultures. In adults, CYP1B1 mRNA was detected in lymphocytes and cells of bronchoalveolar lavage, uterine endometrium, and liver, but not lung. The level of expression was very low in adult liver, and only three of six fetal livers expressed CYP1B1. Fetal tissue other than the liver, especially brain and kidney, expressed high levels of CYP1B1. CYP1B1 mRNA was detected at a low level in first-trimester and full-term placental samples. CYP1B1 mRNA was not induced in placenta by maternal cigarette smoking.

The group also studied the inducibility of CYP1B1 by TCDD in primary fibroblasts and a carcinoma cell line (JEG-3) having different CYP1A1 induction properties. The inducibility of CYP1B1 was found to be regulated independently of CYP1A1. In carcinoma cells, CYP1A1 mRNA was induced up to 9,000-fold, while the expression of CYP1B1 was not affected. Expression of the AhR and Arnt was determined in human placenta and in the carcinoma cell line. Expression of these transcription factors was found to be neither coregulated nor affected by AhR ligands. This study provides evidence that in addition to the AhR complex, other cell-specific factors modulate the response of CYP1B1 and CYP1A1 to AhR ligands. The level of complexity of CYP gene induction continues to increase.

The exposure of two hepatoma cell lines Hep G2 and Hepa-1 to moderate hydrodynamic shear, in microcarrier-attached suspension cultures, resulted in the transient induction of CYP1A1 (Mufti et al., 1995). Both cell lines have been characterized with respect to their AhR concentrations and induce CYP1A1 in response to exposure to xenobiotics such as TCDD. Using an AhR antagonist, α-naphthoflavone, and a protein kinase C inhibitor, staurosporine (ST), in the Hep G2 cell line, the induced CYP1A1 activity was modulated in the same manner as when cells were coexposed to TCDD and either α-NF or ST. Exposure of the Hep G2 cell line to TCDD and shear resulted in enhancement of both the induced CYP1A1 activity and a competitive response. Finally, using wild-type and AhR-defective Hepa-1 cell lines, it was demonstrated that a functional AhR was required for shear-induced CYP1A1 expression. The data obtained in three cell lines indicate a role for the AhR in the induction of CYP1A1 by shear in agitated microcarrier cultures.

Gilday et al. (1996) reported the cloning and sequencing of cDNAs for two catalytically distinct TCDD-induced CYP enzymes in chick embryo liver. One mediates classic CYP1A1 activities, whereas the other has some CYP1A2-like activity and is also responsible for TCDD-induced arachidonic acid epoxygenation. Amino acid sequence analysis shows that although each chick enzyme can be classified in the CYP1A family, both are more like CYP1A1 than CYP1A2, and neither can be said to be directly orthologous to CYP1A1 or CYP1A2. Phylogenetic analysis shows that the two chick enzymes form a separate branch in the CYP1A family tree distinct from mammalian CYP1A1 and CYP1A2 and

from fish CYP1A enzymes. The findings suggest that CYP1A progenitors split independently in evolutionary lines into two CYP enzymes with some parallel functions, which offers evidence for convergent evolution in the CYP1A family. Northern analysis shows that the chick enzymes have a different tissue distribution of CYP1A1 and CYP1A2. PRC and in situ hybridization data show that both chick enzymes are expressed in response to TCDD even before organ morphogenesis. The findings were interpreted to suggest that beyond their role in activating carcinogens, CYP1A enzymes have conferred evolutionary and developmental advantage, perhaps as defenses in maintaining homeostatic responses to toxic chemicals.

According to Christou et al. (1995), rat mammary cells express both CYP1A1 and CYP1B1 in response to PAHs and TCDD exposure, depending on cell type. CYP1B1 protein was scarcely detected in rat mammary cell but was surprisingly active as a participant in 7,12-dimethylbenz[*a*]anthracene (DMBA) metabolism. CYP1B1 was selectively expressed in the stromal fibroblast population of rat mammary cells to the exclusion of CYP1A1. In rat mammary fibroblasts, CYP1B1 protein and associated activity were each present at low levels and were highly induced by benz[*a*]anthracene (BA) to a greater extent than by TCDD (twelve- versus sixfold). However, BA (10 μM) and TCDD (10 nM) stimulated the 5.2-kilobase CYP1B1-specific mRNA equally. These increases are consistent with the involvement of the AhR in the transcription of the CYP1B1 gene and with the additional stabilization of CYP1B1 protein by BA, as previously observed in embryo fibroblasts. The constitutive expression and PAH inducibility of CYP1B1 and CYP1A1 proteins in rat mammary fibroblasts and epithelial cells, respectively, were each decreased approximately 75 percent by a hormonal mixture of 17β-estradiol (0.2 μM), progesterone (1.5 μM), cortisol (1.5 μM) and prolactin (5 μg/ml). Progesterone and cortisol, added singly to fibroblasts suppressed CYP1B1 protein expression in both untreated and BA-induced cells, whereas cortisol also suppressed CYP1B1 mRNA. In contrast, 17β-estradiol stimulated constitutive expression of CYP1B1 protein (50–75 percent) and mRNA level (two- and threefold) but did not affect CYP1B1 expression in BA-treated fibroblasts. The expression of CYP1A1 and CYP1B1 is therefore highly cell specific even though each is regulated through the AhR. Each cytochrome P450 exhibits a surprisingly similar pattern of hormonal regulation even though expressed in different cell types.

In MCF-7 human breast cancer cells, E_2 induction of cat D gene expression is associated with formation of an ER–Sp1 complex within the promoter region (–199/–165) of this gene. E_2-induced cat D gene expression is inhibited by TCDD within 30 minutes in MCF-7 cells. Moreover, using a series of synthetic oligonucleotides, which include the wild-type ER–Sp1 and various mutants, it was shown the nuclear AhR complex binds to an imperfect DRE located between the ER and Sp1 binding sequences. This interaction results in disruption of the ER–Sp1 complex and inhibition of E_2-induced gene expression. These results are

among the first to illustrate that the nuclear AhR complex also exhibits activity as a negative transcription factor via a mechanism similar to that reported for AhR-mediated induction of gene expression.

Wolfle and Marquardt (1996) studied the tumor-promoting activity of TCDD on mouse fibroblasts transformed by certain known carcinogenic chemicals. The promoting effect of TCDD was maximal at a very low concentration (1.5 pM) and comparable to another well-studied tumor promoter TPA (0.25 μg/ml). Chemicals containing reactive oxygen (e.g., scavengers of hydroxyl radicals or antioxidants) hindered the tumor-promoting effect of both TCDD and TPA, suggesting that the promotional effects may involve oxygen radicals.

Induction of CYP1A1 in the hepatoma Hepa 1c1c7 cell line results in elevation of the excretion rate of 8-oxoguanine (oxo8Gua), a biomarker of oxidative DNA damage, and the major repair product of DNA residues 8-oxo-2′-deoxyguanosine (oxo8dG) (Park et al., 1996). Treatment of this cell line with TCDD and ICZ, a metabolite of a natural pesticide found in cruciferous vegetables, induces CYP1A1 activity and elevates excretion rate of oxo8Gua or α-naphthoflavone. An inhibitor of CYP1A1 activity and an antagonist of the AhR reduced the excretion rate of oxo8Gua. The essential role of AhR in this response is shown by the inability of TCDD to induce CYP1A1 and to increase excretion of oxo8Gua in AhR-defective c4 mutant cells. Although there was a significant sevenfold increase over two days in the excretion rate of oxo8Gua into the growth medium of TCDD-treated Hepa 1c1c7 cells compared to controls, no significant increase was detected in the steady-state level of oxo8dG in the DNA presumably due to efficient DNA repair. Thus, the induction of CYP1A1 appears to result in a leak of oxygen radicals and consequent oxidative DNA damage that could lead to mutation and cancer.

Studies by Baker et al. (1995) examined the effect of TCDD in primary cultures of rat hepatocytes. At noncytolethal doses, TCDD inhibited gap junctional intercellular communication (GJIC) in a time- and concentration (10^{-8}–10^{-14} M) dependent manner. This inhibition occurred within 4 hours of treatment at doses of 10^{-8}–10^{-12} M TCDD and persisted up to 48 hours, despite removal of TCDD. Treatment of rat hepatocytes with TCDD resulted in a decrease in hepatocyte connexin 32 mRNA but had no apparent effect on connexin 26 mRNA. Coincubation of rat hepatocytes with TCDD and α-NF abolished down-regulation of GJIC by TCDD. Similarly, co-treatment with a cAMP analogue (8-bromoadenosine 3′,5′-cyclic monophosphate) prevented down-regulation of GJIC by TCDD. Results of this investigation suggest that TCDD inhibits GJIC through the AhR. In addition, this study showed that the inhibition of GJIC by TCDD may be due to transcriptional down-regulation or stability of the connexin 32 gap junction mRNA. In other studies, Warngard et al. (1996) investigated the function, expression, and phosphorylation of different connexins in vitro and in vivo. A good correlation between the ability of TCDD to act as a tumor promoter and to interfere with gap junctional intercellular communication was also reported.

P450RAP protein, a novel adrenocorticotropic hormone-inducible cytochrome P450, is encoded by a rat CYP1B1 gene orthologous to the mouse CYP1B1 gene (Bhattacharyya et al., 1995). Alignment of rat CYP1B1 amino acid sequences with rat CYP1A1 (39 percent identical) indicated eight regions of high identity for each (60–78 percent), interspersed with extensive regions of less than 30 percent similarity. CYP1B1 mRNA was elevated by two-day adrenocorticotropic hormone treatment but much less than CYP11A1 (cytochrome P450 side chain cleavage) mRNA (twofold versus fourfold). Lower levels of the 5.2-kilobase mRNA in other steroidogenic cells (ovary) were consistent with the amount of immunodetectable CYP1B1 protein, and unlike the adrenal, expression in the ovary was stimulated fivefold by β-naphthoflavone, an AhR agonist, in parallel with CYP1A1 induction. In several other tissues (liver > lung > uterus >> kidney), CYP1B1 mRNA and protein were constitutively undetectable but highly induced by β-naphthoflavone, although at much lower levels than CYP1A1. Thus, rat CYP1B1 exhibits regulation through hormonal signaling and the AhR in a cell-specific manner.

In a recent study, Jorgensen and Autrup (1996) used HepG2 and MCF-7 cell lines to examine a possible cell-specific autoregulation of CYP1A1 promotor function. In HepG2 cells coexpression of increasing amounts of CYP1A1 cDNA significantly down-regulated constitutive as well as TCDD-induced CYP1A1 promoter-driven chloramphenicol acetyltransferase (CAT) activity. In contrast, cotransfection of MCF-7 cells with a threefold molar excess of CYP1A1 cDNA relative to the CYP1A1-CAT reporter construct caused a similar twofold increase in TCDD-induced CAT activity, whereas no effect was observed on constitutive promoter activity. This autoregulatory mechanism of the human CYP1A1 gene product was independent of specific 5′-flanking promoter segments tested. RT–PCR analyses did not indicate any changes in mRNA level of AhR and Arnt in the cotransfection studies. Thus, these studies show that the human CYP1A1 gene is exposed to cell-specific autoregulation, probably achieved via different functions of trans-acting factors.

Weiss et al. (1996) described the results of studies in which transient and stable AhR expression analysis in AhR-deficient subclones was carried out. Transiently expressed AhR has a high basal activity on promoters containing AhR binding sites when transfected into receptor-deficient variant cells compared to wild-type cells. Single- and double-hybrid analysis dissociates AhR ligand responsiveness, transactivation, and heterodimerization with Arnt from receptor binding to a xenobiotic response element (XRE). Hybrid receptors also show high basal activity in the absence of exogenous TCDD in AhR-deficient variant cells, indicating that the endogenous AhR activating signal acts directly on the receptor rather than on the XRE-dependent promoters or DNA binding of the receptor. Stable expression of AhR in variant cell clones by retroviral infection fully reconstitutes TCDD responsiveness, including target gene induction and delay of cell cycle progression. These AhR-reconstituted cells, like AhR-

containing wild-type cells, show low basal activity of the transiently expressed AhR hybrid. Thus, the increased basal activity in AhR-deficient cells suggests a negative feedback control of AhR activity. In vitro ligand-binding assays are compatible with the idea that the increased basal activity is due to the accumulation of an AhR binding endogenous ligand.

Gradin and colleagues (1995) presented evidence that induction of CYP1A1 and CYP1B1 gene expression by an AhR ligand is repressed by camptothecin, an inhibitor of topoisomerase I. A transiently transfected reporter construct under control of an XRE containing promoter was not affected by the topoisomerase inhibitor and ligand-dependent activation of the AhR to its DNA binding form is not altered by camptothecin. These results imply that topoisomerase I activity is necessary for the primary CYP1A1 induction response, possibly involving dioxin-dependent alterations in the chromatin structure of the CYP1A1 promoter. The inhibitory effect of camptothecin cannot be exerted once the CYP1A1 gene has been activated.

Walsh et al. (1996) observed little or no TCDD-inducible CYP1A1 mRNA or enzyme activity in high-passage cultures of rat skin cells compared to low-passage cultures. Similarly, transfection of a luciferase reporter construct containing –1,317 to +256 base pairs of the 5′-flanking region of the murine CYP1A1 gene was TCDD-inducible in low- but not high-passage cells. Ligand binding and transfection experiments demonstrated the presence of functional AhR complexes in both high- and low-passage cells. Deletion analysis identified a 26-base pair negative regulatory DNA element contained within the upstream regulatory region of the CYP1A1 gene responsible for this effect. Nuclear extracts from both low- and high-passage cells contain a protein that specifically binds to NeRD-containing DNA. Thus, the loss of PAH sensitivity in high-passage rat epidermal cells appears to be due to decreased expression of CYP1A1, and this effect may be mediated by one or more altered NeRD binding factors present in these cells.

Results from a study by Sadar et al. (1996b) suggest that phenobarbital (PB) induction of CYP1A1 in rainbow trout hepatocytes is regulated by cAMP-dependent pathways (PKA), whereas TCDD induction is not dependent on PKA. This conclusion is consistent with the finding that epinephrine, which increases cAMP levels and activates PKA-dependent pathways, was a potent inhibitor of PB induction but had no effect on TCDD induction of CYP1A1 gene expression. Inhibitors of calcium phospholipid-dependent PKC had modest or no effect on PB and TCDD induction of CYP1A1, respectively.

Estimating Potential Health Risk and Factors Influencing Toxicity

Several approaches have been used to estimate the potential health risks associated with TCDD exposures. These include the use of TEFs, quantitative structure–activity relationships, H4IIE-luc cells, toxicity equivalent concentrations (TECs), and body burdens.

TEF Approach Toxic equivalency factors (TEF) have been used to estimate the potential health risks associated with exposure to TCDD and related chemicals. This approach is described in *Update 1996*. As discussed there, the TEF approach has been criticized because the relative potency of hydrocarbons may be tissue specific and influenced by interactions occurring among the chemicals present in environmental mixtures. For instance, the total toxicity of a mixture of halogenated aromatic hydrocarbons is not necessarily the sum of the toxicities of individual congeners because individual congeners can compete for the same receptor; therefore, nonadditive behavior may occur. Furthermore, TEFs have not been tested for all effects of dioxin and dioxin-like chemicals, nor have all responses for all chemicals of concern been examined.

The validity of the TEF approach in predicting the toxicity of mixtures was investigated by Pohl and Holler (1995). Minimal risk levels (MRLs) were derived based on the data bases available for chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans. The MRL values were then converted to TCDD toxicity equivalents (TEQs). There was good correlation between intermediate-duration oral MRLs for TCDD and 2,3,4,7,8-pentachlorodibenzo-*p*-dioxin when expressed in TEQs (7 and 15 pg/kg per day). Although the studies from which these MRLs were derived used different species (guinea pigs and rats, respectively), the toxicity end points (immunological and hepatic for TCDD and hepatic for 2,3,4,7,8-pentachlorodibenzo-*p*-dioxin) were comparable. Hepatic effects were measured by the same techniques (blood chemistry and histopathology), ensuring similar sensitivity. However, there was a discrepancy between the acute oral MRLs for TCDD and 2,3,4,7,8-pentachlorodibenzo-*p*-dioxin when they were expressed in TEQs (20 and 500 pg/kg per day, respectively). In this case, not only did the studies used for MRL derivation involve different species (mice and guinea pigs, respectively), but the immunotoxicity end points were measured by techniques with different sensitivity (serum complement activity versus histopathology), making comparisons difficult. The correlations presented support the concept that TEFs are valid only if specific criteria for their derivation are met (e.g., a broad data base of information, consistency across end points, additivity of effects, common mechanism of action).

QSAR Models Quantitative structure–activity relationship (QSAR) models have been used to estimate binding affinity of multiple chemical classes. The predictive nature of these approaches has been largely unsuccessful due to a focus on minimum energy conformations to predict the activity of molecules. Using structural conformations other than those of minimum energy, Mekenyan et al. (1996) developed a model for AhR binding affinity based on halogenated aromatic chemicals known to interact with the receptor. Resultant QSAR models showed good utility across multiple classes of halogenated aromatic compounds.

H4IIE-luc Cells Using the wild-type cell line H4IIE, Sanderson et al. (1996) developed a method for rapid screening of environmental samples containing Ah-active polyhalogenated aromatic hydrocarbons (PHAHs). The model consists of a recombinant H4IIE rat hepatoma cell line containing a luciferase reporter gene under the control of dioxin-responsive enhancers (H4IIE-luc cell system). The H4IIE-luc cell system was compared to an H4IIE-wt system that expresses AhR-mediated CYP1A induction. Both cell lines exhibited dose-dependent increases in AhR-mediated response on exposure to known agonists. H4IIE-luc cells were three times as sensitive as H4IIE-wt cells to TCDD. PHAHs tested exhibited similar structure–activity relationships in H4IIE-luc as in H4IIE-wt cells. Binary mixtures of TCDD, PCB-126, and PCB-77 showed no departure from additivity in their combined responses when tested. These findings support the use of H4IIE-luc cells as an alternative bioanalytical tool to H4IIE-wt cells for the detection of Ah agonists in environmental samples.

TEC Approach DeVito et al. (1995) investigated the validity of the TEC approach to predicting the toxicity of mixtures. This approach assumes that hydrocarbons act additively and via a common mechanism to cause toxicity. In their studies, eleven TCDD-like congeners and three non-TCDD-like congeners were combined at ratios found in Lake Michigan trout. Signs of toxicity after exposure of newly fertilized eggs to the mixture or to TCDD were indistinguishable. However, dose–response curves for the mixtures were shifted to the right of TCDD dose–response curves. These data suggest that TCDD-like congeners act via a common mechanism to cause toxicity during early trout development, but may not act strictly additively when combined in a mixture of TCDD- and non-TCDD-like congeners at ratios found in Great Lakes fish. Additive effects predicted by these data deviate less than tenfold from the current safety factor approach used in risk assessments, which suggests that this model is a reasonable approach for assessing the risk posed by complex mixtures of PCDDs, PCDFs, and PCBs. In other studies by Birnbaum and DeVito (1995), mice were dosed to reach steady-state conditions, thus precluding bias of TEF values due to pharmacokinetic factors. Slight differences were found in relative potency of some of the congeners when EROD was compared among tissues (liver, lung, and skin), but these differences were less than fivefold. In addition, there appeared to be slight differences for certain congeners with respect to the relative induction potency of CYP1A1 and CYP1A2.

Body Burdens Walker et al. (1996) have suggested that the body burdens of dioxins that produce effects in experimental animals are comparable to body burdens associated with similar effects in humans. In their studies, human body burdens were estimated from lipid-adjusted serum concentrations of dioxins. In the general population, average background concentrations were estimated at 58 ng TEQ/kg serum lipid, corresponding to a body burden of 13 ng TEQ/kg

body weight. Populations with known exposure to dioxins have body burdens of 96–7,000 ng TEQ/kg body weight. Results of this investigation indicate that chloracne and induction of CYP1A1, effects clearly associated with dioxin, occur at similar body burdens in humans and animals. Induction of cancer in animals occurs at body burdens of 944–137,000 ng TCDD/kg body weight, whereas noncancer effects in animals occur at body burdens of 10–12,500 ng/kg. Based on these correlations, investigators concluded that dioxin exposures may result in cancer and noncancer effects at body burdens within one to two orders of magnitude of those in the general population.

Interspecies and Interindividual Differences in Sensitivity Mass mortalities among marine mammal populations in recent years have raised questions about a possible contributory role of contaminants accumulated through the marine food chain. Ross et al. (1996) carried out a 30-month immunotoxicological experiment in which two groups, each containing 11 harbor seals, were fed TCDD-contaminated herring from the Baltic Sea or relatively uncontaminated herring from the Atlantic Ocean. Seals fed Baltic Sea herring accumulated three to four times higher levels of AhR-mediated TCDDs (measured in TEFs) in blubber than their Atlantic counterparts after two years on their respective diets. Blood was sampled 17 times during the course of the experiment for immunological evaluation, during which time the natural cytotoxic activity of peripheral blood mononuclear cells isolated from seals fed Baltic Sea herring declined to a level approximately 25 percent lower than that observed in seals fed Atlantic herring. Since these cells play an important role in the first line of defense against viruses, their observed inactivity in seals feeding on Baltic Sea herring suggests that exposure to contaminants may have an adverse effect on the defense against viral infections in seals inhabiting polluted waters in Europe.

Ema et al. (1994) observed an insertion mutation that results in a stop codon mutation and a truncated AhR. This receptor polymorphism may result in interindividual differences in responses to dioxin.

Lang et al. (1994) have shown that expression of the AhR in lymphocytes in humans and rodent species is quite different. In mice and rats the AhR is expressed constitutively in lymphocytes, whereas in human peripheral blood lymphocytes, the receptor is not expressed in resting cells and requires culture with mitogens. Differential expression of receptors in target tissues may therefore account for species differences in responsiveness (Lang et al., 1994).

ISSUES IN EVALUATING THE EVIDENCE

A valid surrogate animal model for the study of a human disease must reproduce with some degree of fidelity the manifestations of the disease in humans. Whole-animal studies or animal-based experimental systems continue to be used to study herbicide toxicity because they allow for rigid control of chemi-

cal exposures and close monitoring of health outcomes. Because many of the chemical exposures presently associated with certain diseases in humans have been confirmed in experimental studies (Huff, 1993; Huff et al., 1994), data derived from such studies are generally accepted as a valuable guide in the assessment of biological plausibility.

As discussed in this chapter, many of the toxic effects of the herbicides used in Vietnam have been ascribed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a contaminant of some of the herbicides. This has not, however, simplified the risk assessment process because the toxicologic profile of TCDD is rather complex. In general, there is consensus that most of the toxic effects of TCDD involve interaction with the aryl hydrocarbon receptor, a protein that binds TCDD and other aromatic hydrocarbons with high affinity. The development of AhR knockout mice has helped to establish a definitive association between the AhR and TCDD-mediated toxicity. Formation of an active complex involving the receptor, ligand (the TCDD molecule), and other protein factors is followed by interaction of the activated complex with specific sites on DNA. This interaction results in DNA changes that alter the expression of genes involved in the regulation of cellular processes. In this manner, TCDD and other AhR ligands modulate target cells and presumably exert toxic effects. Attempts to establish correlations between the effects of TCDD in experimental systems and in humans are particularly problematic because species differences in susceptibility to TCDD have been documented. Humans may actually be more resistant than other species to the toxic effects of this chemical (Dickson and Buzik, 1993). Differences in susceptibility involve a toxicokinetic component, since elimination rates in humans may be slower than in rodents. Toxicodynamic interactions are also important because the affinity of TCDD for the AhR is species specific (Lorenzen and Okey, 1991), and responses to occupancy of the receptor vary among different cell types and during different developmental stages.

If TCDD is assumed to be primarily responsible for the harmful effects of herbicides, then toxicity would be predicted to be receptor mediated. Such deductive reasoning, however, has faced considerable challenges, because evidence continues to accumulate that the AhR does not appear to be exclusively responsible for the toxic effects of TCDD, as discussed in *Update 1996* and this chapter. Of particular significance is the recognition that a variable region present in the AhR (Dolwick et al., 1993) may account for the multiple forms of AhR that dictate both species- and cell-specific differences in responsiveness to receptor ligands.

Although studies in which transformed human cell lines are employed to study AhR biology minimize the inherent error associated with species extrapolations, caution must be exercised because the extent to which transformation itself influences toxicity outcomes has yet to be fully defined. It is generally accepted that genetic susceptibility plays a key role in determining the adverse effects of environmental chemicals. In the case of TCDD, the drug-metabolizing enzymes induced in humans are different from those induced in rodents, suggesting that

the impact of different genetic backgrounds on AhR function is not yet completely understood. This issue is particularly central to the assessment of biologic plausibility, because polymorphisms of the AhR in humans similar to those in laboratory animals would place some individuals at greater risk for the toxic and carcinogenic effects of TCDD. Ultimately, the major challenge in the assessment of biologic plausibility for the toxicity of herbicides and TCDD is not restricted to the understanding of receptor-mediated events. The dose–response relationships that arise from multiple toxicokinetic and toxicodynamic interactions must also be considered. Gene regulation models described to date do not consider the intricacies of the multiprotein interactions between the AhR and other proteins. Thus, future attempts to define the quantitative relationship between receptor occupancy and biologic response to TCDD must consider that multiple biochemical changes may influence the overall cellular response.

REFERENCES

Abbott BD. 1995. Review of the interaction between TCDD and glucocorticoids in embryonic palate. Toxicology 105:365–373.

Abbott BD, Birnbaum LS, Diliberto JJ. 1996. Rapid distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to embryonic tissues in C57BL/6N mice and correlation with palatal uptake in vitro. Toxicology and Applied Pharmacology 141:256–263.

Abbott BD, Probst MR. 1995. Developmental expression of two members of a new class of transcription factors: II. Expression of aryl hydrocarbon receptor nuclear translocator in the C57BL-6N mouse embryo. Developmental Dynamics 204(2):144–155.

Abel J, Li W, Dohr O, Vogel C, Donat S. 1996. Dose–response relationship of cytochrome P4501b1 mRNA induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in livers of C57BL/6J and DBA/2J mice. Archives of Toxicology 70(8):510–513.

Aozasa O, Ohta S, Mase Y, Miyata H. 1995. Comparative studies on bioaccumulation of PCDDs and PCDFs in C57BL/6 and DBA/2 mice treated with a mixture by oral administration. Chemosphere 30:1819–1828.

Ayotte P, Carrier G, Dewailly E. 1996. Health risk assessment for Inuit newborns exposed to dioxin-like compounds through breast feeding. Chemosphere 32:531–542.

Bacsi SG, Reisz-Porszasz S, Hankinson O. 1995. Orientation of the heterodimeric aryl hydrocarbon (dioxin) receptor complex on its asymmetric DNA recognition sequence. Molecular Pharmacology 47(3):432–438.

Badesha JS, Maliji G, Flaks B. 1995. Immunotoxic effects of prolonged dietary exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. European Journal of Pharmacology 293(4):429–437.

Baker TK, Kwiatkowski AP, Madhukar BV, Klaunig JE. 1995. Inhibition of gap junctional intercellular communication by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rat hepatocytes. Carcinogenesis 16(10):2321–2326.

Bank PA, Yao EF, Swanson HI, Tullis K, Denison MS. 1995. DNA binding of the transformed guinea pig hepatic Ah receptor complex: identification and partial characterization of two high-affinity DNA-binding forms. Archives of Biochemistry and Biophysics 317(2):439–448.

Beebe LE, Fornwald LW, Diwan BA, Anver MR, Anderson LM. 1995. Promotion of *N*-nitrosodiethylamine-initiated hepatocellular tumors and hepatoblastomas by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or Aroclor 1254 in C57BL/6, DBA/2, and B6D2F1 mice. Cancer Research 55:4875–4880.

Bergesse JR, Balegno HF. 1995. 2,4-Dichlorophenoxyacetic acid influx is mediated by an active transport system in Chinese hamster ovary cells. Toxicology Letters 81:167–173.

Berkers JA, Hassing I, Spenkelink B, Brouwer A, Blaauboer BJ. 1995. Interactive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and retinoids on proliferation and differentiation in cultured human keratinocytes: quantification of cross-linked envelope formation. Archives of Toxicology 69:368–378.

Bhattacharyya KK, Brake PB, Eltom SE, Otto SA, Jefcoate CR. 1995. Identification of a rat adrenal cytochrome P450 active in polycyclic hydrocarbon metabolism as rat CYP1B1. Demonstration of a unique tissue-specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. Journal of Biological Chemistry 270(19):11595–11602.

Birnbaum LS, DeVito MJ. 1995. Use of toxic equivalency factors for risk assessment for dioxins and related compounds. Toxicology 105:391–401.

Blakley BR. 1997. Effect of roundup and Tordon 202C herbicides on antibody production in mice. Veterinary and Human Toxicology 39(4):204–206.

Blakley, PM, Kim ES, Firneisz GD. 1989. Effects of paternal subacture exposure to Tordon 202C on fetal growth and development in CD-1 mice. Teratology 39(3):237–241.

Brodie AE, Azarenko VA, Hu CY. 1996. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibition of fat cell differentiation. Toxicology Letters. 1996 84:55–59.

Brodie AE, Azarenko VA, Hu CY. 1997. Inhibition of increases of transcription factor mRNAs during differentiation of primary rat adipocytes by in vivo 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) treatment. Toxicology Letters 90:91–95.

Brown DJ, Van Beneden RJ, Clark GC. 1995. Identification of two binding proteins for halogenated aromatic hydrocarbons in the hard-shell clam, *Mercenaria mercenaria*. Archives of Biochemistry and Biophysics 319(1):217–224.

Burleson GR, Lebrec H, Yang YG, Ibanes JD, Pennington KN, Birnbaum LS. 1996. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on influenza virus host resistance in mice. Fundamental and Applied Toxicology 29(1):40–47.

Cantrell SM, Lutz LH, Tillitt DE, Hannink M. 1996. Embryotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): the embryonic vasculature is a physiological target for TCDD-induced DNA damage and apoptotic cell death in Medaka (*Orizias latipes*). Toxicology and Applied Pharmacology 141:23–34.

Carrier G, Brunet RC, Brodeur J. 1995a. Modeling of the toxicokinetics of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. Toxicology and Applied Pharmacology 131:253–266.

Carrier G, Brunet RC, Brodeur J. 1995b. Modeling of the toxicokinetics of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. Toxicology and Applied Pharmacology 131:267–276.

Celander M, Weisbrod R, Stegeman JJ. 1997. Glucocorticoid potentiation of cytochrome P4501A1 induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in porcine and human endothelial cells in culture. Biochemical and Biophysical Research Communications 232:749–753.

Chaffin CL, Peterson RE, Hutz RJ. 1996. In utero and lactational exposure of female Holtzman rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: modulation of the estrogen signal. Biology of Reproduction 55:62–67.

Charles JM, Bond DM, Jeffries TK, et al. 1996a. Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxyacetic acid in rodents. Fundamental and Applied Toxicology 33:166–172.

Charles JM, Cunny HC, Wilson RD, Bus JS. 1996b. Comparative subchronic studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in rats. Fundamental and Applied Toxicology 33:161–165.

Charles JM, Dalgard DW, Cunny HC, Wilson RD, Bus JS. 1996c. Comparative subchronic and chronic dietary toxicity studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in the dog. Fundamental and Applied Toxicology 29:78–85.

Chen I, Safe S, Bjeldanes L. 1996. Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (Ah) receptor agonists and antagonists in T47D human breast cancer cells. Biochemical Pharmacology 51(8):1069–1076.

Chen YH, Riby J, Srivastava P, Bartholomew J, Denison M, Bjeldanes L. 1995. Regulation of CYP1A1 by indolo[3,2-b]carbazole in murine hepatoma cells. Journal of Biological Chemistry 270(38): 22548–22555.

Chen YH, Tukey RH. 1996. Protein kinase C modulates regulation of the CYP1A1 gene by the arylhydrocarbon receptor. Journal of Biological Chemistry 271(42):26261–26266.

Cheung YL, Lewis DF, Ridd TI, Gray TJ, Ioannides C. 1997. Diaminonaphthalenes and related aminocompounds: mutagenicity, CYP1A induction and interaction with the Ah receptor. Toxicology 118(2–3):115–127.

Christou M, Savas U, Schroeder S, Shen X, Thompson T, Gould MN, Jefcoate CR. 1995. Cytochromes CYP1A1 and CYP1B1 in the rat mammary gland: cell-specific expression and regulation by polycyclic aromatic hydrocarbons and hormones. Molecular and Cellular Endocrinology 115(1):41–50.

Couture LA, Harris MW, Birnbaum LS. 1990a. Characterization of the peak period of sensitivity for the induction of hydronephrosis in C57B1/6N mice following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundamental and Applied Toxicology 15:142–150.

Couture LA, Abbott BD, Birnbaum LS. 1990b. A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: recent advances toward understanding the mechanism. Teratology 42:619–627.

Corton JC, Moreno ES, Hovis SM, Leonard LS, Gaido KW, Joyce MM, Kennett SB. 1996. Identification of a cell-specific transcription activation domain within the human Ah receptor nuclear translocator. Toxicology and Applied Pharmacology 139(2):272–280.

Coumailleau P, Poellinger L, Gustafsson JA, Whitelaw ML. 1995. Definition of a minimal domain of the dioxin receptor that is associated with hsp90 and maintains wild type ligand binding affinity and specificity. Journal of Biological Chemistry 270(42):25291–25300.

Cummings AM, Metcalf JL, Birnbaum L. 1996. Promotion of endometriosis by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats and mice: time-dose dependence and species comparison. Toxicology and Applied Pharmacology 138:131–139.

de Heer C, Schuurman HJ, Liem AK, Penninks AH, Vos JG, van Loveren H. 1995a. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to the human thymus after implantation in SCID mice. Toxicology and Applied Pharmacology 134(2):296–304.

de Heer C, van Driesten G, Schuurman HJ, Rozing J, van Loveren H. 1995b. No evidence for emergence of autoreactive V beta 6+ T cells in Mls-1a mice following exposure to a thymotoxic dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicology 103(3):195–203.

De Krey GK, Kerkvliet NI. 1995. Suppression of cytotoxic T lymphocyte activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin occurs in vivo, but not in vitro, and is independent of corticosterone elevation. Toxicology 97(1–3):105–112.

DeVito MJ, Birnbaum LS. 1995. The importance of pharmacokinetics in determining the relative potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran. Fundamental and Applied Toxicology 24:145–148.

DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. 1995. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environmental Health Perspectives 103:820–831.

Dickson LC, Buzik SC. 1993. Health risks of "dioxins": a review of environmental and toxicological considerations. Veterinary and Human Toxicology 35(1):68–77.

Diliberto JJ, Jackson JA, Birnbaum LS. 1996. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) disposition following pulmonary, oral, dermal, and parenteral exposures to rats. Toxicology and Applied Pharmacology 138:158–168.

Dohr O, Sinning R, Vogel C, Munzel P, Abel J. 1997. Effect of transforming growth factor-beta1 on expression of aryl hydrocarbon receptor and genes of Ah gene battery: clues for independent down-regulation in A549 cells. Molecular Pharmacology 51(5):703–710.

Dohr O, Vogel C, Abel J. 1995. Different response of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive genes in human breast cancer MCF-7 and MDA-MB 231 cells. Archives of Biochemistry and Biophysics 321(2):405–412.

Dolwick KM, Swanson HI, Bradfield CA. 1993. In vitro analysis of Ah receptor domains involved in ligand-activated DNA recognition. Proceedings of the National Academy of Sciences (USA) 90:8566–8570.

Duffard R, Garcia G, Rosso S, et al. 1996. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicology and Teratology 18:691–696.

Dunn RT II, Ruh TS, Burroughs LK, Ruh MF. 1996. Purification and characterization of an Ah receptor binding factor in chromatin. Biochemical Pharmacology 51(4):437–445.

Ema M, Ohe N, Suzuki M, Mimura J, Sogawa K, Ikawa S, Fujii-Kuriyama Y. 1994. Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. Journal of Biological Chemistry 269(44):27337–27343.

Enan E, Matsumura F. 1995a. Evidence for a second pathway in the action mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Significance of Ah-receptor mediated activation of protein kinase under cell-free conditions. Biochemical Pharmacology 49(2):249–261.

Enan E, Matsumura F. 1995b. Regulation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) of the DNA binding activity of transcriptional factors via nuclear protein phosphorylation in guinea pig adipose tissue. Biochemical Pharmacology 50:1199–1206.

Enan E, Matsumura F. 1996. Identification of c-Src as the integral component of the cytosolic Ah receptor complex, transducing the signal of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) through the protein phosphorylation pathway. Biochemical Pharmacology 52:1599–1612.

Enan E, Lasley B, Stewart D, Overstreet J, Vandevoort CA. 1996a. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) modulates function of human luteinizing granulosa cells via cAMP signaling and early reduction of glucose transporting activity. Reproductive Toxicology 10:191–198.

Enan E, Moran F, VandeVoort CA, Stewart DR, Overstreet JW, Lasley BL. 1996b. Mechanism of toxic action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in cultured human luteinized granulosa cells. Reproductive Toxicology 10:497–508.

Enan E, Overstreet JW, Matsumura F, VandeVoort CA, Lasley BL. 1996c. Gender differences in the mechanism of dioxin toxicity in rodents and in nonhuman primates. Reproductive Toxicology 10:401–411.

Fan F, Rozman KK. 1995. Short- and long-term biochemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Long-Evans rats. Toxicology Letters 75:209–216.

Fan F, Pinson DM, Rozman KK. 1995. Immunomodulatory effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin tested by the popliteal lymph node assay. Toxicologic Pathology 23(4):513–517.

Fan F, Wierda D, Rozman KK. 1996. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on humoral and cell-mediated immunity in Sprague-Dawley rats. Toxicology 106(1–3):221–228.

Fan F, Yan B, Wood G, Viluksela M, Rozman KK. 1997. Cytokines (IL-1beta and TNFalpha) in relation to biochemical and immunological effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicology 116(1–3):9–16.

Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ. 1995. Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor [see comments]. Science 268(5211):722–726.

Fiorella PD, Olson JR, Napoli JL. 1995. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induces diverse retinoic acid metabolites in multiple tissues of the Sprague-Dawley rat. Toxicology and Applied Pharmacology 134:222–228.

Fitzgerald CT, Fernandez-Salguero P, Gonzalez FJ, Nebert DW, Puga A. 1996. Differential regulation of mouse Ah receptor gene expression in cell lines of different tissue origins. Archives of Biochemistry and Biophysics 333(1):170–178.

Fukunaga BN, Probst MR, Reisz-Porszasz S, Hankinson O. 1995. Identification of functional domains of the aryl hydrocarbon receptor. Journal of Biological Chemistry 270(49):29270–29278.

Gaido KW, Maness SC. 1995. Post-transcriptional stabilization of urokinase plasminogen activator mRNA by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a human keratinocyte cell line. Toxicology and Applied Pharmacology 133(1):34–42.

Gasiewicz TA, Kende AS, Rucci G, Whitney B, Willey JJ. 1996. Analysis of structural requirements for Ah receptor antagonist activity: ellipticines, flavones, and related compounds. Biochemical Pharmacology 52(11):1787–1803.

Gassmann M, Kvietikova I, Rolfs A, Wenger RH. 1997. Oxygen- and dioxin-regulated gene expression in mouse hepatoma cells. Kidney International 51(2):567–574.

Gebremichael A, Tullis K, Denison MS, Cheek JM, Pinkerton KE. 1996. Ah-receptor-dependent modulation of gene expression by aged and diluted sidestream cigarette smoke. Toxicology and Applied Pharmacology 141(1):76–83.

Germolec DR, Adams NH, Luster MI. 1995. Comparative assessment of metabolic enzyme levels in macrophage populations of the F344 rat. Biochemical Pharmacology 50(9):1495–1504.

Germolec DR, Henry EC, Maronpot R, Foley JF, Adams NH, Gasiewicz TA, Luster MI. 1996. Induction of CYP1A1 and ALDH-3 in lymphoid tissues from Fisher 344 rats exposed to 2,3,7,8-tetrachlorodibenzodioxin (TCDD). Toxicology and Applied Pharmacology 137(1):57–66.

Gilday D, Gannon M, Yutzey K, Bader D, Rifkind AB. 1996. Molecular cloning and expression of two novel avian cytochrome P450 1A enzymes induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Journal of Biological Chemistry 271(51):33054–33059.

Gohl G, Lehmkoster T, Munzel PA, Schrenk D, Viebahn R, Bock KW. 1996. TCDD-inducible plasminogen activator inhibitor type 2 (PAI-2) in human hepatocytes, HepG2 and monocytic U937 cells. Carcinogenesis 17(3):443–449.

Golub MS, Jacobson SW. 1995. Workshop on perinatal exposure to dioxin-like compounds. IV. Neurobehavioral effects. Environmental Health Perspectives 103 (Suppl) 2:151–155.

Gonzalez FJ, Fernandez-Salguero P, Lee SS, Pineau T, Ward JM. 1995. Xenobiotic receptor knockout mice. Toxicology Letters 82:83117–83121.

Gradin K, McGuire J, Wenger RH, Kvietikova I, Whitelaw ML, Toftgard R, Tora L, Gassmann M, Poellinger L. 1996. Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor. Molecular and Cellular Biology 16(10):5221–5231.

Gradin K, Toftgard R, Berghard A. 1995. Differential effects of a topoisomerase I inhibitor on dioxin inducibility and high-level expression of the cytochrome P450IA1 gene. Molecular Pharmacology 48(4):610–615.

Gray LE Jr, Kelce WR, Monosson E, Ostby JS, Birnbaum LS. 1995. Exposure to TCDD during development permanently alters reproductive function in male Long-Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. Toxicology and Applied Pharmacology 131:108–118.

Gray LE Jr, Ostby JS. 1995. In utero 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. Toxicology and Applied Pharmacology 133:285–294.

Guiney PD, Smolowitz RM, Peterson RE, Stegeman JJ. 1997. Correlation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induction of cytochrome P4501A in vascular endothelium with toxicity in early life stages of lake trout. Toxicology and Applied Pharmacology 143(2):256–273.

Hahn ME, Chandran K. 1996. Uroporphyrin accumulation associated with cytochrome P4501A induction in fish hepatoma cells exposed to aryl hydrocarbon receptor agonists, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and planar chlorobiphenyls. Archives of Biochemistry and Biophysics 329(2):163–174.

Hahn ME, Karchner SI. 1995. Evolutionary conservation of the vertebrate Ah (dioxin) receptor: amplification and sequencing of the PAS domain of a teleost Ah receptor cDNA. Biochemical Journal 310(Pt 2):383–387.

Hakkola J, Pasanen M, Pelkonen O, Hukkanen J, Evisalmi S, Anttila S, Rane A, Mantyla M, Purkunen R, Saarikoski S, Tooming M, Raunio H. 1997. Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. Carcinogenesis 18(2):391–397.

Hanberg A, Kling L, Hakansson H. 1996. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic stellate cell population in the rat. Chemosphere 32:1225–1233.

Hanneman WH, Legare ME, Barhoumi R, Burghardt RC, Safe S, Tiffany-Castiglioni E. 1996. Stimulation of calcium uptake in cultured rat hippocampal neurons by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicology 112:19–28.

Harper N, Steinberg M, Thomsen J, Safe S. 1995. Halogenated aromatic hydrocarbon-induced suppression of the plaque-forming cell response in B6C3F1 splenocytes cultured with allogenic mouse serum: Ah receptor structure activity relationships. Toxicology 99(3):199–206.

Hassoun EA, Bagchi D, Stohs SJ. 1995. Evidence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced tissue damage in fetal and placental tissues and changes in amniotic fluid lipid metabolites of pregnant CF1 mice. Toxicology Letters 76:245–250.

Hassoun EA, Stohs SJ. 1996. Comparative teratological studies on TCDD, endrin and lindane in C57B/6J and DBA/2J mice. Comparative Biochemistry and Physiology: C. Pharmacology, Toxicology, and Endocrinology 113(3)393–398.

Hayes HM, Tarone RE, Cantor KP, Jessen CR, McCurnin DM, Richardson RC. 1991. Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. Journal of the National Cancer Institute 83(17):1226–1231.

Hayes HM, Tarone RE, Cantor KP. 1995. On the association between canine malignant lymphoma and opportunity for exposure to 2,4-dichlorophenoxyacetic acid. Environmental Research 70: 119–125.

Henry EC, Kent TA, Gasiewicz T. 1997a. DNA binding and transcriptional enhancement by purified TCDD cntdot Ah receptor complex. Archives of Biochemistry and Biophysics 339(2):305–314.

Henry TR, Spitsbergen JM, Hornung MW, Abnet CC, Peterson RE. 1997b. Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish (*Danio rerio*). Toxicology and Applied Pharmacology 142:56–68.

Henshel DS, Martin JW, Norstrom R, Whitehead P, Steeves JD, Cheng KM. 1995. Morphometric abnormalities in brains of great blue heron hatchlings exposed in the wild to PCDDs. Environmental Health Perspectives 103 (Suppl 4):61–66.

Hoffer A, Chang CY, Puga A. 1996. Dioxin induces transcription of *fos* and *jun* genes by Ah receptor-dependent and -independent pathways. Toxicology and Applied Pharmacology 141(1): 238–247.

Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, Brooks BA, Hankinson O. 1991. Cloning of a factor required for activity of the Ah (dioxin) receptor, Science 252(5008):954–958.

Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray-Grant M, Perdew GH, Bradfield CA. 1997. Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. Journal of Biological Chemistry 272(13):8581–8593.

Hossain A, Kikuchi H, Ikawa S, Sagami I, Watanabe M. 1995a. Identification of a 120-kDa protein associated with aromatic hydrocarbon receptor nuclear translocator. Biochemical and Biophysical Research Communications 212(1):144–150

Hossain A, Kikuchi H, Ikawa S, Sagami I, Watanabe M. 1995b. Identification of cellular protein that can interact specifically with the basic helix-loop-helix domain of the aromatic hydrocarbon receptor. Biochemical and Biophysical Research Communications 215(1):405–411.

Huang Y, Harper PA, Okey AB. 1995. Aromatic hydrocarbon receptor in cultured fetal cells from C57BL/6J and DBA/2J mice: similarity in molecular mass to receptors in adult livers. Canadian Journal of Physiology and Pharmacology 73(1):18–26.

Huff J. 1993. Chemicals and cancer in humans: first evidence in experimental animals. Environmental Health Perspectives 100:201–210.

Huff J, Lucier G, Tritscher A. 1994. Carcinogenicity of TCDD: experimental, mechanistic, and epidemiologic evidence. Annual Review of Pharmacology and Toxicology 34:343–372.

Hughes MF, Mitchell CT, Edwards BC, Rahman MS. 1995. In vitro percutaneous absorption of dimethylarsinic acid in mice. Journal of Toxicology and Environmental Health 45:279–290.

Huisman M, Koopman-Esseboom C, Fidler V, et al. 1995a. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Human Development 41:111–127.

Huisman M, Koopman-Esseboom C, Lanting CI, et al. 1995b. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Human Development 43:165–176.

Hushka DR, Greenlee WF. 1995. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inhibits DNA synthesis in rat primary hepatocytes. Mutation Research 333:89–99

Ilian MA, Sparrow BR, Ryu BW, Selivonchick DP, Schaup HW. 1996. Expression of hepatic pyruvate carboxylase mRNA in C57BL/6J Ah(b/b) and congenic Ah(d/d) mice exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Journal of Biochemical Toxicology 11:51–56.

Janz DM, Bellward GD. 1996a. In ovo 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure in three avian species. 1. Effects on thyroid hormones and growth during the perinatal period. Toxicology and Applied Pharmacology 139:281–291.

Janz DM, Bellward GD. 1996b. In ovo 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure in three avian species. 2. Effects on estrogen receptor and plasma sex steroid hormones during the perinatal period. Toxicology and Applied Pharmacology 139:292–300.

Jorgensen ECB, Autrup H. 1995. Effect of a negative regulatory element (NRE) on the human CYP1A1 gene expression in breast carcinoma MCF-7 and hepatoma HepG2 cells. FEBS Letters 365(2–3):101–107.

Jorgensen ECB, Autrup H. 1996. Autoregulation of human CYP1A1 gene promoter activity in HepG2 and MCF-7 cells. Carcinogenesis 17(3):435–441.

Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S, Tasie BO, Kale R, Sohni YR. 1995. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. Environmental and Molecular Mutagenesis 25(2):148–153.

Karras JG, Morris DL, Matulka RA, Kramer CM, Holsapple MP. 1996. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) elevates basal B-cell intracellular calcium concentration and suppresses surface Ig- but not CD40-induced antibody secretion. Toxicology and Applied Pharmacology 137(2):275–284.

Kerkvliet NI. 1995. Immunological effects of chlorinated dibenzo-*p*-dioxins. Environmental Health Perspectives 103 (Suppl 9):47–53.

Kerkvliet NI, Baecher-Steppan L, Shepherd DM, Oughton JA, Vorderstrasse BA, DeKrey GK. 1996. Inhibition of TC-1 cytokine production, effector cytotoxic T lymphocyte development and alloantibody production by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Journal of Immunology 157(6):2310–2319.

Kharat I, Saatcioglu F. 1996. Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor. Cross-talk between aryl hydrocarbon- and estrogen-mediated signaling. Journal of Biological Chemistry 271(18):10533–10537.

Kim CS, Binienda Z, Sandberg JA. 1996. Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid dosimetry in the developing rabbit brain. Toxicology and Applied Pharmacology 136:250–259.

Kleman M, Gustafsson JA. 1996. Interactions of procarcinogenic heterocyclic amines and indolocarbazoles with the dioxin receptor. Biological Chemistry 377(11):741–762.

Ko HP, Okino ST, Ma Q, Whitlock JP Jr. 1996. Dioxin-induced CYP1A1 transcription in vivo: the aromatic hydrocarbon receptor mediates transactivation, enhancer-promoter communication, and changes in chromatin structure. Molecular and Cellular Biology 16(1):430–436

Kohn MC, Lucier GW, Clark GC, Sewall C, Tritscher AM, Portier CJ. 1993. A mechanistic model of effects of dioxin on gene expression in the rat liver. Toxicology and Applied Pharmacology 120(1):138–154.

Kohn MC, Sewall CH, Lucier GW, Portier CJ. 1996. A mechanistic model of effects of dioxin on thyroid hormones in the rat. Toxicology and Applied Pharmacology 136:29–48.

Kraemer SA, Arthur KA, Denison MS, Smith WL, DeWitt DL. 1996. Regulation of prostaglandin endoperoxide H synthase-2 expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Archives of Biochemistry and Biophysics 330(2):319–328.

Kremer J, Lai ZW, Esser C. 1995. Evidence for the promotion of positive selection of thymocytes by Ah receptor agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. European Journal of Pharmacology 293(4):413–427.

Krishnan V, Porter W, Santostefano M, Wang X, Safe S. 1995. Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 cells. Molecular and Cellular Biology 15(12):6710–6719.

Lamb JC, Moore JA, Marks TA. 1980. Evaluation of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity in C57BL/6 mice. Reproduction and Fertility in Treated Male Mice and Evaluation of Congenital Malformations in Their Offspring. National Toxicology Program.

Lang DS, Becker S, Clark GC, Devlin RB, Koren HS. 1994. Lack of direct immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on human peripheral blood lymphocyte subsets in vitro. Archives of Toxicology 68(5):296-302.

Lawrence BP, Leid M, Kerkvliet NI. 1996. Distribution and behavior of the Ah receptor in murine T lymphocytes. Toxicology and Applied Pharmacology 138(2):275–284.

Lee IJ, Jeong KS, Roberts BJ, Kallarakal AT, Fernandez-Salguero P, Gonzalez FJ, Song BJ. 1996. Transcriptional induction of the cytochrome p4501a1 gene by a thiazolium compound, yh439. Molecular Pharmacology 49(6):980–988.

Lesca P, Peryt B, Larrieu G, Alvinerie M, Galtier P, Daujat M, Maurel P, Hoogenboom L. 1995. Evidence for the ligand-independent activation of the ah receptor. Biochemical and Biophysical Research Communications 209(2):474–482.

Li SY, Dougherty JJ. 1997. Inhibitors of serine/threonine-specific protein phosphatases stimulate transcription by the Ah receptor/Arnt dimer by affecting a step subsequent to XRE binding. Archives of Biochemistry and Biophysics 340(1):73–82.

Li W, Donat S, Dohr O, Unfried K, Abel J. 1994. Ah receptor in different tissues of C57BL/6J and DBA/2J mice: use of competitive polymerase chain reaction to measure Ah-receptor mRNA expression. Archives of Biochemistry and Biophysics 315(2):279–284.

Li X, Johnson DC, Rozman KK. 1997. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) increases release of luteinizing hormone and follicle-stimulating hormone from the pituitary of immature female rats in vivo and in vitro. Toxicology and Applied Pharmacology 142:264–269.

Li X, Rozman KK. 1995. Subchronic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and their reversibility in male Sprague-Dawley rats. Toxicology 97:133–140.

Lindebro MC, Poellinger L, Whitelaw ML. 1995. Protein-protein interaction via PAS domains: role of the PAS domain in positive and negative regulation of the bHLH/PAS dioxin receptor-Arnt transcription factor complex. EMBO Journal 14(14):3528–35239.

Liu H, Safe S. 1996. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on insulin-induced responses in MCF-7 human breast cancer cells. Toxicology and Applied Pharmacology 138(2): 242–250.

Liu PC, Matsumura F. 1995. Differential effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the "adipose-type" and "brain-type" glucose transporters in mice. Molecular Pharmacology 47:65–73.

Lorenzen A, Kennedy SW, Bastien LJ, Hahn ME. 1997. Halogenated aromatic hydrocarbon-mediated porphyrin accumulation and induction of cytochrome P4501A in chicken embryo hepatocytes. Biochemical Pharmacology 53:373–384.

Lorenzen A, Okey AB. 1991. Detection and characterization of Ah receptor in tissue and cells from human tonsils. Toxicology and Applied Pharmacology 107:203–214.

Lu YF, Santostefano M, Cunningham BD, Threadgill MD, Safe S. 1996a. Substituted flavones as aryl hydrocarbon (Ah) receptor agonists and antagonists. Biochemical Pharmacology 51(8): 1077–1087.

Lu YF, Sun G, Wang X, Safe S. 1996b. Inhibition of prolactin receptor gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in MCF-7 human breast cancer cells. Archives of Biochemistry and Biophysics 332(1):35–40.

Luebke RW, Copeland CB, Andrews DL. 1995. Host resistance to *Trichinella spiralis* infection in rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Fundamental and Applied Toxicology 24(2):285–289.

Luebke RW, Copeland CB, Diliberto JJ, Akubue PI, Andrews DL, Riddle MM, Williams WC, Birnbaum LS. 1994. Assessment of host resistance to *Trichinella spiralis* in mice following preinfection exposure to 2,3,7,8-TCDD. Toxicology and Applied Pharmacology 125(1):7–16.

Ma Q, Whitlock JP Jr. 1996. The aromatic hydrocarbon receptor modulates the Hepa 1c1c7 cell cycle and differentiated state independently of dioxin. Molecular and Cellular Biology 16(5):2144–2150

Ma X, Stoffregen DA, Wheelock GD, Rininger JA, Babish JG. 1997. Discordant hepatic expression of the cell division control enzyme p34cdc2 kinase, proliferating cell nuclear antigen, p53 tumor suppressor protein, and p21Waf1 cyclin-dependent kinase inhibitory protein after WY14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid) dosing to rats. Molecular Pharmacology 51:69–78.

Mahon MJ, Gasiewicz TA. 1995. Ah receptor phosphorylation: localization of phosphorylation sites to the C-terminal half of the protein. Archives of Biochemistry and Biophysics 318(1): 166–174.

Masten SA, Shiverick KT. 1995. The Ah receptor recognizes DNA binding sites for the B cell transcription factor, BSAP: a possible mechanism for dioxin-mediated alteration of CD19 gene expression in human B lymphocytes. Biochemical and Biophysical Research Communications 212(1):27–34.

Masten SA, Shiverick KT. 1996. Characterization of the aryl hydrocarbon receptor complex in human B lymphocytes: evidence for a distinct nuclear DNA-binding form. Archives of Biochemistry and Biophysics 336(2):297–308.

McGrath LF, Cooper KR, Georgopoulos P, Gallo MA. 1995. Alternative models for low dose–response analysis of biochemical and immunological endpoints for tetrachlorodibenzo-*p*-dioxin. Regulatory Toxicology and Pharmacology 21:382–396.

McGuire J, Whitelaw ML, Pongratz I, Gustafsson JA, Poellinger L. 1994. A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-helix dioxin receptor. Molecular and Cellular Biology 14(4):2438–2446.

Mekenyan OG, Veith GD, Call DJ, Ankley GT. 1996. A QSAR evaluation of Ah receptor binding of halogenated aromatic xenobiotics. Environmental Health Perspectives 104(12):1302–1310.

Merchant M, Safe S. 1995. In vitro inhibition of 2 ,3 ,7 ,8 -tetrachlorodibenzo-*p*-dioxin-induced activity by alpha-naphthoflavone and 6-methyl-1,3,8-trichlorodibenzofuran using an aryl hydrocarbon (Ah)-responsive construct. Biochemical Pharmacology 50(5):663–668.

Michalek JE, Caudill SP, Tripathi RC. 1997. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. Erratum. Journal of Toxicology and Environmental Health 52(6):557–558.

Michalek JE, Pirkle JL, Caudill SP, Tripathi RC, Patterson DG Jr, Needham LL. 1996a. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. Journal of Toxicology and Environmental Health 47:209–220.

Michalek JE, Tripathi RC, Kulkarni PM, Pirkle JL. 1996b. The reliability of the serum dioxin measurement in veterans of Operation Ranch Hand. Journal of Exposure Analysis and Environmental Epidemiology 6:327–338.

Miranda S, Vollrath V, Wielandt AM, Loyola G, Bronfman M, Chianale J. 1997. Overexpression of mdr2 gene by peroxisome proliferators in the mouse liver. Journal of Hepatology 26(6): 1331–1339.

Moos AB, Kerkvliet NI. 1995. Inhibition of tumor necrosis factor activity fails to restore 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced suppression of the antibody response to sheep red blood cells. Toxicology Letters 81(2–3):175–181.

Mufti NA, Bleckwenn NA, Babish JG, Shuler ML. 1995. Possible involvement of the Ah receptor in the induction of cytochrome P-450IA1 under conditions of hydrodynamic shear in microcarrier-attached hepatoma cell lines. Biochemical and Biophysical Research Communications 208(1):144–152.

Münzel P, Bock-Hennig B, Schieback S, Gschaidmeier H, Beck-Gschaidmeier S, Bock KW. 1996. Growth modulation of hepatocytes and rat liver epithelial cells (WB-F344) by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Carcinogenesis 17(2):197–202.

Neubert R, Maskow L, Delgado I, Helge H, Neubert D. 1995. Chlorinated dibenzo-*p*-dioxins and dibenzofurans and the human immune system. 2. In vitro proliferation of lymphocytes from workers with quantified moderately-increased body burdens. Life Sciences 56(6): 421–436.

Nodland KI, Wormke M, Safe S. 1997. Inhibition of estrogen-induced activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the MCF-7 human breast cancer and other cell lines transfected with vitellogenin A2 gene promoter constructs. Archives of Biochemistry and Biophysics 338(1):67–72.

Ochi T, Nakajima F, Sakurai T, Kaise T, Oya-Ohta Y. 1996. Dimethylarsinic acid causes apoptosis in HL-60 cells via interaction with glutathione. Archives of Toxicology 70:815–821.

Okino ST, Whitlock JP Jr. 1995. Dioxin induces localized, graded changes in chromatin structure: implications for Cyp1A1 gene transcription. Molecular and Cellular Biology 15(7):3714–3721.

Oliveira GH, Palermo-Neto J. 1995. Toxicology of 2,4-dichlorophenoxyacetic acid (2,4-D) and its determination in serum and brain tissue using gas chromatography-electron-capture detection. Journal of Analytical Toxicology 19:251–255.

Olnes MJ, Verma M, Kurl RN. 1996. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin modulates expression of the prostaglandin G/H synthase-2 gene in rat thymocytes. Journal of Pharmacology and Experimental Therapeutics 279(3):1566–1573.

Ou X, Ramos KS. 1995. Regulation of cytochrome P4501A1 gene expression in vascular smooth muscle cells through aryl hydrocarbon receptor-mediated signal transduction requires a protein synthesis inhibitor. Archives of Biochemistry and Biophysics 316(1):116–122.

Palmeira CM, Moreno AJ, Madeira VM. 1995a. Effects of paraquat, dinoseb and 2,4-D on intracellular calcium and on vasopressin-induced calcium mobilization in isolated hepatocytes. Archives of Toxicology 69:460–466.

Palmeira CM, Moreno AJ, Madeira VM. 1995b. Thiols metabolism is altered by the herbicides paraquat, dinoseb and 2,4-D: a study in isolated hepatocytes. Toxicology Letters 81:115–123.

Park JY, Shigenaga MK, Ames BN. 1996. Induction of cytochrome P4501A1 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or indolo(3,2-b)carbazole is associated with oxidative DNA damage. Proceedings of the National Academy of Sciences of the United States of America 93(6): 2322–2327.

Paulino CA, Guerra JL, Oliveira GH, Palermo-Neto J. 1996. Acute, subchronic and chronic 2,4-dichlorophenoxyacetic acid (2,4-D) intoxication in rats. Veterinary and Human Toxicology 38:348–352.

Paulino CA, Palermo-Neto J. 1995. Effects of acute 2,4-dichlorophenoxyacetic acid on cattle serum components and enzyme activities. Veterinary and Human Toxicology 37:329–332.

Peters JM, Wiley LM. 1995a. Murine preimplantation embryos express aryl hydrocarbon receptor nuclear translocator (Arnt)mRNA. Teratology 51(3):193.

Peters JM, Wiley LM. 1995b. Evidence that murine preimplantation embryos express aryl hydrocarbon receptor. Toxicology and Applied Pharmacology 134(2):214–221.

Phillips M, Enan E, Liu PC, Matsumura F. 1995. Inhibition of 3T3-L1 adipose differentiation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Journal of Cell Science 108(Pt 1):395–402.

Pohl H, Holler J. 1995. Halogenated aromatic hydrocarbons and toxicity equivalency factors (TEFs) from the public health assessment perspective. Chemosphere 31(1):2547–2559.

Pollenz RS, Sullivan HR, Holmes J, Necela B, Peterson RE. 1996. Isolation and expression of cDNAs from rainbow trout (*Oncorhynchus mykiss*) that encode two novel basic helix-loop-Helix/PER-ARNT-SIM (bHLH/PAS) proteins with distinct functions in the presence of the aryl hydrocarbon receptor. Evidence for alternative mRNA splicing and dominant negative activity in the bHLH/PAS family. Journal of Biological Chemistry 271(48):30886–30896.

Prell RA, Kerkvliet NI. 1997. Involvement of altered B7 expression in dioxin immunotoxicity: B7 transfection restores the CTL but not the autoantibody response to the P815 mastocytoma. Journal of Immunology 158(6):2695–2703.

Prell RA, Oughton JA, Kerkviet NI. 1995. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on anti-CD3-induced changes in T-cell subsets and cytokine production. International Journal of Immunopharmacology 17(11):951–961.

Rhile MJ, Nagarkatti M, Nagarkatti PS. 1996. Role of Fas apoptosis and MHC genes in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced immunotoxicity of T cells. Toxicology 110(1–3):153–167.

Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. 1993. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [see comments]. Fundamental and Applied Toxicology 21(4):433–441.

Rin K, Kawaguchi K, Yamanaka K, Tezuka M, Oku N, Okada S. 1995. DNA-strand breaks induced by dimethylarsinic acid, a metabolite of inorganic arsenics, are strongly enhanced by superoxide anion radicals. Biological and Pharmaceutical Bulletin 18:45–48.

Roman BL, Sommer RJ, Shinomiya K, Peterson RE. 1995. In utero and lactational exposure of the male rat to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: impaired prostate growth and development without inhibited androgen production. Toxicology and Applied Pharmacology 134:241–250.

Ross PS, De Swart RL, Timmerman HH, Reijnders PJH, Vos JG, Van Loveren H, Osterhaus ADME. 1996. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. Aquatic Toxicology 34(1):71–84.

Rowlands JC, McEwan IJ, Gustafsson JA. 1996. Trans-activation by the human aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator proteins: direct interactions with basal transcription factors. Molecular Pharmacology 50(3):538–548.

Sadar MD, Ash R, Sundqvist J, Olsson PE, Andersson TB. 1996a. Phenobarbital induction of CYP1A1 gene expression in a primary culture of rainbow trout hepatocytes. Journal of Biological Chemistry 271(30):17635–17643.

Sadar MD, Blomstrand F, Andersson TB. 1996b. Phenobarbital induction of cytochrome P4501A1 is regulated by cAMP-dependent protein kinase-mediated signaling pathways in rainbow trout hepatocytes. Biochemical and Biophysical Research Communications 225(2):455–461.

Sadar MD, Westlind A, Blomstrand F, Andersson TB. 1996c. Induction of CYP1A1 by GABA receptor ligands. Biochemical and Biophysical Research Communications 229(1):231–237.

Sandberg JA, Duhart HM, Lipe G, Binienda Z, Slikker W Jr, Kim CS. 1996. Distribution of 2,4-dichlorophenoxyacetic acid (2,4-D) in maternal and fetal rabbits. Journal of Toxicology and Environmental Health 49:497–509.

Sanderson JT, Aarts JM, Brouwer A, Froese KL, Denison MS, Giesy JP. 1996. Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-O-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. Toxicology and Applied Pharmacology 137(2):316–325.

Santostefano M, Safe S. 1996. Characterization of the molecular and structural properties of the transformed and nuclear aryl hydrocarbon (Ah) receptor complexes by proteolytic digestion. Chemico-Biological Interactions 100(3):221–240.

Sastry BV, Janson VE, Clark CP, Owens LK. 1997. Cellular toxicity of 2,4,5-trichlorophenoxyacetic acid: formation of 2,4,5-trichlorophenoxyacetylcholine. Cellular and Molecular Biology 43(4):549–557.

Schmidt JV, Bradfield CA. 1996. Ah receptor signaling pathways. Annual Review of Cell and Developmental Biology 12:55–89.

Schmidt JV, Su, GH, Reddy JK, Simon MC, Bradfield CA. 1996. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proceedings of the National Academy of Sciences of the United States of America 93(13):6731–6736.

Schuetz EG, Schuetz JD, Thompson MT, Fisher RA, Madariage JR, Strom SC. 1995. Phenotypic variability in induction of P-glycoprotein mRNA by aromatic hydrocarbons in primary human hepatocytes. Molecular Carcinogenesis 12(2):61–65.

Selmin O, Lucier GW, Clark GC, et al. 1996. Isolation and characterization of a novel gene induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. Carcinogenesis 17:2609–2615.

Sewall CH, Clark GC, Lucier GW. 1995a. TCDD reduces rat hepatic epidermal growth factor receptor: comparison of binding, immunodetection, and autophosphorylation. Toxicology and Applied Pharmacology 132:263–272.

Sewall CH, Flagler N, Vanden Heuvel JP, et al. 1995b. Alterations in thyroid function in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicology and Applied Pharmacology 132:237–244.

Smialowicz RJ, Williams WC, Riddle MM. 1996. Comparison of the T cell-independent antibody response of mice and rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundamental and Applied Toxicology 32(2):293–297.

Sommer RJ, Ippolito DL, Peterson RE. 1996. In utero and lactational exposure of the male Holtzman rat to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: decreased epididymal and ejaculated sperm numbers without alterations in sperm transit rate. Toxicology and Applied Pharmacology 140:146–153.

Stahl BU. 1995. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin blocks the physiological regulation of hepatic phosphoenolpyruvate carboxykinase activity in primary rat hepatocytes. Toxicology 103:45–52.

Stegeman JJ, Hahn ME, Weisbrod R, Woodin BR, Joy JS, Najibi S, Cohen RA. 1995. Induction of cytochrome P4501A1 by aryl hydrocarbon receptor agonists in porcine aorta endothelial cells in culture and cytochrome P4501A1 activity in intact cells. Molecular Pharmacology 47(2):296–306.

Swanson HI, Chan WK, Bradfield CA. 1995. DNA binding specificities and pairing rules of the Ah receptor, ARNT, and SIM proteins. Journal of Biological Chemistry 270(44):26292–26302.

Tritscher AM, Seacat AM, Yager JD, et al. 1996. Increased oxidative DNA damage in livers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treated intact but not ovariectomized rats. Cancer Letters 98:219–225.

Tuomisto J, Andrzejewski W, Unkila M, et al. 1995. Modulation of TCDD-induced wasting syndrome by portocaval anastomosis and vagotomy in Long-Evans and Han/Wistar rats. European Journal of Pharmacology 292:277–285.

Unkila M, Pohjanvirta R, Tuomisto J. 1995a. Biochemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds on the central nervous system. International Journal of Biochemistry and Cell Biology 27:443–455.

Unkila M, Ruotsalainen M, Pohjanvirta R, et al. 1995b. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on tryptophan and glucose homeostasis in the most TCDD-susceptible and the most TCDD-resistant species, guinea pigs and hamsters. Archives of Toxicology 69:677–683.

Van Birgelen AP, Van der Kolk J, Fase KM, et al. 1995. Subchronic dose-response study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague-Dawley rats. Toxicology and Applied Pharmacology 132:1–13.

Vasiliou V, Kozak CA, Lindahl R, Nebert DW. 1996. Mouse microsomal class 3 aldehyde dehydrogenase: AHD3 cDNA sequence, inducibility by dioxin and clofibrate, and genetic mapping. DNA and Cell Biology 15(3):235–245.

Viluksela M, Stahl BU, Rozman KK. 1995. Tissue-specific effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the activity of phosphoenolpyruvate carboxykinase (PEPCK) in rats. Toxicology and Applied Pharmacology 135:308–315.

Voskoboinik I, Ooi SG, Drew R, Ahokas JT. 1997. Peroxisome proliferators increase the formation of BPDE-DNA adducts in isolated rat hepatocytes. Toxicology 122(1–2):81–91.

Walker MK, Cook PM, Butterworth BC, Zabel EW, Peterson RE. 1996. Potency of a complex mixture of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in causing fish early life stage mortality. Fundamental and Applied Toxicology 30:178–186.

Walker MK, Pollenz RS, Smith SM. 1997. Expression of the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator during chick cardiogenesis is consistent with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced heart defects. Toxicology and Applied Pharmacology 143(2):407–419.

Walsh AA, Tullis K, Rice RH, Denison MS. 1996. Identification of a novel cis-acting negative regulatory element affecting expression of the CYP1A1 gene in rat epidermal cells. Journal of Biological Chemistry 271(37):22746–22753.

Wang X, Thomsen JS, Santostefano M, Rosengren R, Safe S, Perdew GH. 1995. Comparative properties of the nuclear aryl hydrocarbon (Ah) receptor complex from several human cell lines. European Journal of Pharmacology 293(3):191–205.

Wanibuchi H, Yamamoto S, Chen H, et al. 1996. Promoting effects of dimethylarsinic acid on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in rats. Carcinogenesis 17:2435–2439.

Wanner R, Brommer S, Czarnetzki BM, Rosenbach T. 1995. The differentiation-related upregulation of aryl hydrocarbon receptor transcript levels is suppressed by retinoic acid. Biochemical and Biophysical Research Communications 209(2):706–711.

Wanner R, Panteleyev A, Henz BM, Rosenbach T. 1996. Retinoic acid affects the expression rate of the differentiation-related genes aryl hydrocarbon receptor, ARNT and keratin 4 in proliferative keratinocytes only. Biochimica et Biophysica Acta 1317(2):105–111.

Warngard L, Bager Y, Kato Y, Kenne K, Ahlborg UG. 1996. Mechanistical studies of the inhibition of intercellular communication by organochlorine compounds. Archives of Toxicology Supplement 18:149–159.

Watson MA, Devereux TR, Malarkey DE, Anderson MW, Maronpot RR. 1995. H-ras oncogene mutation spectra in B6C3F1 and C57BL/6 mouse liver tumors provide evidence for TCDD promotion of spontaneous and vinyl carbamate-initiated liver cells. Carcinogenesis 16(8): 1705–1710.

Weber LW, Lebofsky M, Stahl BU, Smith S, Rozman KK. 1995. Correlation between toxicity and effects on intermediary metabolism in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated male C57BL/6J and DBA/2J mice. Toxicology and Applied Pharmacology 131:155–162.

Weiss C, Kolluri SK, Kiefer F, Gottlicher M. 1996. Complementation of Ah receptor deficiency in hepatoma cells: negative feedback regulation and cell cycle control by the Ah receptor. Experimental Cell Research 226(1):154–163.

Weston WM, Nugent P, Greene RM. 1995. Inhibition of retinoic-acid-induced gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochemical and Biophysical Research Communications 207(2):690–694.

White TE, Rucci G, Liu Z, Gasiewicz TA. 1995. Weanling female Sprague-Dawley rats are not sensitive to the antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicology and Applied Pharmacology 133(2):313–320.

Whitelaw ML, McGuire J, Picard D, Gustafsson JA, Poellinger L. 1995. Heat shock protein hsp90 regulates dioxin receptor function in vivo. Proceedings of the National Academy of Sciences of the United States of America 92(10):4437–4441.

Wilker C, Johnson L, Safe S. 1996. Effects of developmental exposure to indole-3-carbinol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproductive potential of male rat offspring. Toxicology and Applied Pharmacology 141(1):68–75.

Wolfle D, Marquardt H. 1996. Antioxidants inhibit the enhancement of malignant cell transformation induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Carcinogenesis 17:1273–1278.

Xiao GH, Pinaire JA, Rodrigues AD, Prough RA. 1995. Regulation of the Ah gene battery via Ah receptor-dependent and independent processes in cultured adult rat hepatocytes. Drug Metabolism and Disposition 23(6):642–650.

Yamaguchi Y, Kuo MT. 1995. Functional analysis of aryl hydrocarbon receptor nuclear translocator interactions with aryl hydrocarbon receptor in the yeast two-hybrid system. Biochemical Pharmacology 50(8):1295–1302.

Yamamoto S, Wanibuchi H, Hori T, Yano Y, Matsui-Yuasa I, Otani S, Chen H, Yoshida K, Kuroda K, Endo G, Fukushima S. 1997. Possible carcinogenic potential of dimethylarsinic acid as assessed in rat in vivo models: a review. Mutation Research 386(3):353–361.

Yao Y, Hoffer A, Chang CY, Puga A. 1995. Dioxin activates HIV-1 gene expression by an oxidative stress pathway requiring a functional cytochrome P450 CYP1A1 enzyme. Environmental Health Perspectives 103(4):366–371.

Zhao W, Ramos KS. 1995. Inhibition of DNA synthesis in primary cultures of adult rat hepatocytes by benzo[*a*]pyrene and related aromatic hydrocarbons: role of Ah receptor-dependent events. Toxicology 99(3):179–189.

Zorn NE, Russell DH, Buckley AR, Sauro MD. 1995. Alterations in splenocyte protein kinase C (PKC) activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in vivo. Toxicology Letters 78:93–100.

4

Methodologic Considerations in Evaluating the Evidence

QUESTIONS TO BE ADDRESSED

The committee was charged with 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 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 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.

The law establishing the committee (Public Law 102-4, codified as 38 USC Sec. 1116) did not provide a specific list of diseases and conditions suspected to be associated with herbicide exposure. The committee staff and members responsible for the 1994 report *Veterans and Agent Orange* (hereafter *VAO*) (IOM, 1994) developed such a list based on diseases and conditions that had been mentioned in the scientific literature or in other documents that came to their attention through extensive literature searches. The *VAO* list has been supplemented over time in response to developments in the literature. The information used by the committee was developed through a comprehensive search of relevant data bases. Public and commercial data bases covering biological, medical,

toxicological, chemical, historical, and regulatory information were examined. The majority of these data bases were bibliographic, providing citations to scientific literature. 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 pertinent citations. Internet search engines were used to scan for information posted on the Internet. Literature identification continued through September 30, 1997. The input received both in written and oral form from veterans and other interested persons at public hearings and in written submissions served as a valuable source of additional information. Appendix A gives additional detail on the search strategies used to generate reference sources. Information submitted to the committee by interested persons is also listed in Appendix A.

This second biennial update concentrates on evaluating the evidence published following the completion of work on *Veterans and Agent Orange: Update 1996* (hereafter *Update 1996*) (IOM, 1996) and *VAO*. For each health outcome, the new evidence is reviewed in detail. Conclusions are based on the totality of the accumulated evidence, not just on recently published studies. In other words, new evidence is not interpreted alone but is put into the context of evidence addressed in the two previous reports.

In addition to bringing earlier work up to date, the committee has addressed other areas of concern identified by the Department of Veterans Affairs (DVA). Specifically, the committee was asked to do the following:

1. Pay particular attention to the relationship between exposure to herbicides and the subsequent development of diabetes. Chapter 11 contains an extended discussion of this topic. Special attention has also been given to adverse reproductive outcomes, which are addressed in Chapter 9. This area attracted heightened interest after a finding, reported in *Update 1996*, of limited/suggestive evidence of an association between herbicide exposure and spina bifida in the children of veterans.

2. Examine the issue of the latency between exposure to herbicides and development of adverse health outcomes. In response to DVA's request, Chapter 8 (1) proposes a methodology to address issues concerning the timing of herbicide exposure and the risk of cancer; (2) reviews the literature on herbicide exposure and cancers classified in the *sufficient* and *limited/suggestive evidence of an association* categories for results that describe how the timing of exposure affects relative risk; and (3) describes timing-of-exposure characteristics of the Vietnam veterans and summarizes the implications of these factors for their risk of cancer.

3. Discuss the classification of chondrosarcomas of the skull. This subject is addressed in Chapter 7 as part of the discussion of bone cancer.

4. Offer advice on herbicide exposure assessment for Vietnam veterans. Chapter 5 of the report contains an extended discussion of this issue. In addition, a separate effort by another Institute of Medicine (IOM) committee is facilitating

the development and evaluation of models of herbicide exposure for use in studies of Vietnam veterans. That committee authored and disseminated a Request for Proposals for exposure assessment research in 1997 (IOM, 1997) and began scientific oversight of the research in 1998.

5. Address the potential for using data combination methodologies to informatively reexamine existing data on the health effects of herbicide or dioxin exposure. The committee conducted a workshop on this topic in August 1997, which brought together experts in these methodologies and researchers who have developed and analyzed data sets evaluating the health of Vietnam veterans and individuals exposed to herbicides or dioxin. The results of this effort will be addressed in a separate publication.

The committee's judgments have both quantitative and qualitative aspects; they reflect both the evidence examined and the approach taken to evaluate it. In *VAO*, the committee delineated how it approached its task, so that readers would be able to assess and interpret the committee's findings. By offering this information, the committee wished to make the report useful to those seeking to update its conclusions as new information was obtained. The committees responsible for *Update 1996* and the present effort have adopted the original committee's approach.

The remainder of this chapter outlines the types of evidence that the committee identified; the approaches used in evaluating published reports, both singly and collectively; and the nature of the committee's conclusions. Details of the analysis and specific conclusions concerning each health outcome appear in subsequent chapters. Descriptions of methodology and specific information on how the committee interpreted the questions asked in the original legislation can be found in Chapter 5 of *VAO*.

Is Herbicide Exposure Statistically Associated with the Health Outcome?

The committee necessarily focused on a pragmatic question: What is the nature of the relevant evidence for or against a statistical association between exposure and the health outcome? 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 relationship between exposure and disease in defined populations or groups. The committee reports the measure of effect used by the study. However, the use of terms such as "relative risk," "odds ratio," or "estimate of relative risk" is not consistent in the literature. In this report, the committee intends *relative risk* to refer to the results of cohort studies and *odds ratio* (an estimate of relative risk) to refer to the results of case-control

studies. Values of relative risk greater than 1 may indicate a positive or direct association—that is, a harmful association—whereas values between 0 and 1 may indicate a negative or inverse association—that is, a protective association. A "statistically significant" difference is one that, under the assumptions made in the study and the laws of probability, would be fairly unlikely to occur if there was no true difference.

Determining whether an observed statistical association between exposure and a health outcome is "real" requires additional scrutiny because there may be explanations for the observed association other than the exposure. These include *error* in the design, conduct, or analysis of the investigation; *bias*, or a systematic tendency to distort the measure of association so that it may not represent the true relation between exposure and outcome; *confounding*, or distortion of the measure of association because another factor related to both exposure and outcome has not been recognized or taken into account in the analysis; and *chance*, the effect of random variation, which produces spurious associations that can, with a known probability, sometimes depart widely from the true relation.

Therefore, in deciding whether an association between herbicide exposure and a particular outcome existed, the committee examined the quantitative estimates of risk and evaluated whether these estimates might be due to error, bias, confounding, or chance, or were likely to represent a true association.

In pursuing the question of statistical association, 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 never occur. Any instrument of observation, including epidemiologic studies, has a limit to its resolving power. Hence, in a strict technical sense, the committee could not prove the absolute absence of a health outcome associated with herbicide or dioxin exposure. Nevertheless, for some outcomes examined, there was limited or suggestive evidence consistent with *no* association. The committee was able to conclude in some cases that *within the limits of the current resolving power of the existing studies,* there is no association with herbicide exposure.

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

This question, which is pertinent principally (but not exclusively) if there is evidence for a positive association between exposure and a health outcome, concerns the likely magnitude of the association in Vietnam veterans exposed to herbicides. The most desirable evidence in 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 exposure levels among Vietnam veterans have not been adequately determined, which has been the case in most studies, this question becomes difficult to answer. The committee found the available evidence sufficient for drawing conclusions about the association between herbicide exposure and a number of health outcomes. However, the lack of good data on Vietnam veterans per se, especially with regard to exposure, had complicated the assessment of the increased risk of disease among individuals exposed to herbicides during service in Vietnam. By considering the magnitude of the association observed in other cohorts, the quality and results of studies that have been made of veterans, and other principles of epidemiologic research, the committee has formulated a qualitative judgment regarding the risk of disease among Vietnam veterans. 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.

When the available data do not permit a meaningful statement regarding risk among Vietnam veterans, no conclusion is drawn and the reader is referred to the appropriate section in an earlier report for additional discussion.

Is There a Plausible Biologic Mechanism?

Chapter 3 details the cellular and animal experimental evidence that provides the basis for the assessment of biologic plausibility, that is, the extent to which a statistical association is consistent with existing biological or medical knowledge. As with the epidemiologic evidence, the chapter concentrates on studies published during 1995–1997 but considers all relevant studies in drawing conclusions. The likelihood that a given chemical exposure–health outcome relationship reflects a true association in humans is addressed in the context of research regarding the mechanism of interaction between the chemical and biological systems, evidence of tumorigenicity in animal studies, evidence of an association between exposure and health outcome occurrence in humans, and/or evidence that a given outcome is associated with occupational or environmental chemical exposures. It must be recognized, however, that given the limitations of existing biological and medical knowledge, the lack of data in support of a plausible biologic mechanism does not rule out the possibility that a causal relationship does exist.

ISSUES IN EVALUATING THE EVIDENCE

Toxicologic Studies

A valid surrogate animal model for the study of a human disease must reproduce with some degree of fidelity the manifestations of the disease in hu-

mans. Whole animal studies or animal-based experimental systems continue to be used to study herbicide toxicity because they allow for rigid control of chemical exposures and close monitoring of health outcomes. Because many of the chemical exposures presently associated with certain diseases in humans have been confirmed in experimental studies (Huff, 1993; Huff et al., 1994), data derived from such studies are generally accepted as a valuable guide in the assessment of biological plausibility.

As discussed in Chapter 3, many of the toxic effects of the herbicides used in Vietnam have been ascribed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant of some of these herbicides. This has not, however, simplified the risk assessment process because the toxicologic profile of TCDD is rather complex. In general, there is consensus that most of the toxic effects of TCDD involve interaction with the aryl hydrocarbon receptor (AhR), a protein that binds TCDD and other aromatic hydrocarbons with high affinity. Attempts to establish correlations between the effects of TCDD in experimental systems and in humans are particularly problematic, because species differences in susceptibility to TCDD have been documented. Although studies in which transformed human cell lines are employed to study AhR biology minimize the inherent error associated with species extrapolations, caution must be exercised because the extent to which transformation itself influences toxicity outcomes has yet to be fully defined. In addition, while it is generally accepted that genetic susceptibility plays a key role in determining the adverse effects of environmental chemicals, the impact of different genetic backgrounds on AhR function is not yet completely understood.

Evidence continues to accumulate that the AhR is not exclusively responsible for the toxic effects of TCDD. Thus, the dose–response relationships that arise from multiple toxicokinetic and toxicodynamic interactions must also be considered. Future attempts to define the quantitative relationship between receptor occupancy and biologic response to TCDD must consider that multiple biochemical changes may influence the overall cellular response.

These considerations, and the available scientific information regarding non-TCDD biological effects, are discussed in greater detail in Chapter 3.

Epidemiologic Studies

Environmental and/or occupational exposures to herbicides or TCDD provide data on human responses that can be compared directly to data obtained in experimental studies. Higher-than-background body burdens of dioxin have been documented in many of these groups, and details describing the major findings from these studies are reviewed in Chapters 7–11 of this report. In general, the elevated risks of cancers at various sites reported in epidemiologic studies are consistent with the known biological actions of the agents present in herbicide formulations. Although its full potential has yet to be realized, the application of

molecular and cellular measurements to epidemiologic research promises to increase our understanding of the association between herbicide exposure and disease occurrence. This may provide a significant advantage in the assessment of biologic plausibility, because biologically based epidemiologic data allow for more accurate identification and quantification of exposures. For instance, the analytical data available from individuals known to have been exposed to herbicides during the Vietnam War constitute a valuable resource for the study of TCDD-related disease, with documented TCDD body burdens providing a quantitative bridge between experimental studies and human epidemiology. Taken together, experimental studies and epidemiologic investigations provide complementary perspectives from which to view human health effects of exposure to herbicides. However, it must be recognized that the ultimate test of associations between exposure and disease occurrence lies in data obtained from human populations.

To obtain additional information pertinent to the evaluation of the potential effects of herbicide exposure of veterans, this and previous committees decided to review studies of other groups potentially exposed to the herbicides contained in Agent Orange, to other herbicides, and to dioxin, the contaminant believed to cause many of the purported adverse effects of Agent Orange. These study populations include industrial and agricultural workers, Vietnamese citizens, and people exposed environmentally as a result of residing near the site of an accident or a toxic waste dump. The committee felt that reviewing the studies of such groups would help in determining (1) whether these compounds could be associated with particular health outcomes in veterans and (2) the nature of any dose–response relationships, although the committee acknowledged that such findings may have only an indirect bearing on the association in veterans themselves. It is also important to note that the categories of association described below relate to the association between exposure to chemicals and health outcomes in human populations, not to the likelihood that any individual's health problem is associated with or caused by the herbicides in question.

The Role of Case Studies and Other Studies with No Comparison Groups

With the exception of one condition, the committee did not specifically consider case studies or other published studies lacking a control or comparison group. The one exception involved studies of acute and subacute transient peripheral neuropathy. The committee elected to consider case histories from occupational cohorts and descriptive reports of the Seveso accident when evaluating the association between exposure and these conditions because their transient nature precluded using case-control and other types of studies with comparison populations.

Publication Bias

The phenomenon known as publication bias is also of concern to the committee. It has been well documented (Begg and Berlin, 1989; Berlin et al., 1989; Dickersin, 1990; Dickersin et al., 1992; Easterbrook et al., 1991; Stern and Simes, 1997) 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 that are based solely on the published literature could be biased in favor of a positive association. In general, however, for reports of overall associations with exposure, the committee did not consider the risk of publication bias to be high among studies of herbicide exposure and health risks. The committee took this position because there are numerous published studies showing no positive association; because it examined a substantial amount of unpublished material; and because the committee felt that 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 considerable.

Nevertheless, publication bias of a more specific and subtle form may still have had a bearing on the committee's evaluation of the evidence. In particular, the relationship between timing and duration of exposure and subsequent changes in risk of disease was a major concern. This more subtle bias would arise if decisions to publish *specific* findings relative to timing of exposure were based on the statistical significance of those findings. For example, a study of production workers by the National Institute for Occupational Safety and Health (NIOSH) (Fingerhut et al., 1991) found a more substantial increase in risk among those whose exposure had begun more than 20 years previously and had lasted for more than a year. Many other studies did not publish data relevant to the issues of duration of, and time since, exposure. In most cases, it is impossible to know whether or not such issues were examined and simply not discussed in a publication solely because no "interesting" associations were found. Even decisions to examine or not examine such relationships may have been based on the investigators' perception that such analyses would or would not lead to any statistically significant findings.

The Role of 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, confounding, or chance on the reported results; integrating the overall evidence within and across diverse areas of research; and formulating the conclusions themselves. These aspects of the committee's review required thoughtful

consideration of alternative approaches at several points. They could not be accomplished by adherence to a narrowly 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 about association expressed in this report are based on the committee's collective judgment. The committee has endeavored to express its judgments as clearly and precisely as the data allowed.

Integration of New Evidence

As stated above, this second biennial update concentrates on evaluating the evidence published following the completion of work on *Update 1996* and *VAO*. For each disease, the new evidence is evaluated, and conclusions are based on the totality of accumulated evidence, not just on recently published studies. For one health outcome—urinary bladder cancer—the committee found new evidence that was sufficient to change the conclusion reached in previous reports. Although there is no evidence that exposure to herbicides is related to bladder cancer, relative risks in largest groups of exposed individuals under study tended to be greater than 1. This new information led the committee to change the classification of bladder cancer from *limited/suggestive evidence of* no *association* to *inadequate or insufficient evidence to determine whether an association exists*. For all other health outcomes, evidence appearing since the publication of *Update 1996* reinforced, or was not considered strong enough to change, the previous conclusions.

SUMMARY OF THE EVIDENCE

Categories of Association

The categories of association used by the committee are the same as those used in the previous reports. Consistent with the charge to the Secretary of Veterans Affairs in P.L. 102-4, the distinctions between the categories are based on "statistical association," not on causality. Thus, standard criteria used in epidemiology for assessing causality (Hill, 1971) do not strictly apply. The distinctions between the categories 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 well characterized and appropriately measured. The categories of association are as follows:

- *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, this may constitute sufficient evidence for an association.

- *Limited/Suggestive Evidence of an Association* Evidence is suggestive of an association between herbicides and the outcome, but it is limited because chance, bias, and confounding could not be ruled out with confidence. For example, if at least one high-quality study shows a positive association but the results of other studies are inconsistent, this may constitute limited/suggestive evidence of an association.

- *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, if studies fail to control for confounding, contain inadequate exposure assessment, or have inadequate sample size, this may constitute inadequate/insufficient evidence to determine whether an association exists.

- *Limited/Suggestive Evidence of* No *Association* There are several adequate studies, covering the full range of exposure levels that humans 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.

REFERENCES

Begg CB, Berlin JA. 1989. Publication bias and dissemination of clinical research. Journal of the National Cancer Institute 81:107–115.

Berlin JA, Begg CB, Louis TA. 1989. An assessment of publication bias using a sample of published clinical trials. Journal of the American Statistical Association 84:381–392.

Dickersin K, Min Y-I, Meinert CL. 1992. Factors influencing publication of research results: follow-up of applications submitted to two institutional review boards. Journal of the American Medical Association 267:374–378.

Dickersin K. 1990. The existence of publication bias and risk factors for its occurrence. Journal of the American Medical Association 263:1385–1389.

Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR. 1991. Publication bias in clinical research. Lancet 337:867–872.

Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. 1991. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New England Journal of Medicine 324:212–218.

Hill, AB. 1971. Principles of Medical Statistics, 9th ed. New York: Oxford University Press.

Huff J, Lucier G, Tritscher A. 1994. Carcinogenicity of TCDD: experimental, mechanistic, and epidemiologic evidence. Annual Review of Pharmacology and Toxicology 34:343–372.

Huff J. 1993. Chemicals and cancer in humans: first evidence in experimental animals. Environmental Health Perspectives 100:201–210.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Institute of Medicine. 1997. Characterizing Exposure of Veterans to Agent Orange and Other Herbicides Used in Vietnam: Scientific Considerations Regarding a Request for Proposals for Research. Washington, DC: National Academy Press.

Stern JM, Simes RJ. 1997. Publication bias: evidence of delayed publication in a cohort study of clinical research projects. British Medical Journal 315:640–645.

5

Exposure Assessment

MILITARY USE OF HERBICIDES IN VIETNAM

Background

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. 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). 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 river boats 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).

Four major compounds were used in the Ranch Hand herbicide formulations—2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 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.

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.

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 (see Table 5-1). From 1962 to 1965, small quantities of Agents Purple, Blue, Pink, and Green were used. From 1965 to 1970, Agents Orange, White, and Blue were employed; from 1970 to 1971, only Agents White and Blue were used in the defoliation program (Young and Reggiani, 1988).

Agent Purple was a 5:3:2 mixture of the *n*-butyl ester of 2,4-D and the *n*-butyl and isobutyl esters of 2,4,5-T that was used on broadleaf plants. 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

TABLE 5-1 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 and Reggiani, 1988.

Phytar 560-G. Cacodylic acid is a highly soluble organic arsenic compound that is readily broken down in soil. Approximately one-half of all Agent Blue was used for crop destruction missions; it was the agent of choice for destruction of rice crops. 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. Agent Green was a single-component formulation of the *n*-butyl ester of 2,4,5-T, used 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. 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).

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. 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.

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).

The military use of 2,4,5-T, and thus Agent Orange, was suspended by the U.S. Department of Defense in April 1970 (Young and Reggiani, 1988). 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).

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 river boats, 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, for example, around depots, airfields, and outposts (Collins, 1967). However, because these helicopter and ground sprays were less rigidly controlled than fixed-wing aerial spraying, 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 river 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." 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 (DoD) 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, 1979). 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 had been biodegraded or photo degraded (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, 1979).

A very different picture arose when the U.S. General Accounting Office (U.S. GAO, 1979) 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 during this period. 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, 1979).

Level of Dioxin (TCDD) in Herbicides Used in Vietnam

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, TCDD, or dioxin) is a contaminant of 2,4,5-T. Small quantities of other dioxins are present in 2,4-D. 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.

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 either had 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 the 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; the estimate for Agents Pink and Green was 65.6 ppm (Young et al., 1978). As a result of TCDD contamination in the herbicides, it has been estimated that about 368 pounds of dioxin was sprayed in Vietnam over a six-year period (Gough, 1986).

OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES TO HERBICIDES AND DIOXIN

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 pasture lands to enable desirable grasses to grow. 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. Railroads, utility companies, and certain government agencies have used 2,4,5-T to limit the growth of weeds along railroad tracks, next to power lines, and along highways. 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).

In investigating the possible health effects of exposure to herbicides in Vietnam, the committee also looked at available information on occupational and environmental exposures to dioxin, the contaminant found in 2,4,5-T. These studies included residents living in and around Seveso, Italy, who were exposed during industrial accidents; chemical plant workers who were occupationally exposed to TCDD during the production of 2,4,5-T or other phenoxy herbicides or chlorophenols such as hexachlorophene or trichlorophenol; sawmill workers exposed to higher chlorinated dioxins that contaminated wood preservatives; pulp and paper workers exposed to dioxin through the pulp bleaching process; and residents of China exposed to dioxin as a contaminant in a pesticide used to prevent schistosomiasis.

2,4-D, the other herbicide used in Agent Orange and a constituent of Agents Purple and White, has attracted less interest from researchers because published studies do not indicate it is contaminated with 2,3,7,8-TCDD. Rather little research has been conducted on exposures to the two other primary herbicides used in Vietnam, picloram and cacodylic acid.

In Vietnam and in most of the occupational and environmental studies examined by the committee, subjects could have been exposed to a number of other chemicals besides herbicides or TCDD. In some cases the exposure mixture included a variety of dioxin and dibenzofuran congeners. Attempts to assess the

toxicity of these mixtures are discussed later in this chapter as well as in Chapter 3. In other cases the exposure mixtures include a range of herbicides, fungicides, insecticides, wood preservatives, insect repellents, and other chemicals that might be used in the industry being studied. In such situations it is possible that these co-exposures could be confounding the association of herbicides or TCDD with the disease. Alternatively, these co-exposures may act synergistically with the herbicides, increasing the risk from exposure.

EXPOSURE ASSESSMENT FOR EPIDEMIOLOGY

The committee was asked to 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. This and previous committees have all found that the weakest methodologic aspect complicating the interpretation of the available epidemiologic studies is the definition and quantification of exposure.

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 that substance. Accurate estimation of any risk associated with exposure depends on the ability to 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 only to assess the presence or absence of exposure, but also to characterize the *degree* of exposure—its *intensity* and *duration*. Exposure assessment contributes to the epidemiologic study process in several ways. First, well-defined contrasts in the exposure of groups being studied increase the validity of individual and group risk assessments. A poorly defined contrast could result, for example, if a group of people assumed to be exposed to a particular agent contained many individuals who were not, in fact, truly exposed. Second, very large groups must be studied to identify the small risks associated with low levels of exposure, whereas 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* or statistical power is also linked to the extent of the exposure and the accuracy of its measurement.

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 veter-

ans 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. 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, 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, as opposed to the simple exposed/unexposed comparison described above, should (if the classification of exposure is done without serious errors) yield less diluted risk estimates and provide support for a dose–response trend. This method also does 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 epidemiologic studies, quantitative exposure data are sometimes developed through a process called *historic exposure reconstruction*. In occupational cohort studies, work records and industrial hygiene data may be available that cover the entire history of the factory being studied. In this way 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, including the use of expert judgment, development of physical process models, and the extrapolation of sampling or production data. An analogous approach for Vietnam veterans might, for example, distinguish individuals by dates of service, proximity to herbicide spraying, and job responsibilities relative to herbicides.

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 exposure subgroups of the cohort, so that the total exposure (sometimes called *cumulative exposure*) is proportional to its duration. Based on these assumptions, one would hypothesize that a true risk would increase with the duration of exposure.

Another form of quantitative exposure assessment involves the use of biomarkers for the agent of interest. TCDD and other chlorinated dibenzo-*p*-dioxins and dibenzofurans are found in blood and tissues of nonoccupationally exposed humans at part-per-trillion (nanogram-per-kilogram) levels. After 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).

Although exposure to TCDD from environmental sources occurs on a continuing basis (Geyer et al., 1986; Byard, 1987), 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. This means that, in theory, TCDD levels in the body long after exposure could be used to estimate TCDD levels at the end of exposure using a pharmacokinetic model and the clearance rate (half-life) of TCDD in the body. Several epidemiologic studies have tried this approach to estimating exposures in the cohort under study (Flesch-Janys et al., 1995; Ott and Zober, 1996; Ramlow et al., 1996; and others). However, various authors have reported different estimates of TCDD half-life (Pirkle et al., 1989; Needham et al., 1994; Michalek et al., 1996a; Flesch-Janys et al., 1996; Michalek et al., 1997), and TCDD half-life is likely to change as a persons weight and percentage of body fat change when the person ages (Flesch-Janys et al., 1996). In addition, serum TCDD levels have been shown to vary with several other personal characteristics, including age, race, body mass, region of residence, and smoking status (Devine et al., 1990; Flesch-Janys et al., 1996). Although quantitative measures of exposure are highly desirable, a biomarker, especially one gathered years after exposure, is not necessarily better than qualitative exposure measures. For example, Fingerhut et al. (1989) reported that "years exposed" was correlated with both current TCDD level ($r = .82$) and estimated TCDD level at the end of exposure ($r = .80$). Group differences in serum TCDD levels can be useful in confirming that occupational exposure measures reflect true differences in exposure; this has been done in studies by the National Institute for Occupational Safety and Health (NIOSH) (Fingerhut et al., 1989, 1991; Sweeney et al., 1990) and others.

To summarize, epidemiologists generally think of the various exposure assessment strategies described above in a hierarchy of increasing accuracy: the exposed/unexposed approach is 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.

The strength of association between an exposure and a disease is only one of the criteria used in evaluating epidemiologic evidence. 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 the degree of exposure among different cohorts or subcohorts of the study can be determined. 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.

Once an exposure–disease association has been established, it is often desirable to consider the implications for some exposed population other than the population in which the study was performed. In making this inference, it is important to have exposure assessments that allow valid comparisons of exposure of the different study populations. For example, if an increased risk of a particular disease has been demonstrated in workers occupationally exposed to an herbicide for a long period, 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 (integrating both level and duration), with risk assessed per unit of exposure. If the exposure levels are unknown or poorly characterized, then extrapolating from one population to another may be difficult.

The types of occupational and environmental exposure situations studied, and the likely intensity and duration of the exposures to herbicides and TCDD, are diverse. In principle, this provides an opportunity to compare results between studies to determine whether certain diseases are more common in populations likely to have higher exposures. However, because of the complex pattern of exposures to 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 a particular herbicide or with TCDD in the mixed exposure setting. Attempts to assess the toxicity of mixtures of dioxin and dibenzofuran congeners are discussed later in this chapter as well as in Chapter 3.

In August of 1997, the committee hosted a workshop for many of the researchers involved in studies of Vietnam veterans and individuals exposed to herbicides or dioxin. The goal of the workshop was to discuss the feasibility of using current data combination techniques (such as meta-analysis and data pooling) with existing data bases to further investigate the health effects of herbicide and dioxin exposures. The question of which analysis techniques, which data sets, and which health outcomes might be best suited for such an approach was

also discussed. A review of that workshop will be released as a National Academy of Sciences report.

EXPOSURE ASSESSMENT IN STUDIES OF VIETNAM VETERANS

Different approaches have been used to estimate the exposure of Vietnam veterans, including self-reported exposures, records-based exposure estimates, or biomarkers of TCDD exposure. Each approach 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 combat experience—as proxies for exposure to herbicides. Studies of this type include the Centers for Disease Control and Prevention's (CDC's) Vietnam Experience Study and Selected Cancers Study, the Department of Veterans Affairs' (DVA's) mortality studies, and most studies of veterans conducted by states. This approach almost surely dilutes whatever health effects of herbicides exist, because many members of the cohort presumed to be exposed to herbicides may, in reality, not have been.

Ranch Hands and Army Chemical Corps

Military occupation has been shown to be a valid exposure classification for two specific occupations that involved the direct handling and distribution of herbicides: the Air Force Ranch Hands, who were responsible for aerial spraying of herbicides, and 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 for jobs that involved more frequent handling of herbicides. The median TCDD level for enlisted ground crew (24 parts per trillion [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 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 on 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 duration 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 an individual's tour of duty, divided by the number of Ranch Hands sharing such duties during this individual's tour. Each Ranch Hand was placed in an exposure category (high, medium, or low) based on the value of the individual's exposure index. The index included 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.

More recently, Michalek et al. (1995) developed several indices of herbicide exposure for members of the Ranch Hand cohort and tried to relate these to the levels of serum TCDD measured between 1987 and 1992. Self-administered questionnaires completed by veterans of Operation Ranch Hand were used to develop three indices for herbicide or TCDD exposure: (1) the number of days of skin exposure; (2) the percentage of skin area exposed; and (3) the number of days of skin exposure, times the percentage of skin exposed, times a factor for the concentration of TCDD in the herbicide. A fourth index used no information gathered from individual subjects. It was calculated as the volume of herbicide sprayed during a specific individual's tour of duty, times the concentration of TCDD in herbicides sprayed in that period, divided by the number of crew members at that time in each job specialty.

Each of the four models tested was significantly related to the serum TCDD level, although each explained only between 19 and 27 percent of the variability in serum TCDD. Days of skin exposure had the highest correlation. Military job classification (non-Ranch Hand combat troops, Ranch Hand administrators, Ranch Hand flight engineers, and Ranch Hand ground crew), which is separate from the four indices, explained 60 percent of the variance in serum TCDD concentrations. When the questionnaire-derived indices were applied within each job classification, days of skin exposure added significantly, but not substantially, to the variability explained by job alone.

Other Vietnam Veterans

Surveys of Vietnam veterans who were not part of the Ranch Hands or Chemical Corps groups indicate that 25 to 55 percent believe they were exposed to herbicides (Erickson et al., 1984 a,b; Stellman and Stellman, 1986; CDC, 1989). A few attempts have been made to estimate exposures of the Vietnam veterans who were not part of the Ranch Hand or Chemical Corps groups. The CDC was involved in two such studies: the CDC Agent Orange Study (CDC, 1985) and the CDC Birth Defects Study which developed an exposure opportunity index (EOI) to score Agent Orange exposures (Erickson et al., 1984 a,b).

As part of a case-control study to determine if there was an increased risk of birth defects among the offspring of Vietnam veterans, an Agent Orange exposure assessment was done (Erickson et al., 1984 a,b). 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 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.

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. 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.

In 1983, the CDC was assigned by the U.S. government to conduct a study of the possible long-term health effects of Vietnam veterans' exposures to Agent Orange. The Agent Orange Study 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 spraying using military records to track troop movement and the HERBS tapes to locate herbicide spraying patterns. The original study was to involve three cohorts, each containing approximately 8,500 men.

The DoD Environmental Services Group 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" were calculated at the company level (about 250 men), based on a reported unit location occurring within a specified time and distance from a known herbicide application. Three exposure scores were proposed—short, intermediate, and chronic—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. 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" and that there was substantial variability in exposure scores among units and among individual veterans (CDC, 1985).

To test the validity of several indirect methods for estimating exposure of ground troops to Agent Orange in Vietnam, in 1987 the CDC Agent Orange Validation Study measured serum TCDD levels in a nonrandom sample of Vietnam veterans and Vietnam era veterans who did not serve in Vietnam (CDC, 1988). 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 the "high" exposure group included 191 veterans. 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, 1988).

The median TCDD level in Vietnam veterans was 4 ppt, with a range from less than 1 to 45 ppt and two veterans having levels greater than 20 ppt; the distributions of these measurements were nearly identical to those for the control group of 97 non-Vietnam veterans. In other words, the CDC's Validation Study found that study subjects could not be distinguished from controls based on serum TCDD levels. In addition, 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, 1988). 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.

In addition, these serum TCDD levels in Vietnam veterans suggest that the exposure to TCDD in Vietnam was substantially less, *on average*, than that of occupationally exposed workers, of persons exposed as a result of the industrial explosion in Seveso, Italy, or of the heavily exposed occupational workers that are the focus of many of the studies evaluated by the committee. As noted above,

this estimation of *average* exposure does not preclude the existence of a heavily exposed subgroup of Vietnam veterans.

In 1997, a committee convened by the Institute of Medicine developed a Request for Proposals (RFP) seeking individuals and organizations capable of conducting research to develop one or more historic exposure reconstruction approaches suitable for epidemiologic studies of herbicide exposure among U.S. veterans during the Vietnam War (IOM, 1997). These approaches were to incorporate information from, for example, existing data bases, biomarker data, and supplemental material gathered from surveys of military personnel, governmental and nongovernmental organizations, and other sources. Work funded under this RFP began in 1998.

EXPOSURE ASSESSMENT IN OCCUPATIONAL AND ENVIRONMENTAL 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 using 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, sawmill workers, 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.

Studies of environmental exposures are related primarily to unintentional releases of TCDD into the environment at Seveso, Italy, or in an area where herbicides or antiparasitic pesticides were applied. 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.

The studies described below are also addressed in detail in Chapter 6 of this report, in Chapter 6 of *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (henceforth called *VAO*) (IOM, 1994), and Chapter 5 of *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996).

Production Workers

The Netherlands

Two plants in the Netherlands that manufactured phenoxy herbicides and chlorophenols between 1955 and 1986 were studied by Bueno de Mesquita et al.

(1993). In one of the plants an accident in 1963 resulted in the release of polychlorinated dibenzo-*p*-dioxins (PCDDs). Cohort exposure was based on employment records and interviews. Workers were considered "exposed" if they worked in any of several departments, had been exposed as a result of the 1963 accident, or entered an exposed department at least once a week on a regular basis. This initial study was part of the first International Agency for Research on Cancer (IARC) analysis (Saracci et al., 1991). A follow-up of the cohort included a more detailed exposure assessment using serum TCDD levels of a subset of workers to model historical exposure levels (Hooiveld et al., 1996). This expanded follow-up cohort was included in the second follow up IARC analysis (Kogevinas et al., 1997).

United Kingdom

Workers from four factories that manufactured phenoxy herbicides and chlorophenols between 1963 and 1985 were studied by Coggon et al. (1991) and Kauppinen et al. (1994). No air or biological sampling was done. Subjects were classified according to their potential exposure to phenoxy herbicides and chlorophenols based on job histories. These cohorts were included in the first IARC analysis (Saracci et al., 1991) and the second follow-up IARC analysis (Kogevinas et al., 1997).

First IARC Study

A multisite study by IARC involved 18,390 production workers and herbicide sprayers from 20 cohorts in 10 countries (Saracci et al., 1991). Included in this analysis are the cohorts from the Netherlands and the United Kingdom described above. Four of the cohorts included workers who sprayed 2,4,5-T, 2,4-D, or 2-methyl-4-chlorophenoxyacetic acid (MCPA). Exposure for this study was estimated from a combination of factory records, work histories, and questionnaires. The cohort was subdivided 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 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 MCPA

or mecoprop (MCPP), which were unlikely to contain significant quantities of TCDD.

Two nested case-control studies were conducted using this cohort (Kogevinas et al., 1995). For the case-control studies, exposures to 21 chemicals were estimated on a relative scale by three industrial hygienists. A cumulative exposure score was calculated for each subject for each chemical, based on the individual's work history and knowledge of the determinants of exposure such as department or job, use of personal protective equipment, contact with chemicals and other factors (Kauppinen et al., 1994). In the case-control analysis, the cumulative exposure scores for each chemical were divided into low-, medium-, and high-exposure categories (Kogevinas et al., 1995).

United States

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 that contained TCDD) to vent out through the chimney and into the inside of the building (Gough, 1986). The workers have been studied since the 1980s to evaluate the health impact of their exposures to TCDD (Zack and Suskind, 1980; Zack and Gaffey, 1983; Collins et al., 1993). No exposure measurements were made of this cohort; exposure was determined by their having chloracne, by their presence in the plant during a 9-month period after the accident, or in one study, by their having worked in the 2,4,5-T production area.

The Dow Chemical Company plant in Midland, Michigan was another widely studied U.S. plant. This plant produced 2,4,5-T and its esters—trichlorophenol and pentachlorophenol—(Cook et al., 1986; Ott et al., 1987; Bond et al., 1989). By using wipe samples, process streams, and intermediate product information, jobs in the various departments during different time periods were ranked on a scale of 0 to 4 for intensity of exposure to TCDD, with each unit indicating an increase on a logarithmic scale. Cumulative exposure indices were calculated for each "exposed" job by multiplying the duration of each job by the estimated intensity of exposure and then summing across all exposed jobs. Recently, Ramlow et al. (1996) have done an update study of this cohort, using the cumulative exposure index as part of the analysis.

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. This study of 12 chemical companies, included the Nitro, West Virginia, and Midland, Michigan, plants described above as well as others. 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 the length of time working in processes involving TCDD contamination and the 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 the duration of employment. For the full cohort, the duration of exposure in a process involving TCDD contamination was used as the primary exposure metric. It should be noted that workers were exposed concurrently to other chlorophenols and phenoxy herbicides that were contaminated with TCDD, as well as to numerous other chemicals during their employment.

Germany

In 1953 the BASF production unit at Ludwigshafen, Germany, which produced trichlorophenol, had an accidental release. The primary exposure classification scheme used was to classify workers exposed during the accident or during subsequent cleanup and repair and those not exposed (Zober et al., 1990). Also analyzed were workers who developed chloracne and those who did not. Later studies of this cohort estimated the TCDD dose for the entire cohort using a statistical model describing the relationship between various exposure conditions and TCDD blood levels for a subset of 138 workers. Then, using work history information and TCDD half-life data the authors estimated the TCDD levels of each worker in the cohort at the time of exposure (Ott et al., 1993; Ott and Zober, 1996). There were subsets of this cohort that were also exposed to aromatic amines, such as β-naphthylamine, and to asbestos; these data were collected during the exposure assessment. This cohort was not included in any of the IARC studies.

Another German cohort studied involved workers from a herbicide manufacturing plant of Boehringer-Ingelheim in Hamburg, Germany. Assessment of exposure to TCDD was based on samples of materials, products, waste, and soil from the plant. Based on this information, workers were classified as having high, medium, or low exposure according to the production departments in which they worked (Manz et al., 1991). Flesch-Janys et al. (1995) did an update of this cohort and added quantitative exposure assessment based on blood or adipose measurements of polychlorinated dibenzo-*p*-dioxin and furan (PCDD/F). Using a first-order kinetics model, half-lives from an elimination study in 48 workers from this cohort, and background levels for the German population, the authors estimated PCDD/F levels for the 190 workers with serum or adipose measurements of PCDD/F. Then regressing the estimated PCDD/F level of these workers at the end of their exposure against the time they worked in each production department in the plant, the authors estimated the contribution of the working time in each

production department to the PCDD/F level at the end of exposure. These production department working time "weights" were then used, along with the work histories of the remainder of the cohort, to estimate the PCDD/F level for each cohort member at the end of the person's exposure. The epidemiologic analysis used these estimated TCDD doses.

Becher et al. (1996) report an analysis of several German cohorts including the Boehringer-Ingelheim cohort described above, a cohort from the BASF Ludwigshafen plant that did not include those involved in the 1953 accident, and a cohort from a Bayer plant in Uerdingen and a Bayer plant in Dormagen. All of the plants were involved in the production of phenoxy herbicides or chlorophenols. Exposure assessment involved the estimation of duration of employment from the start of work in a department with suspected exposure until the end of employment at the plant. This may include some periods without exposure. Analysis was based upon time since first exposure.

Second IARC Study

Kogevinas et al. (1997) updated and expanded the international cohort studied by Saracci et al. (1991) to include the data of Fingerhut et al. (1991) and Becher et al. (1996). The study population was classified as exposed to phenoxy herbicides or chlorophenols based primarily on individual job records and company exposure questionnaires. The great majority of workers were classified as exposed if they had ever worked in the production or spraying of phenoxy herbicides or chlorophenols (four cohorts were an exception with minimum employment periods of 1 to 12 months). The exposed workers were aggregated into five groups: main production, maintenance, other exposed jobs, unspecified tasks, and sprayers. Based on these categories and on information about production processes and the composition of the materials used, the exposed workers were further classified into three categories: (1) exposed to TCDD or higher chlorinated dioxins; (2) unexposed to TCDD or higher chlorinated dioxins; and (3) unknown exposure to TCDD or higher chlorinated dioxins.

Agricultural, Forestry, Outdoor Workers, and Environmental Exposures

Agricultural 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 the duration or intensity of exposure or to determine the specific type of herbicide or chemical to which a worker was

exposed. Because agricultural workers are often exposed to a wide variety of herbicides, pesticides, and other chemicals, it is difficult to associate diseases with any particular exposure in this setting.

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., 1988; Hansen et al., 1992; Ronco et al., 1992). Other studies have used county of residence as a surrogate of exposure, relying on 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 exposure according to 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).

Herbicide and Pesticide Sprayers

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 specifically to phenoxy herbicides or TCDD. Individual estimates of the intensity and frequency of exposure were rarely quantified in the studies the committee examined, however, and applicators often were known to have sprayed 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.

Paper and Pulp Mill Workers

An occupational group thought to be exposed to TCDD and chlorinated phenols consists of paper and pulp mill workers. When free chlorine gas is used to bleach pulp, TCDD can be produced. Although TCDD has been found in chlorine-bleached pulp, studies of pulp and paper mill workers have not, to date, shown elevated levels of dioxins in their biological tissues (Rosenberg et al.,

1994, 1995; Mouradian et al., 1995). 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).

Sawmill Workers

Workers in sawmills may be exposed to pentachlorophenates, which are contaminated with higher chlorinated PCDDs (Cl_6–Cl_8) or tetrachlorophenates, which are less contaminated with higher chlorinated PCDDs. The wood is dipped in these chemicals and then cut and planed in the mills. Most exposure is dermal, although some exposure can occur via inhalation (Teschke et al., 1994; Hertzmann et al., 1997).

Seveso, Italy

One of the largest industrial accidents involving environmental exposures to TCDD occurred in Seveso, Italy, 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: 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 the 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. The Seveso cohort continues to be monitored, including a 15-year follow-up of mortality (Bertazzi et al., 1997) and a continuing examination of serum TCDD levels (Pesatori, 1995; Needham et al., 1997).

Vietnamese Studies

Several studies have investigated exposure to herbicides among the residents of southern Vietnam, comparing unexposed residents of the South to residents of the North (Constable and Hatch, 1985). Other studies have attempted to identify North Vietnam veterans 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).

REVIEW OF THE SCIENTIFIC LITERATURE

Several studies published since the release of *Update 1996* provided useful information for the refinement of exposure assessment strategies. Although none of these studies led the committee to revise its basic view of the role of exposure assessment in the evaluation of epidemiologic studies, several recent reports warrant brief discussion here. The studies fall into three categories: (1) investigations of the clearance of TCDD from the human body, (2) evaluations of other types (congeners) of dioxins found in humans exposed to herbicides and related chemicals, and (3) the development of several exposure indices for epidemiologic studies.

TCDD Half-Life Investigations

The pharmacokinetics of TCDD in humans—its absorption, 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 that rely on these measures of exposure. A complex, poorly understood process distributes dioxins among body tissues and slowly clears them 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 backwards to estimate the likely concentration of TCDD in fat or blood in the past. It is also often 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. Furthermore, the metabolic processes governing this movement and disposition may not be relevant in the determination of the dose of TCDD to specific areas, such as the brain or reproductive organs. In the epidemiologist's view, the "causal pathway" linking exposure to the biomarker (serum or fat TCDD) may be different from that linking exposure to disease. This can also be complicated by the role of individual susceptibility factors, which may be polymorphic in humans and hence increase or decrease the risk of TCDD-related disease (Pesatori, 1995).

A number of studies have tried to estimate the half-life of TCDD in humans. These have been reported in *VAO* or *Update 1996*. For example, in a study of 36 Ranch Hand veterans, the median half-life of TCDD was estimated to be 7.1 years (Pirkle et al., 1989). 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 (Wolfe et al., 1994). Using 213 Ranch Hands veterans with

three repeated blood serum measurements from 1982, 1987, and 1992, Michalek et al. (1996a) estimated a half-life of 8.7 years. An erratum published for this study changed that estimate to 8.5 years (Michalek et al., 1997).

A study of 27 persons exposed during the 1976 TCDD release in Seveso, Italy, and followed for 15.9 years, yielded a mean half-life estimate of 8.2 years and a median half-life estimate of 7.8 years (Needham et al., 1994).

A recently published study of 48 German workers exposed to TCDD in a plant producing herbicides showed a median half-life of 7.2 years (Flesch-Janys et al., 1996). The time between the first and last analysis was 6.3 years. Increasing age and percentage of body fat were associated with increasing half-life for most congeners. Smokers in general had a faster decay rate than non- or ex-smokers.

Also during this review a study investigating the reliability of serum TCDD measurements using paired samples from 46 Ranch Hands veterans was reported. The coefficient of reliability for these repeated measurements was 0.87 when the measurement was made at 50 ppt dioxin or less. When it was more than 50 ppt dioxin, the coefficient of reliability was 0.93, but only if the measurements were analyzed after logarithmic transformation (Michalek et al., 1996b).

Other Dioxin Congeners

In addition to 2,3,7,8-TCDD, other congeners of dioxin and dibenzofuran contaminated the herbicides sprayed in Vietnam, as well as the products used and manufactured by the occupational cohorts whose health experience forms the basis for many of the committee's conclusions. Because these may contribute to cancer risk, dioxin "toxic equivalent factors" (Teq factors) have been estimated for the various other congeners of dioxin and dibenzofuran (U.S. EPA, 1989). A Teq factor for each dioxin or furan congener is estimated by comparing its toxicity to that of 2,3,7,8-TCDD, which is arbitrarily assigned a Teq factor of 1.0. Other congeners have lower Teq factors, some as much as 1,000 times lower. In principle, it is possible to measure each congener and calculate a toxic equivalent for the entire mixture, but this is costly. Most studies of dioxin-exposed individuals have related health effects to TCDD levels only and have not considered other associated dioxins or furans.

The use of 2,3,7,8-TCDD alone as a measure of risk when exposure includes many congeners must be considered cautiously. Different sources of dioxin contamination may have different distributions of congeners. Also, the stability of the different congeners in the environment differs, so that human exposures occurring long after spraying may differ from those at the time of spraying. Finally, the half-lives of different congeners in the body differ, so that an exposed individual will have varying patterns of exposure to each congener over time. Therefore, although it is probably not feasible to conduct a total congener analysis in every study, the use of TCDD measurements alone may represent an oversimplification of the full exposure picture.

Since *Update 1996*, Flesch-Janys et al. (1996) have published a study reporting the half-lives for various dioxin congeners. These ranged from 3.7 years for heptachlorinated dibenzodixoin (1,2,3,4,6,7,8-HpCDD) to 15.7 years for pentachlorinated dibenzodioxin (1,2,3,7,8-PCDD). For the furans, the median half-lives were between 3.0 and 19.6 years.

Schecter et al. (1996a) measured TCDD and its congeners among 50 Vietnam veterans from the state of Michigan, chosen on the basis of their likely exposure to Agent Orange in Vietnam. They measured the levels of dioxin and dibenzofuran congeners in blood samples. They found average 1,2,3,4,6,7,8-HpCDD levels 13 times higher than the average TCDD levels and average 1,2,3,4,6,7,8,9-OCDD levels 88 times higher than average TCDD levels. The dioxin total Teq level averaged 24.7 for these veterans, with an average total Teq level of 31.8 when dibenzofuran congeners were also included. This report described semen sample levels for a subset of this population ($N = 17$) as well; these were pooled into 3 sets of samples for analysis. Average 1,2,3,4,6,7,8-HpCDD levels 30 times higher than average TCDD levels were found, along with average 1,2,3,4,6,7,8,9-OCDD levels that were more than 260 times higher than average TCDD levels. The dioxin total Teq level for these semen samples averaged 0.010, with an average total Teq level of 0.013 when dibenzofuran congeners were also included. DeVito et al. (1995) report that background Teq blood levels of dibenzodioxins and dibenzofurans vary from 28 to 41 ng/kg (lipid adjusted).

Sodium pentachlorophenol (NaPCP) has been widely used in the control of schistosomiasis. Dioxin is a contaminant in NaPCP. During 1972, 1973, and 1978 more than 1,300 tons of 5-ppm NaPCP were sprayed in problem areas in central China. Samples were collected from sprayers or handlers of NaPCP, from persons living for more than 30 years (or their whole lives, if younger) in areas where NaPCP was sprayed, and persons living in unsprayed area (Schecter et al., 1996b). Samples were pooled for analysis, so that for each category there is only one sample result. The dioxin total Teq levels in blood and breast milk samples from residents who lived and/or worked in the sprayed areas were about two times the levels of those from nonsprayed areas. Those living in the area when spraying was done had a total Teq level of 16.3, which is 2.6 times higher than that in nonsprayed areas (6.4 Teq). Analysis for dioxin congeners was done for a bulk sample of the NaPCP product that was sprayed, four sediment samples from a lake where NaPCP was sprayed, and personal blood and breast milk samples. A similar pattern of dioxin congener levels was found in all samples, suggesting a "fingerprint" that may represent NaPCP exposures in this area of China.

TCDD Exposure Levels for Selected Studies

Flesch-Janys et al. (1995) reported updated results for a cohort of workers ($N = 1,184$) in a German plant where herbicides and insecticides were produced (2,4,5-T, trichlorophenol, bromophos, and lindane). The original study (Manz et

al., 1991) used exposure surrogates such as duration of employment, time of entry into the plant, and qualitative exposure groups in the mortality analysis. The update increased the follow-up period of this cohort and added quantitative exposure assessment based on blood or adipose measurements of PCDD/F. Using a first-order kinetic model, half-lives from an elimination study in 48 workers from this cohort, and background levels for the German population, the authors estimated PCDD/F levels for the 190 workers with serum or adipose measurements of PCDD/F. Then regressing the estimated PCDD/F level of these workers at the end of their exposure against the time they worked in each production department in the plant, the contribution of the working time in each production department to the PCDD/F level at the end of exposure was estimated. These production department working time "weights" were then used, along with the work histories of the remainder of the cohort, to estimate the PCDD/F level for each cohort member at the end of his or her exposure. This yielded a mean estimated TCDD level at last exposure of 141.4 ng/kg (median, 38.2 ng/kg) for the cohort. The estimated TCDD level at last exposure ranged from 0 to 3890.2 ng/kg for the cohort. A total toxic equivalency was also computed for all dioxins and furans combined by summing the levels of all congeners weighted by their toxic equivalency factor relative to TCDD. The mean of toxic equivalencies for the cohort (without TCDD) was 155 ng/kg (median, 69.2 ng/kg). The mean of the total toxic equivalencies for the cohort (with TCDD) was 296.5 ng/kg (median, 118.3 ng/kg; range 1.2–4361.9 ng/kg).

Schecter et al. (1996a) measured TCDD and its congeners among 50 Vietnam veterans from the state of Michigan who were selected for having a high likelihood of exposure to Agent Orange based on self-report or history of cancer or on children with birth defects. They found TCDD levels greater than 5 ppt in 16 (32 percent) of these Vietnam veterans. Six (12 percent) had TCDD levels higher than 20 ppt 23–24 years after their potential Agent Orange exposure. The authors report the mean of the U.S. population TCDD level in blood or adipose tissue as 3.5 ppt (median, 3.1; range 1.0–7.7 ppt).

Needham et al. (1997) report TCDD levels from serum samples taken at Seveso in 1976 after the accident there. In zone A, presumed to be the area of highest exposure, serum TCDD levels for 296 residents were determined. Seven percent (N = 22) of these samples were below the limit of detection of the method. Using one-half of the limit of detection for these values resulted in a median TCDD level of 447 ppt (129–1860 ppt) for the 25th and 75th percentiles. For zone B the median value for 80 samples, with 45 percent (N = 36) below the limit of detection, was 94 ppt (51–153 ppt) for the 25th and 75th percentiles. For zone R the median value for 48 samples, with 23 percent (N = 11) below the limit of detection, was 48 ppt (22–118 ppt for the 25th and 75th percentiles).

Almost 20 years later, plasma TCDD levels were assayed among 62 individuals living in the region of Seveso, Italy, at the time of the industrial accident there (Landi et al., 1997). Among the seven from the highly exposed zone A, the

geometric mean level was 53 ppt. In zone B, 55 persons were measured and the geometric mean level was 11 ppt, with women having significantly higher TCDD levels than men. Neither presence in the contaminated area at the time of the accident, number of years spent in the zone, occupation, nor distance from the accident site within the zone, explained the gender difference. When zone B measurements were pooled, gender, distance from the accident site, and meat consumption were significantly associated with TCDD concentration. In the noncontaminated area zone non-ABR, the geometric mean TCDD levels were 4.9 ppt. Although this represents a typical background level of TCDD, the authors found that women had a significantly higher TCDD concentrations than men.

Development of Exposure Indices

In an epidemiologic study, it would be ideal to have a measure of the dose at the target organ to use in the dose–response analysis. This would necessitate the development of a pharmacokinetic model for the estimation of tissue dose over the period of interest. In a number of studies the authors have attempted to estimate dose of tetrachlorinated dibenzo-*p*-dioxin and furan (TCDD/F) at the time of last exposure by extrapolating back from more current serum TCDD measurements (Fingerhut et al., 1989; Ott et al., 1993; Flesch-Janys et al., 1995). In each case the authors chose a half-life estimate for TCDD that applied to all members of the cohort and assumed a one-compartment first-order kinetics model of TCDD levels.

As stated earlier, the caveats with the pharmacokinetic modeling done to date are several: (1) it is assumed that among all potential toxic agents in Agent Orange, TCDD is the agent responsible for the health effects of interest; (2) the congeners of TCDD are not accounted for; (3) it is assumed that a single-compartment model is sufficient for dose estimation; (4) to date, no parameters for the effects of age, smoking, percentage of body fat, or weight on clearance have been included in these models; and (5) it is also assumed that the dose received prior to the end of exposure (the "peak") was accumulated at a constant rate. Despite these caveats, developments in this area of exposure assessment may prove fruitful in attempts to pool diverse data sets relevant to evaluating the risks of exposures to Agent Orange and other herbicides in Vietnam.

When the issues of toxicokinetic modeling of dose have been addressed, a question will still remain regarding what dose metric should be used. It could be the peak dose, the average dose over a lifetime or the period of exposure, or the cumulative dose over either the individual's lifetime or the period of exposure. Many of these dose metrics will be highly correlated and will also be correlated with more simple metrics such as duration of exposure. Nevertheless, if such dose metrics could be developed, they might provide a means to pool data across studies where exposures have been measured simply in categorical terms or in terms of duration of exposure.

Scheuplein and Bowers (1995) and Aylward et al. (1996) have modeled exposure to TCDD as a simple single-compartment constant infusion process with exponential decline post-exposure. They estimate the peak concentration of TCDD—assumed to be at the end of exposure—as well as the area under the curve (AUC)—which is the cumulative dose of TCDD in parts-per-trillion-years—and the average concentration—which divides the AUC by the number of years. Aylward and colleagues used this model with the NIOSH worker cohort (Fingerhut et al., 1989) and found a great overlap in the exposures estimated for the four NIOSH exposure duration categories, regardless of the exposure metric used. It should be pointed out that in the NIOSH study, blood samples were available on only about 5 percent of those in the study cohort so that estimation of dose using blood TCDD was not possible for the whole cohort. However, because the NIOSH analysis did find a high correlation between measured TCDD levels and duration (years) of exposure (r = .72) and between estimated TCDD levels at the end of exposure (peak) and duration (years) of exposure (r = .80), exposure duration was assumed to be a good surrogate for TCDD level. Aylward has estimated the peak, average, and AUC TCDD levels of the Ranch Hand (Aylward et al., 1997) and Seveso (Hays et al., 1997) populations as well. Again, regardless of the dose metric used, there is great overlap in the estimated exposure levels for all the analysis categories used in these studies (zones in Seveso and exposure groups in Ranch Hands).

REFERENCES

Air Force Health Study (AFHS). 1991. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Serum Dioxin Analysis of 1987 Examination Results. Brooks AFB, TX: USAF School of Aerospace Medicine. 9 vols.

Australian Senate Standing Committee on Science and the Environment. 1982. Pesticides and the Health of Australian Vietnam Veterans. First report. 240 pp.

Aylward LL, Hays SM, Karch NJ, Paustenbach DJ. 1996. Relative susceptibility of animals and humans to the cancer hazard posed by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using internal measures of dose. Environmental Science and Technology 30: 3534–3543. (Erratum published in Environmental Science and Technology 1997; 31:1252.)

Aylward LL, Hays SM, Czernec J, Brien B, Paustenbach DJ, Karch NJ. 1997. Relative doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) using alternate dosimetrics: comparison of the NIOSH and Ranch Hand Populations. Organohalogen Compounds 34:6–9.

Becher H, Flesch-Janys D, Kauppinen T, Kogevinas M, Steindorf K, Manz A, Wahrendorf J. 1996. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. Cancer Causes and Control 7(3):312–21.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989. Ten-year mortality study of the population involved in the Seveso incident in 1976. American Journal of Epidemiology 129:1187–1200.

Bertazzi PA, Zochetti C, Guercilena S, Consonni D, Tironi A, Landi MT, Pesatori AC. 1997. Dioxin exposure and cancer risk: a 15-year mortality study after the "Seveso Accident," Epidemiology 8(6):646–652.

Blair A, White DW. 1985. Leukemia cell types and agricultural practices in Nebraska. Archives of Environmental Health 40:211–214.

Bond GG, McLaren EA, Lipps TE, Cook RR. 1989. Update of mortality among chemical workers with potential exposure to the higher chlorinated dioxins. Journal of Occupational Medicine 31:121–123.

Brown JW. 1962. Vegetational Spray Test in South Vietnam. Fort Detrick, MD: U.S. Army Chemical Corps Biological Laboratories. DDC Number AD 476961. 119 pp.

Buckingham WA. 1982. Operation Ranch Hand: The Air Force and Herbicides in Southeast Asia 1961–1971. Washington, DC: U.S. Air Force Office of Air Force History.

Bueno de Mesquita HB, Doornbos G, van der Kuip DA, Kogevinas M, Winkelmann R. 1993. Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in the Netherlands. American Journal of Industrial Medicine 23:289–300.

Burmeister LF. 1981. Cancer mortality in Iowa farmers: 1971–1978. Journal of the National Cancer Institute 66:461–464.

Byard JL. 1987. The toxicological significance of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds in human adipose tissue. Journal of Toxicology and Environmental Health 22: 381–403.

Cantor KP. 1982. Farming and mortality from non-Hodgkin's lymphoma: a case-control study. International Journal of Cancer 29:239–247.

Carmelli D, Hofherr L, Tomsic J, Morgan RW. 1981. A Case-Control Study of the Relationship Between Exposure to 2,4-D and Spontaneous Abortions in Humans. SRI International. Prepared for the National Forest Products Association and the U.S. Department of Agriculture, Forest Service.

Centers for Disease Control (CDC). 1985. Agent Orange Projects Interim Report Number 2: Exposure Assessment for the Agent Orange Study. Atlanta: CDC, Center for Environmental Health, Division of Chronic Disease Control, Agent Orange Projects.

Centers for Disease Control (CDC). 1988. Serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in U.S. Army Vietnam era veterans. Journal of the American Medical Association 260:1249–1254.

Centers for Disease Control (CDC). 1989. Health Status of Vietnam Veterans. Vietnam Experience Study. Atlanta: U.S. Department of Health and Human Services. Vols. I–V, Supplements A–C.

Checkoway H. 1986. Methods of treatment of exposure data in occupational epidemiology. Medicina del Lavoro 77:48–73.

Coggon D, Pannett B, Winter P. 1991. Mortality and incidence of cancer at four factories making phenoxy herbicides. British Journal of Industrial Medicine 48:173–78.

Collins CV. 1967. Herbicide Operations in Southeast Asia, July 1961–June 1967. San Francisco: Headquarters, Pacific Air Forces. NTIS AD-779 796.

Collins JJ, Strauss ME, Levinskas GJ, Conner PR. 1993. The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a trichlorophenol process accident. Epidemiology 4:7–13.

Constable JD, Hatch MC. 1985. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250.

Cook RR, Bond GG, Olson RA. 1986. Evaluation of the mortality experience of workers exposed to the chlorinated dioxins. Chemosphere 15:1769–1776.

Craig DA. 1975. Use of Herbicides in Southeast Asia. Historical Report. Kelly AFB, TX: San Antonio Logistics Center, Directorate of Energy Management. 58 pp.

Dai LC, Phuong NTN, Thom LH, Thuy TT, Van NTT, Cam LH, Chi HTK, Thuy LB. 1990. A comparison of infant mortality rates between two Vietnamese villages sprayed by defoliants in wartime and one unsprayed village. Chemosphere 20:1005–1012.

Darrow RA, Irish KR, Minarik CD. 1969. Herbicides Used in Southeast Asia. Kelly AFB, TX. Technical Report SAOQ-TR-69-11078. 60 pp.

Devine OJ, Karon JM, Flanders WD, Needham LL, Patterson DG Jr. 1990. Relationships between concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin serum and personal characteristics in U.S. Army Vietnam veterans. Chemosphere 20:681–691.

DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. 1995. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environmental Health Perspectives 103(9):820–831.

Dux J, Young PJ. 1980. Agent Orange: The Bitter Harvest. Sydney: Hodder and Stoughton.

Erickson JD, Mulinare J, Mcclain PW. 1984a. Vietnam veterans' risks for fathering babies with birth defects. Journal of the American Medical Association 252:903–912.

Erickson JD, Mulinare J, Mcclain PW, Fitch TG, James LM, McClearn AB, Adams MJ. 1984b. Vietnam Veterans' Risks for Fathering Babies with Birth Defects. Atlanta: U.S. Dept. of Health and Human Services, Centers for Disease Control.

Fingerhut MA, Sweeney MH, Patterson DG Jr, Piacitelli LA, Morris JA, Marlow DA, Hornung RW, Cameron LW, Connally LB, Needham LL, Halperin WE. 1989. Levels of 2,3,7,8-TCDD in the serum of U.S. chemical workers exposed to dioxin contaminated products: interim results. Chemosphere 19:835–840.

Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. 1991. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New England Journal of Medicine 324:212–218.

Flesch-Janys D, Berger J, Gurn P, Manz A, Nagel S, Waltsgott H, Dwyer JH. 1995. Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. American Journal of Epidemiology 142:1165–1175.

Flesch-Janys D, Becher H, Gurn P, Jung D, Konietzko J, Manz A, Papke O. 1996. Elimination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in occupationally exposed persons. Journal of Toxicology and Environmental Health 47(4):363–378.

Geyer H, Scheunert I, Korte F. 1986. Bioconcentration potential of organic environmental chemicals in humans. Regulatory Toxicology and Pharmacology 6:313–347.

Gonzales J. 1992. List of Chemicals Used in Vietnam. Presented to the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. Illinois Agent Orange Committee, Vietnam Veterans of America.

Gordon JE, Shy CM. 1981. Agricultural chemical use and congenital cleft lip and/or palate. Archives of Environmental Health 36:213–221.

Gough M. 1986. Dioxin, Agent Orange: The Facts. New York: Plenum Press.

Hansen ES, Hasle H, Lander F. 1992. A cohort study on cancer incidence among Danish gardeners. American Journal of Industrial Medicine 21:651–660.

Hays SM, Aylward LL, Mocarelli P, Needham LL, Brambilla P, Gerthoux P, Patterson DG, Czernec J, Paustenbach DJ, Karch NJ. 1997. Comparative dose-response of the NIOSH and Seveso populations to the carcinogenic hazard of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) using alternate dosimetrics. Organohalogen Compounds 34:305–310.

Henneberger PK, Ferris BG Jr, Monson RR. 1989. Mortality among pulp and paper workers in Berlin, New Hampshire. British Journal of Industrial Medicine 46:658–664.

Hertzman C, Teschke K, Ostry A, Hershler R, Dimich-Ward H, Kelly S, Spinelli JJ, Gallagher RP, McBride M, Marion SA. 1997. Mortality and cancer incidence among sawmill workers exposed to chlorophenate wood preservatives. American Journal of Public Health 87(1):71–79.

Hill, AB. 1971. Principles of Medical Statistics, 9th ed. New York: Oxford University Press.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. Journal of the American Medical Association 256:1141–1147.

Hooiveld M, Heederik D, Bueno de Mesquita HB. 1996. Preliminary results of the second follow-up of a Dutch cohort of workers occupationally exposed to phenoxy herbicides, chlorophenols and contaminants. Organohalogen Compunds 30:185–189.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine (IOM). 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Institute of Medicine (IOM). 1997. Characterizing Exposure of Veterans to Agent Orange and Other Herbicides Used in Vietnam: Scientific Considerations Regarding a Request for Proposals for Research. Washington, DC: National Academy Press.

Irish KR, Darrow RA, Minarik CE. 1969. Information Manual for Vegetation Control in Southeast Asia. Misc. Publication 33. Fort Detrick, MD: Department of the Army, Plant Sciences Laboratories, Plant Physiology Division. NTIS AD-864-443.

Jappinen P, Pukkala E. 1991. Cancer incidence among pulp and paper workers exposed to organic chlorinated compounds formed during chlorine pulp bleaching. Scandinavian Journal of Work, Environment, and Health 17:356–359.

Kauppinen TP, Pannett B, Marlow DA, Kogevinas M. 1994. Retrospective assessment of exposure through modeling in a study on cancer risks among workers exposed to phenoxy herbicides, chlorophenols and dioxins. Scandinavian Journal of Work, Environment and Health 20:262–271.

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Bas B, Coggon D, Green L, Johnson E, Littorin M, Lynge E, Marlow DA, Mathews JD, Neuberger M, Benn T, Pannett B, Pearce N, Saracci R. 1995. Soft tissue sarcoma and non Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols and dioxins: two nested case control studies. Epidemiology 6:396–402.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno de Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Pearce N, Saracci R. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. American Journal of Epidemiology 145(12):1061–1075.

Landi MT, Needham LL, Lucier G, Mocarelli P, Bertazzi PA, Caporaso N. 1997. Concentrations of dioxin 20 years after Seveso [letter]. Lancet 349:1811.

Manz A, Berger J, Dwyer JH, Flesch-Janys D, Nagel S, Waltsgott H. 1991. Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 338:959–964.

Michalek JE, Wolfe WH, Miner JC, Papa TM, Pirkle JL. 1995. Indices of TCDD exposure and TCDD body burden in veterans of Operation Ranch Hand. Journal of Exposure Analysis and Environmental Epidemiology 5(2):209–223.

Michalek JE, Pirkle JL, Caudill SP, Tripathi RC, Patterson DG Jr, Needham LL. 1996a. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. Journal of Toxicology and Environmental Health 47(3):209–220.

Michalek JE, Tripathi RC, Kulkarni PM, Pirkle JL. 1996b. The reliability of the serum dioxin measurement in veterans of Operation Ranch Hand. Journal of Exposure Analysis and Environmental Epidemiology 6(3):327–338.

Michalek JE, Caudill SP, Tripathi RC. 1997. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up [Published Erratum]. Journal of Toxicology and Environmental Health 52(6):557–558.

Midwest Research Institute (MRI). 1967. Assessment of Ecological Effects of Extensive or Repeated Use of Herbicides. MRI Project No. 3103-B. Kansas City, MO: MRI. NTIS AD-824-314.

Military Assistance Command, Vietnam (MACV). Military History Branch. 1972. Chronology of Events Pertaining to U.S. Involvement in the War in Vietnam and Southeast Asia.

Morrison HI, Semenci RM, Morison D, Magwood S, Mao Y. 1992. Brain cancer and farming in western Canada. Neuroepidemiology 11: 267–276.

Mouradian R, Burt S, Tepper A, Hanley K. 1995. Boise Cascade, United Paperworkers International Union, Rumford, ME (HE 88-0140-2517), Cincinnati, OH, United States National Institute for Occupational Safety and Health.

Musicco M, Sant M, Molinari S, Filippini G, Gatta G, Berrino F. 1988. A case-control study of brain gliomas and occupational exposure to chemical carcinogens: the risks to farmers. American Journal of Epidemiology 128:778–785.

National Academy of Sciences (NAS). National Research Council, Assembly of Life Sciences. 1974. The Effects of Herbicides in South Vietnam. Washington, DC: National Academy Press

Needham LL, Gerthoux PM, Patterson DG, Brambilla P, Pirkle JL, Tramacere PI, Turner WE, Beretta C, Sampson EJ, Mocarelli P. 1994. Half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in serum of Seveso adults: interim report. Organohalogen Compounds 21:81–85.

Needham LL, Gerthoux PM, Patterson DG Jr, Brambilla P, Turner WE, Beretta C, Pirkle JL, Colombo L, Sampson EJ, Tramacere PL, Signorini S, Meazza L, Carreri V, Jackson RJ, Mocarelli P. 1997. Serum dioxin levels in Seveso, Italy, population in 1976. Teratogenesis, Carcinogenesis, and Mutagenesis 17(4–5):225–240.

Ott MG, and Zober A. 1996. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occuptional and Environmental Medicine 53:606–612.

Ott MG, Olson RA, Cook RR, Bond GG. 1987. Cohort mortality study of chemical workers with potential exposure to the higher chlorinated dioxins. Journal of Occupational Medicine 29:422–429.

Ott MG, Messerer P, Zober A. 1993. Assessment of past occupational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using blood lipid analyses. International Archives of Occupational and Environmental Health 65:1–8.

Pesatori AC. 1995. Dioxin contamination in Seveso: the social tragedy and the scientific challenge. Medicina del Lavoro 86(2):111–124.

Pirkle JL, Wolfe WH, Patterson DG, Needham LL, Michalek JE, Miner JC, Peterson MR, Phillips DL. 1989. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam veterans of Operation Ranch Hand. Journal of Toxicology and Environmental Health 27:165–171.

Ramlow JM, Spadacene NW, Hoag SR, Stafford BA, Cartmill JB, Lerner PJ. 1996. Mortality in a cohort of pentachlorophenol manufacturing workers, 1940–1989. American Journal of Industrial Medicine 30:180–194.

Robinson CF, Waxweiler RJ, Fowler DP. 1986. Mortality among production workers in pulp and paper mills. Scandinavian Journal of Work, Environment, and Health 12:552–560.

Ronco G, Costa G, Lynge E. 1992. Cancer risk among Danish and Italian farmers. British Journal of Industrial Medicine 49:220–225.

Rosenberg C, Kontsas H, Tornaeus J, Mutanen P, Jappinen P, Patterson DG Jr, Needham LL, Vainio H. 1994. PCDD/PCDF levels in the blood of workers in a pulp and paper mill. Organohalogen Compounds 21:101–104.

Rosenberg C, Kontsas H, Tornaeus J, Mutanen P, Jappinen P, Vainio H, Patterson DG Jr, Needham LL. 1995. PCDD/PCDF levels in the blood of workers at a pulp and paper mill. Chemosphere 31(8):3933–3944.

Ryan JJ, Schecter A, Sun W-F, Lizotte R. 1986. Distribution of chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans in human tissues from the general population. In: Rappe C, Choudhary G, Keith L, eds. Chlorinated Dioxins and Dibenzofurans in Perspective. Chelsea, MI: Lewis Publishers. 3–16.

Saracci R, Kogevinas M, Bertazzi PA, Bueno De Mesquita BH, Coggon D, Green LM, Kauppinen T, L'Abbe KA, Littorin M, Lynge E, Mathews JD, Neuberger M, Osman J, Pearce N, Winkelmann R. 1991. Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. Lancet 338:1027–1032.

Schecter A, Ryan JJ, Constable JD. 1986. Chlorinated dibenzo-*p*-dioxin and dibenzofuran levels in human adipose tissue and milk samples from the north and south of Vietnam. Chemosphere 15:1613–1620.

Schecter A, McGee H, Stanley JS, Boggess K, Brandt-Rauf P. 1996a. Dioxins and dioxin-like chemicals in blood and semen of American Vietnam veterans from the state of Michigan. American Journal of Industrial Medicine 30(6):647–654.

Schecter AJ, Li L, Ke J, Furst P, Furst C, Papke O. 1996b. Pesticide application and increased dioxin body burden in male and female agricultural workers in China. Journal of Occupational and Environmental Medicine 38(9):906–911.

Scheuplein RJ, Bowers JC. 1995. Dioxin—an analysis of the major human studies: comparison with animal-based cancer risks. Risk Analysis 15(3):319–333.

Smith TJ. 1987. Exposure assessment for occupational epidemiology. American Journal of Industrial Medicine 126:249–268.

Solet D, Zoloth SR, Sullivan C, Jewett J, Michaels DM. 1989. Patterns of mortality in pulp and paper workers. Journal of Occupational Medicine 31:627–630.

Stellman SD, Stellman JM. 1986. Estimation of exposure to Agent Orange and other defoliants among American troops in Vietnam: a methodological approach. American Journal of Industrial Medicine 9:305–21.

Sweeney MH, Fingerhut MA, Patterson DG, Connally LB, Piacitelli L, Morris JA, Greife AL. 1990. Comparison of serum levels of 2,3,7,8-TCDD in TCP production workers and in an unexposed comparison group. Chemosphere 20:993–1000.

Teschke K, Hertzman C, Fenske RA, Jin A, Ostry A, van Netten C, Leiss W. 1994. A history of process and chemical changes for fungicide application in the western Canadian lumber industry: what can we learn? Applied Occupational and Environmental Hygiene 9:984–993.

Thomas TL, Kang HK. 1990. Mortality and morbidity among Army Chemical Corps Vietnam veterans: a preliminary report. American Journal of Industrial Medicine 18:665–673.

U.S. Army. 1972. Herbicides and Military Operations. Vols. I and II. Washington, DC: Department of the Army, Engineer Strategic Studies Group, Office, Chief of Engineers.

U.S. Environmental Protection Agency (EPA). 1989. Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans (CDDs and CDFs) and 1989 Update. Springfield: U.S. Department of Commerce: National Technical Information Service. PB90 145756.

U.S. General Accounting Office (GAO). 1979. U.S. Ground Troops in South Vietnam Were in Areas Sprayed with Herbicide Orange. Report by the Comptroller General of the United States, FPCD 80 23. Washington, DC: GAO.

Vineis P, Terracini B, Ciccone G, Cignetti A, Colombo E, Donna A, Maffi L, Pisa R, Ricci P, Zanini E, Comba P. 1986. Phenoxy herbicides and soft-tissue sarcomas in female rice weeders. A population-based case-referent study. Scandinavian Journal of Work, Environment, and Health 13:9–17.

Warren WF. 1968. A Review of the Herbicide Program in South Vietnam. San Francisco: Scientific Advisory Group. Working Paper No. 10-68. NTIS AD-779-797.

Wigle DT, Semenciw RB, Wilkins K, Riedel D, Ritter L, Morrison HI, Mao Y. 1990. Mortality study of Canadian male farm operators: non-Hodgkin's lymphoma mortality and agricultural practices in Saskatchewan. Journal of the National Cancer Institute 82:575–582.

Wiklund K, Holm L-E. 1986. Soft tissue sarcoma risk in Swedish agricultural and forestry workers. Journal of the National Cancer Institute 76:229–234.

Wiklund K, Lindefors BM, Holm L-E. 1988. Risk of malignant lymphoma in Swedish agricultural and forestry workers. British Journal of Industrial Medicine 45:19–24.

Wolfe WH, Michalek JE, Miner JC, Pirkle JL, Caudill SP, Patterson DG Jr, Needham LL. 1994. Determinants of TCDD half-life in veterans of Operation Ranch Hand. Journal of Toxicology and Environmental Health 41:481–488.

Woods JS, Polissar L. 1989. Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in western Washington State. Chemosphere 18:401–406.

Young AL, Reggiani GM, eds. 1988. Agent Orange and Its Associated Dioxin: Assessment of A Controversy. Amsterdam: Elsevier.

Young AL, Thalken CE, Arnold EL, Cupello JM, Cockerham LG. 1976. Fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the Environment: Summary and Decontamination Recommendations. Colorado Springs: U.S. Air Force Academy. USAFA TR 76 18.

Young AL, Calcagni JA, Thalken CE, Tremblay JW. 1978. The Toxicology, Environmental Fate, and Human Risk of Herbicide Orange and Its Associated Dioxin. Brooks AFB, TX: Air Force Occupational and Environmental Health Lab. USAF OEHL TR 78 92.

Young AL. 1992. The Military Use of Herbicides in Vietnam. Presentation to the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. December 8, 1992. Washington, DC.

Zack JA, Gaffey WR. 1983. A mortality study of workers employed at the Monsanto company plant in Nitro, West Virginia. Environmental Science Research 26:575–591.

Zack JA, Suskind RR. 1980. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. Journal of Occupational Medicine 22:11–14.

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349–356.

Zober A, Messerer P, Huber P. 1990. Thirty-four-year mortality follow up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. International Archives of Occupational and Environmental Health 62:139–157.

6

Epidemiologic Studies

In seeking evidence for associations between health outcomes and exposure to herbicides and TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), many 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 7, 9, 10, and 11. The three main groups of individuals studied with respect to herbicide exposure are those with occupational, environmental, and military exposures. The historical basis for the groups studied was examined in Chapter 2 of *Veterans and Agent Orange* (henceforth called *VAO*) (IOM, 1994). A discussion of the criteria for inclusion in the review is detailed in Appendix A of *VAO*.

This chapter summarizes the epidemiologic studies and reports reviewed by the committee. Included are new studies published after *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996), studies that were not reviewed by the committees that wrote the prior reports; and studies that have been updated since publication of *Update 1996*. Tables 6-1, 6-2, and 6-3 give a brief overview of the epidemiologic studies reviewed for both the prior reports and this document. The summaries include the study method used and, if available, how the study subjects were selected; how the data were collected; the inclusion criteria; and how exposure was determined. The tables also list the numbers of subjects in the study and comparison populations and provide a brief description of the study. No studies are evaluated in this chapter; rather, a methodologic framework is provided for the health outcome chapters that follow. Qualitative critique of the study design, population size, methods of data collection, case and control ascertainment, or quality of exposure assessment has been

(text continues on page 218)

TABLE 6-1 Epidemiologic Studies—Occupational Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
PRODUCTION WORKERS				
NIOSH				
New Studies				
Sweeney et al., 1996, 1997	Cross-sectional	Study of numerous noncancer endpoints for liver function, gastrointestinal disorders, chloracne, serum glucose, hormone and lipid levels, and diabetes in same group as Calvert et al. (1991)	281	260
Halperin et al., 1995	Cross-sectional	Study of surrogates for cytochrome P-450 induction in same group as Calvert et al. (1991)	281	260
Studies Reviewed in *Update 1996*				
Calvert et al., 1994	Cross-sectional	Study of porphyria cutanea tarda in same group as Calvert et al. (1991)	281	260
Egeland et al., 1994	Cohort	Study of total serum testosterone and gondadotropin levels in chemical production workers exposed to dioxin, in same group as Calvert et al. (1991)	248	231
Studies Reviewed in *VAO*				
Sweeney et al., 1993	Cohort	Peripheral neuropathy 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

Calvert et al., 1992	Cohort	Assessment of liver and gastrointestinal systems in same group as Calvert et al. (1991)	281	260
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
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	—
Monsanto				
Studies Reviewed in *VAO*				
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	—
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
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

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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	—
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	—
Dow				
New Studies				
Ramlow et al., 1996	Cohort	Study of mortality in a cohort of workers exposed to pentachlorophenol (PCP)	770	(1) U.S. population (2) 36,804 unexposed workers
Studies Reviewed in *Update 1996*				
Bloeman et al., 1993	Cohort	Additional years of follow-up of Bond et al. (1988) study cohort through 1986	878	(1) U.S. population (2) 36,804 unexposed workers
Studies Reviewed in *VAO*				
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., 1989b	Cohort	Extension of Ott et al. (1987) study through 1984	2,187	—
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

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
Ott et al., 1987 Cook et al., 1987	Cohort	Expanded Cook et al. (1986) study an additional three years, through 1982	2,187	—
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
Cook et al., 1986	Cohort	Mortality experience (1940–1979) of men manufacturing chlorinated phenols compared to U.S. white men	2,189	—
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
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
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	—

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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	—
BASF				
New Studies				
Ott and Zober, 1996	Cohort	Cancer incidence and mortality experience (through 1992) of workers exposed to TCDD after the BASF accident, during reactor cleanup, maintenance, or demolition; based on the cohort of Zober et al. (1990)	243	—
Studies Reviewed in *Update 1996*				
Zober et al., 1994	Cohort	Morbidity experience in the same group as Zober et al. (1990)	158	161
Studies Reviewed in *VAO*				
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	—
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: (1) 180,000 town (2) 1.8 million district (3) 60.5 million Federal Republic of Germany (4) Two groups of 74 each from other cohort studies

IARC				
New Studies				
Kogevinas et al., 1997	Cohort	Mortality study (through 1992) of workers engaged in the production or application of phenoxy herbicides and composed of (1) the Saracci et al. (1991) cohorts, (2) the German cohorts of Becher et al. (1996), and (3) the NIOSH cohorts of Fingerhut et al. (1991)	26,615 total (21,863 exposed; 4,160 probably exposed; 592 unknown exposure)	—
Becher et al., 1996	Cohort	Cancer mortality (through 1989) among German workers in four chemical factories exposed to 2,4,5-T and/or trichlorophenol (subcohorts I and II), and phenoxy herbicides and chlorophenols (subcohorts III and IV)	2,479	—
Flesch-Janys et al., 1995	Cohort	Cancer and circulatory system mortality among workers in a chemical plant in Hamburg, Germany, exposed in varying degrees to herbicides contaminated with PCDD/F	1,189	(1) population (2) 2,528 gas workers
Studies Reviewed in *Update 1996*				
Kogevinas et al., 1995	Case-control	Two nested case-control studies of the relationship between STS and NHL and occupational exposures in members of the IARC cohort	STS: 11 cases NHL: 32 cases	5 controls per case
Kogevinas et al., 1993	Cohort	Cancer incidence and mortality experience of female workers in seven countries, potentially exposed to chlorophenoxy herbicides, chlorophenols, and dioxin compared to national death rates and cancer incidence rates	701	—

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Lynge, 1993	Cohort	Cancer incidence in the same group as Lynge (1985), with follow-up extended through 1987	3,390 men 1,071 women	—
Kogevinas et al., 1992	Cohort	Study of mortality from STS and malignant lymphomas in an international cohort of production workers and herbicide sprayers (same group as Saracci et al., 1991)	14,439 (13,482 exposed 416 probably exposed 541 unknown exposure)	3,951 non-exposed employees
Studies Reviewed in *VAO*				
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	—
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	—
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	—
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	—
Other Chemical Plants				
New Studies				
Tonn et al., 1996	Cohort	Study of the long-term immune system effects of TCDD in industrial workers involved in production and maintenance operations at a German chemical factory producing 2,4,5-T between 1966 and 1976	11	10
Studies Reviewed in *VAO*				
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
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	—

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
Pazderova-Vejlupkova et al., 1981	Descriptive	Study of development of TCDD intoxication among men in Prague (1965–1968)	55	No comparison group
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
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)
AGRICULTURAL/FOREST PRODUCTS ***Cohort Studies of Agricultural Workers*** **New Studies**				
Gambini et al., 1997	Cohort	Cancer mortality (1957–1992) among a cohort of rice growers in the Novara Province of northern Italy	958	—
Kristensen et al., 1997	Cohort	Birth defects among the offspring of Norwegian farmers born after 1924	192,417 births	61,351 births

Faustini et al., 1996	Cohort	Study of immune system components and functions among farmers who mixed and applied commercial formulations containing the chlorophenoxy herbicides 2,4-D and MCPA	10	Internal comparison
Studies Reviewed in *Update 1996*				
Dean, 1994	Cohort	Study of mortality from brain and hematopoietic cancers of agricultural workers compared to non-agricultural workers in Ireland (1971–1987)	(population size unclear)	—
Morrison et al., 1994	Cohort	Update of mortality experience in Wigle et al. (1990) cohort, through 1987, with addition of farmers from Alberta and Manitoba.	155,547	—
Semenciw et al., 1994	Cohort	Study of leukemia mortality in same group as Morrison et al. (1993)	155,547	—
Blair et al., 1993	Cohort	Study of causes of death, including cancer, among farmers in 23 states (1984–1988)	119,648 white men 2,400 white women 11,446 nonwhite men 2,066 nonwhite women	—
Semenciw et al., 1993	Cohort	Study of multiple myeloma mortality of male farmers compared to male population of the three prairie provinces of Canada (1971–1987)	155,547	—
Senthilselvan et al., 1992	Cross-sectional	Study of the association between pesticide exposure and asthma in male farmers	1,939	No comparison group

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Studies Reviewed in *VAO*				
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	—
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	—
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	—
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 I talian farmers 18 to 74 years old compared to persons in other occupational groups	No *N*s given	No *N*s given

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
Wigle et al., 1990	Cohort	Mortality experience from non-Hodgkin's lymphoma 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	—
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
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
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, 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	—
Burmeister, 1981	Cohort	Study of mortality of farmers compared to nonfarmers in Iowa (1971–1978)	6,402	13,809

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Cohort Studies of Forestry Workers				
Studies Reviewed in *VAO*				
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	—
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	—
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
Cohort Studies of Herbicide/Pesticide Sprayers				
New Studies				
Heacock et al., 1998	Cohort	Fertility study among British Columbia workers potentially exposed chlorophenate wood preservatives in 14 sawmillls between 1955–1988; Includes the cohort of Hertzman et al. (1997)	18,016 births	1,668 births
Hertzman et al., 1997	Cohort	Mortality study among British Columbia workers potentially exposed chlorophenate wood preservatives in 11 sawmillls between 1950–1985	23,829	2,658

Dimich-Ward et al., 1996	Cohort; Nested case-control	Analysis of birth defects among the offspring (born between 1952–1988) of the Hertzman et al. (1997) cohort	19,675 births among 9,512 fathers	5 non-defect births controls per case
Garry et al., 1996a	Cohort	Study of chromosome abnormalities based on the cohort of Garry et al. (1994)	23 fumigant appliers; 18 insecticide appliers 20 herbicide appliers	33
Garry et al., 1996b	Cohort	Birth defects among the offspring born between 1989–1992 of male pesticide appliers in Minnesota	4,935 births among 34,772 pesticide appliers 125 with birth anomalies	3,666 births with anomalies in the general population
Zhong and Rafnsson, 1996	Cohort	Cancer mortality among various subgroups of pesticide users in Iceland	2,449 (1,860 male and 589 female)	—
Studies Reviewed in *Update 1996*				
Asp et al., 1994	Cohort	Mortality and cancer morbidity experience of male chlorophenoxy herbicide appliers (same cohort as Riihimaki et al., 1982 and 1983) in Finland (1955–1971), through 1989, compared to general population rates for morbidity and mortality.	1,909	—
Garry et al., 1994	Cross-sectional	Evaluation of health outcomes resulting from exposure to pesticides by male pesticide appliers in Minnesota	719	No comparison group
Studies Reviewed in *VAO*				
Swaen et al., 1992	Cohort	Cancer mortality experience (through 1987) among Dutch male herbicide appliers licensed before 1980, compared to the total male Dutch population	1,341	—

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Bender et al., 1989	Cohort	Cancer mortality of Minnesota highway maintenance workers compared to expected numbers based on white Minnesota men	4,849	—
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	—
Wiklund et al., 1988b	Cohort	Risk of STS in Wiklund et al. (1987) cohort through 1984	20,245	—
Wiklund et al., 1987	Cohort	Risk of HD and NHL among Swedish pesticide appliers from date of license through 1982, compared to expected number of cases in the total population	20,245	
Blair et al., 1983	Cohort	Mortality experience of white male Florida pesticide appliers compared to U.S. and Florida men	3,827	—
Riihimaki et al., 1983	Cohort	Cancer morbidity and mortality in cohort (Riihimaki et al., 1982) through 1980.	1,926	—
Riihimaki et al., 1982	Cohort	Study of mortality among herbicide appliers exposed to 2,4-D and 2,4,5-T in Finland compared to mortality expected in the population	1,926	—

Smith et al., 1982	Cohort	Study of adverse reproductive outcomes among chemical appliers and agricultural contractors by category of exposure: none; chemicals not 2,4,5-T; 2,4,5-T 486 pregnancies (2,4,5-T)	113 pregnancies (chemicals not 2,4,5-T)	401 pregnancies (not exposed)
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	—
Smith et al., 1981	Cohort	Study of chemical appliers (1973–1979) in New Zealand compared to agricultural contractors for differences in adverse reproductive outcomes	459	422
Axelson et al., 1980	Cohort	Additional years of follow-up to cohort established in Axelson and Sundell (1974)	348	—
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	—
Case-Control Studies				
New Studies				
Blatter et al., 1997	Case-control	Multicenter Dutch study of paternal occupation and risk of spina bifida in offspring (1980–1992)	222	764

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Liou et al., 1997	Case-control	Study of occupational and environmental risk factors and Parkinson's disease (PD) in Taiwan (1993–1995)	120	240
Tatham et al., 1997	Nested case-control	Population based study of the occupational risk factors for subgroups of NHL patients based on the CDC's Selected Cancers Study (CDC, 1990a–d)	1,048	1,659
Nanni et al., 1996	Case-control	Population-based study in northeastern Italy of occupational and chemical risk factors for chronic lymphocytic leukemia (CLL) and NHL lymphomas (1987–1990)	187	977
Schulte et al., 1996	PMR analysis with nested case-control	Study of neurodegenerative diseases and occupational risk factors from 27 states		
Seidler et al., 1996	Case-control	Study of Parkinson's disease and various rural factors, including exposure to herbicides and wood preservatives in Germany	380	(1) 379 neighborhood controls (2) 376 regional controls
Studies Reviewed in *Update 1996*				
Hardell et al., 1994	Case-control	Study of the association between occupational exposures and parameters related to NHL in white males in Sweden	105	335

Mellemgaard et al., 1994	Case-control	Study of cases of renal-cell carcinoma (20–79 years) in Denmark, compared to population-based sample without cancer for identification of occupational risk factors	365	396
Nurminen et al., 1994	Case-control	Study of structural defect infants born to mothers engaged in agricultural work during the first trimester of pregnancy, compared to infants with structural defects born to mothers who did not engage in agricultural work during the first trimester	1,306	1,306
Brown et al., 1993	Case-control	Population-based case-control study of multiple myeloma in Iowa men for association with pesticide exposures	173	650
Persson et al., 1993	Case-control	Study of risk factors potentially associated with HD and NHL in males identified from the Regional Cancer Registry in Sweden	HD: 31 NHL: 93	204
Semchuk et al., 1993	Case-control	Study of cases of Parkinson's disease (36–90 years) in Canada, compared to population-based sample for association with occupational exposure to herbicides and other exposures	75 men 55 women	150 men 110 women
Zahm et al., 1993	Case-control	Study of NHL and exposure to pesticides in white women diagnosed with NHL between July 1, 1983, and June 30, 1986	206	824
McDuffie et al., 1990	Case-control	Study of pesticide exposure in male cases of primary lung cancer in Saskatchewan, compared to control subjects matched by age, sex, and location of residence	273	187

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Studies Reviewed in *VAO*				
Cantor et al., 1992	Case-control	Population-based case-control study of NHL in Iowa and Minnesota men for association with farming exposures	622	1,245
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
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
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
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

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
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	—
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
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
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
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

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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	—
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
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
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
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

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
Pearce et al., 1987	Case-control	Expanded study (Pearce et al., 1986b) of NHL to include ICD 200 diagnosed cases, and additional controls for association with farming exposures	183	338
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
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
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
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

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
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
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
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
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
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

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
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
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
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
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

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
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
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
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
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

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, 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., 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 HNL	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
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

continued

TABLE 6-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
PAPER/PULP WORKERS				
Studies Reviewed in *VAO*				
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
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	—
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	—

NOTE: COPD = chronic obstructive pulmonary disease; HD = Hodgkin's disease; IARC = International Agency for Research on Cancer; ICD = International Classification of Diseases; NHL = non-Hodgkin's lymphoma; PMR = proportionate mortality ratio; SEER = surveillance, epidemiology, and end results; STS = soft-tissue sarcoma; *Update 1996* = *Veterans and Agent Orange: Update 1996* (IOM, 1996); and *VAO* = *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (IOM, 1994).

[a]The dash (—) indicates the comparison group is based on a population (e.g., U.S. white males, country rates), and details are given in the text for specifics of the actual population.

TABLE 6-2 Epidemiologic Studies—Environmental Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
SEVESO				
New Studies				
Bertazzi et al., 1997	Cohort	Study of cancer incidence among Seveso residents in contaminated zones (A, B, R) after 15 years of follow-up through 1991	45,373 total: 805 in zone A; 5,943 in zone B; 38,625 in zone R	232,747
Mocarelli et al., 1996	Cohort	Study of sex ratio among the offspring of Seveso residents born in zone A from (1) 1977–1984 and (2) 1985–1994	(1) 74 births (28 male, 48 female) (2) 124 births (60 male, 48 female)	—
Studies Reviewed in *Update 1996*				
Bertazzi et al., 1993	Cohort	Study of cancer incidence in Seveso residents (aged 20 to 74 years) in contaminated zones (A, B, R) exposed to TCDD on July 10, 1976, compared to neighboring residents in unexposed areas	724 zone A 4,824 zone B 31,647 zone R	181,579
Pesatori et al., 1993	Cohort	Evaluation of cancer incidence in Seveso residents aged 1–19 years in the first post-accident decade compared to age-matched residents of neighboring unexposed areas	Approximately 20,000	167,391
Studies Reviewed in *VAO*				
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

continued

TABLE 6-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
Assennato et al., 1989a	Cohort	Comparison of dermatologic and laboratory findings in children during periodic exams following accident in Seveso	193 with chloracne	123
Assennato et al., 1989b	Cohort	Study of health outcomes in workers assigned to cleanup or referent group following Seveso accident	36	36
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
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

Mastroiacovo et al., 1988	Cohort	Comparison of birth defects occurring among zone A, B, and 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, or -R)
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
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
Tenchini et al., 1983	Cross-sectional	Cytogenetic analysis of maternal and fetal tissue among Seveso exposed compared to control sample	19	16
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
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
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

continued

TABLE 6-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
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
TIMES BEACH/QUAIL RUN **Studies Reviewed in *VAO***				
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
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
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
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

Stehr et al., 1986	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
Vietnam				
Studies Reviewed in *Update 1996*				
Cordier, et al. 1993	Case-control	Study of cases of hepatocellular carcinoma (1989–1992) in males living in Vietnam, compared to other hospitalized patients for association with a range of exposures including herbicides	152	241
Studies Reviewed in *VAO*				
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., 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
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

continued

TABLE 6-2 *Continued*

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				
New Studies				
Gallagher et al., 1996	Case-control	Community-based study of primary basal cell carcinoma (BCC) and patients with primary squamous cell carcinoma (SCC) in Alberta, Canada	BCC: 226 SCC: 180	406
Lovik et al., 1996	Cohort	Study of immune system parameters in hobby fishermen in the Frierfjord in southeastern Norway	24	10
Masala et al., 1996	Case-control	Multicenter study of NHL, HD, multiple myelomas (MM), and acute myeloid leukemias (AML) in Italy by region	HD: 421 NHL: 1822 MM: 325 AML: 263	Internal comparison by region
Waterhouse et al., 1996	PMR analysis with nested case-control	Study of NHL, HD, and CLL in a rural Michigan community	42 males 32 females	4 controls per case
Svensson et al., 1995	Cohort	Mortality and cancer incidence experience in two cohorts of Swedish fishermen	East coast: 2,896	West coast: 8,477
Weisglas-Kuperus et al., 1995	Cohort	Study of the immunological effects of pre- and postnatal PCB or TCDD exposure in 207 Dutch infants from birth to 18 months	105 breast-fed	102 bottle-fed

Wolf and Karmaus, 1995	Cross-sectional	Study of the effects of inhalative exposure to TCDD and related compounds in wood preservatives on cell-mediated immunity in German day care center employees	221	189
Studies Reviewed in *Update 1996*				
Butterfield et al., 1993	Case-control	Study of possible environmental risk factors associated with young-onset Parkinson's disease	63	68
Peper et al., 1993	Descriptive	Study of environmental exposure to dioxins and furans and potential association with adverse neuropsychological effects in Germany	19	None
Studies Reviewed in *VAO*				
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
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 rice weeding and defined in two categories	63 HD 253 NHL 49 STS	No control/ unexposed
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	—

continued

TABLE 6-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
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
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
Michigan Department of Public Health, 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
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
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)	—	—
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

NOTE: HD = Hodgkin's disease; NHL = non-Hodgkin's lymphoma; STS = soft-tissue sarcoma; *Update 1996* = *Veterans and Agent Orange: Update 1996* (IOM, 1996); and *VAO* = *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (IOM, 1994).

[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.

TABLE 6-3 Epidemiologic Studies—Vietnam Veterans

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
UNITED STATES STUDIES				
Ranch Hands				
New Studies				
Michalek et al., 1998a	Cohort	Paternal serum dioxin levels and infant death among the offspring of Ranch Hands	859 children: 323 background exposure, 267 low exposure, 269 high exposure	1,223 children
Henriksen et al., 1997	Cohort	Study of the relationship between serum dioxin and glucose and insulin levels and diabetes mellitus in Ranch Hands through 1992	989	1,276
AFHS, 1996 Michalek et al., 1998b	Cohort	Mortality update of Ranch Hands through the end of 1993 in the same cohort as AFHS (1983, 1984b, 1985, 1986, 1989, 1991a, 1995)	1,261	19,080
Henriksen et al., 1996	Cohort	Study of serum dioxin and reproductive hormones in Ranch Hands in 1982, 1985, 1987, and 1992	1,045 (participants, 1982) 474 (provided semen)	1,224 (participants, 1982) 532 (provided semen)
Studies Reviewed in *Update 1996*				
AFHS, 1995	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)

Wolfe et al., 1995	Cohort	Paternal serum dioxin levels and reproductive outcomes of Ranch Hand veterans compared with Air Force veterans from Southeast Asia who did not participate in herbicide spraying missions	932	1202
Studies Reviewed in *VAO*				
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, 1995	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 followup, compared with Air Force C-130 air and ground crew veterans in Southeast Asia	995	1,299
Centers for Disease Control (CDC)				
Studies Reviewed in *VAO*				
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

continued

TABLE 6-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
O'Brien et al., 1991	Cohort	Interview report and mortality for NHL based on Vietnam Experience Study	8,170	7,564
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 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
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
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
Department of Veterans Affairs (DVA)				
New Studies				
Dalager and Kang, 1997	Cohort	Morbidity and mortality experience (1968–1987) of Army Chemical Corps Vietnam veterans compared to U.S. men; Extension of Thomas and Kang (1990)	2,872	2,737
Mahan et al., 1997	Case-control	Study of lung cancer among Vietnam veterans (1983–1990)	329	269 111
McKinney et al., 1997	Cross-sectional	Study of the smoking behavior of veterans and nonveterans using the 1987 National Medical Expenditure Survey (NMES)	15,000	—
Bullman and Kang, 1996	Cohort	Mortality study of veterans with nonlethal (combat and noncombat) wounds sustained during the Vietnam war	34,534	—

continued

TABLE 6-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Watanabe and Kang, 1996	Cohort	Mortality experience (1965–1988) of Army and Marine Corps Vietnam veterans; Extension of Breslin et al. (1988) and Watanabe et al. (1991)	33,833	36,797
Dalager et al., 1995b	Case-control	Cases of HD diagnosed 1969–1985 among Vietnam-era veterans	283	404
Watanabe and Kang, 1995	Cohort	Post service mortality among Marine Vietnam veterans	10,716	9,346
Studies Reviewed in *Update 1996*				
Dalager et al., 1995a	Cohort	Update of Thomas et al. (1991) through December 31, 1995	4586	5325
Bullman et al., 1994	Case-control	Study of the association between testicular cancer and surrogate measures of exposure to Agent Orange in male Vietnam veterans	97	311
Studies Reviewed in *VAO*				
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
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

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
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
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
Bullman et al., 1990	Cohort	Mortality experience of Army I Corps Vietnam veterans compared to Army Vietnam era veterans	6,668 deaths	27,917 deaths
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
Thomas and Kang, 1990	Cohort	Morbidity and mortality experience (1968–1987) of Army Chemical Corps Vietnam veterans compared to U.S. men	894	—
True et al., 1988	Cross-sectional	PTSD and Vietnam combat experience evaluated among Vietnam-era veterans	775	1,012

continued

TABLE 6-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Breslin et al., 1988 Burt et al., 1987	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
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
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
American Legion				
Studies Reviewed in *VAO*				
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				
New Studies				
Clapp, 1997	Case-control	Selected cancers identified (1988–1993) among Massachusetts Vietnam veterans, compared to Massachusetts Vietnam-era veterans with cancers of other sites; Update of Clapp et al. (1991)	245	999
Studies Reviewed in *Update 1996*				
Visintainer et al., 1995	Cohort	Mortality experience (1965–1971) among male Michigan Vietnam veterans, compared to non-Vietnam veterans from Michigan	3,364 deaths	5,229 deaths
Studies Reviewed in *VAO*				
Fiedler and Gochfeld, 1992 Kahn et al., 1992a–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
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
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

continued

TABLE 6-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Levy, 1988	Cross-sectional	Study of PTSD in chloracne as indicator of exposure to TCDD and control Vietnam veterans in Massachusetts	6	25
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 Vietnam 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	—
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
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	—
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

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
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
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
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
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
Other U.S. Veteran Studies				
Studies Reviewed in *VAO*				
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

continued

TABLE 6-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
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
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
AUSTRALIAN STUDIES				
New Studies				
Crane et al., 1997a	Cohort	Mortality experience (through 1994) of Australian veterans who served in Vietnam	59,036 males 484 females	—
Crane et al., 1997b	Cohort	Mortality experience (through 1994) of Australian national servicemen who served in Vietnam	18,949	24,646
O'Toole et al., 1996a–c	Cross-sectional	Survey of self-reported health status (1989–1990) of Australian Army Vietnam veterans	641	—
Studies Reviewed in *VAO*				
Field and Kerr, 1988	Cohort	Study of Tasmanian Vietnam veterans compared to neighborhood controls for adverse reproductive and childhood health outcomes	357	281

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
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
OTHER VIETNAM VETERANS STUDIES				
New Studies				
Chinh et al., 1996	Cohort	Study of antinuclear antibodies and sperm autoantibodies among Vietnamese veterans who served 5–10 years in a "dioxin-sprayed zone"	25	63 36

NOTE: HD = Hodgkin's disease; NHL = non-Hodgkin's lymphoma; PTSD = posttraumatic stress disorder; STS = soft-tissue sarcoma; *Update 1996* = *Veterans and Agent Orange: Update 1996* (IOM, 1996); and *VAO* = *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (IOM, 1994).
[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.

reserved for the individual health outcome chapters in which the results of these studies are discussed.

The text and tables in this chapter are organized in three basic sections—occupational studies, environmental studies, and studies in Vietnam veterans—with subsections included under each heading. The studies address exposures to 2,4-D (2,4-dichlorophenoxyacetic acid); 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and its contaminant TCDD; cacodylic acid; and picloram. In some cases, the committee examined studies addressing compounds chemically related to the herbicides used in Vietnam, such as 2-methyl-4-chlorophenoxyacetic acid (MCPA), hexachlorophene, and chlorophenols, including trichlorophenol. In other instances, investigators did not indicate specific herbicides to which study participants were exposed, or the level of exposure. These complicating factors were considered when the committee weighed the relevance of a study to its findings. Where available, details are given with regard to exposure assessment and how exposure was subsequently used in the analysis.

The occupational section includes studies of production workers, agricultural and forestry workers (including herbicide and pesticide appliers), and paper and pulp workers, as well as case-control studies of specific cancers and the association with exposures to herbicides or related compounds. The environmental section includes studies of populations accidentally exposed to unusual levels of herbicides or dioxin as a result of the location in which they live, for example, the residents of Seveso, Italy; Times Beach, Missouri; and the southern portion of Vietnam. The section on Vietnam veterans includes studies conducted in the United States by the Air Force; the Centers for Disease Control and Prevention (CDC), the Department of Veterans Affairs (DVA; formerly the Veterans Administration [VA]); the American Legion; and the State of Michigan, as well as other groups. Studies of Australian Vietnam veterans are also presented there.

Many cohorts potentially exposed to dioxin and the herbicides used in Vietnam are monitored on an ongoing basis. Studies of the groups that are assessed regularly include the National Institute for Occupational Safety and Health (NIOSH), International Agency for Research on Cancer (IARC), National Cancer Institute (NCI), Seveso, and Ranch Hand cohorts. Typically, the risks between exposure to herbicides and specific health outcomes are updated every three to five years. For example, the health of the Ranch Hands cohort was assessed in 1982, 1987, 1992, and 1997. *For such studies, the committee has chosen to focus on the most recent update available when multiple comparisons are made across years.* For the sake of thoroughness, the discussion of specific health outcomes in Chapters 7–10 includes reference to all studies, including those subsumed by the most recent update.

Similarly, researchers investigating the constituent cohorts used in some large multicenter studies may publish reports based solely on the individuals they monitor. Examples include the IARC and NCI cohort studies. *The committee has chosen to focus on the studies of the larger multicenter cohorts.* However, for the

sake of thoroughness, Chapters 7–11 reference all of these studies, including those subsumed by the larger multicenter cohorts.

OCCUPATIONAL STUDIES

Several occupational groups in the United States and elsewhere have been exposed to the types of herbicides used in Vietnam and, more specifically, to TCDD, a contaminant of some herbicides and other products. Occupational groups exposed to these chemicals include farmers, agricultural and forestry workers, herbicide sprayers, workers in chemical production plants, and workers involved in paper and pulp manufacturing. In addition, studies that use job titles as broad surrogates of exposure and studies that rely on disease registry data have been conducted. Exposure characterization varies widely in these studies in terms of measurement, quantification, level of detail, confounding by other exposures, and individual versus surrogate or group (ecological) measures.

Production Workers

National Institute for Occupational Safety and Health (NIOSH)

In 1978, NIOSH began a study to identify all U.S. workers potentially exposed to TCDD between 1942 and 1984 (Fingerhut et al., 1991). In a total of 12 chemical companies, 5,000 workers were identified from personnel and payroll records as having been involved in production or maintenance processes associated with TCDD contamination. Their exposure resulted from working with certain chemicals in which TCDD was a contaminant, including 2,4,5-trichlorophenol (TCP) and 2,4,5-T, Silvex, Erbon, Ronnel, and hexachlorophene. An additional 172 workers identified previously by their employers as being exposed to TCDD were also included in the study cohort. The 12 plants involved were large manufacturing sites of major chemical companies. Thus, many of the study subjects probably were exposed to many other chemicals, some of which could be carcinogenic.

Prior to this study, NIOSH conducted a cross-sectional study that included a comprehensive medical history, medical examination, and measurement of pulmonary function of workers employed in the manufacture of chemicals with TCDD contamination at chemical plants in Newark, New Jersey, from 1951 to 1969, and in Verona, Missouri, from 1968 to 1969 and from 1970 to 1972 (Sweeney et al., 1989, 1993; Calvert et al., 1991, 1992; Alderfer et al., 1992). The plant in New Jersey manufactured TCP and 2,4,5-T; the Missouri plant manufactured TCP, 2,4,5-T, and hexachlorophene.

A number of studies were later conducted that looked at specific health outcomes among the larger cohort, including pulmonary function (Calvert et al., 1991), liver and gastrointestinal function (Calvert et al., 1992), mood (Alderfer et

al., 1992), the peripheral nervous system (Sweeney et al., 1993), porphyria cutanea tarda (Calvert et al., 1994), and reproductive hormones (Egeland et al., 1994). *VAO* and *Update 1996* describe the details of each of these studies.

Based on a previous exposure characterization study (Sweeney et al., 1990) of this cohort, Halperin et al. (1995) conducted a cross-sectional medical survey of chemical workers at the New Jersey and Missouri plants. Of the 586 workers eligible for participation, 357 completed an occupational history, and 281 of these participated in a medical exam. Urine and serum samples were collected from 58 of the latter individuals, who served as cases. Two hundred sixty non-exposed individuals from neighborhoods near the plants participated in the medical exam. Of these 125 had urine samples collected and served as controls. Surrogates for cytochrome P-450 induction (5-acetylamino-6-formylamino-3-methyluracil [AMFU] and cotinine levels) were measured in the urine samples of cases and controls. Blood serum TCDD levels were measured in all 58 cases and a subset of the 260 individual who participated in the medical exam. The median TCDD level in these 260 participants was used for the controls that did not themselves give blood samples.

Sweeney et al. (1996, 1997) evaluated other noncancer end points for liver function, gastrointestinal disorders, chloracne, serum glucose, hormone and lipid levels, and diabetes in 281 of the 586 workers first identified by Calvert et al. (1991) in New Jersey and Missouri. In addition, 260 controls were examined.

Monsanto

Included in the study cohort of Fingerhut et al. (1991) are a number of individual cohort members from Monsanto's production facilities. These are discussed in more detail in *VAO*. One set of Monsanto studies are based on a violent reaction that occurred on March 8, 1949, in the trichlorophenol (TCP) production process at the Nitro, West Virginia, plant of Monsanto (Zack and Suskind, 1980; Moses et al., 1984; Collins et al., 1993). Other studies focused on exposure of Monsanto workers involved in numerous aspects of producing 2,4,5-T (Suskind and Hertzberg, 1984; Moses et al., 1984; Zack and Gaffey, 1983).

Dow Chemical Company

Several studies of Dow Chemical Company production workers are summarized in *VAO* and *Update 1996*. These study populations, except for one article by Bond and colleagues (1988), is included in the NIOSH study (Fingerhut et al., 1991). Originally, Dow Chemical Company conducted a study on the work force engaged in the production of 2,4,5-T (Ott et al., 1980) and later on TCP manufacturing workers exhibiting chloracne (Cook et al., 1980). A extension and follow-up study compared medical examination results and morbidity (Bond et al., 1983) as well as reproductive outcomes from paternal TCDD (Townsend et al., 1982).

Dow employees with chloracne, established on the basis of past diagnosis or clinical description were later enrolled in a prospective mortality study (Bond et al., 1987).

Dow Chemical Company assembled a large cohort at the Midland, Michigan, plant (Cook et al., 1986; Cook et al., 1987; Bond et al., 1989b). Based on this large Midland cohort, a cohort study of women (Ott et al., 1987) and a case-control study of STS (Sobel et al., 1987) was conducted. Exposure to TCDD was better characterized in this cohort based on chloracne diagnosis (Bond et al., 1989a). Dow Chemical Company has also undertaken a large-scale cohort mortality study of workers exposed to herbicides in several Dow plants (Bond et al., 1988; Bloemen et al., 1993).

As part of this ongoing Dow study, Ramlow et al. (1996) examined mortality in a cohort of workers exposed to pentachlorophenol (PCP). The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential polychlorinated dibenzodioxin (PCDD) exposure between 1937 and 1980. From this cohort, 770 workers were identified who were considered to have potential PCP exposure based on work history records. Exposure to PCP was assessed using historical industrial hygiene and process data, which resulted in a strategy for ranking jobs by exposure intensity on a scale of 1 to 3. Exposure assessment to PCDD was performed using the process described by Ott et al. (1987), in which semiquantitative, logarithmic exposure intensity scores ranging from 1 to 4 for TCDD and 0 to 2 for hexa- or octachlorodibenzodioxins (H/OCDD) were assigned to each job title. Cumulative exposure indices for PCP and dioxin were calculated using these assigned scores. In the study analysis, the U.S. white male death rates (5-year age and calendar time specific) and the non-PCP and PCDD male Dow Michigan employees for 1940 to 1989 were both used as reference values to calculate expected deaths. Four exposure groups were developed for TCDD (1 unit = very low; 1–1.9 = low; 2–2.9 = medium; 3 = high). Standardized mortality ratios (SMRs) were calculated with exposure lagged by 15 years, using both the U.S. and the Dow referent populations.

BASF

In Germany, an accident on November 17, 1953, during the manufacture of trichlorophenol at BASF Aktiengesellschaft, resulted in the exposure of some workers in the plant to TCDD. *VAO* and *Update 1996* summarize studies of these workers, including a mortality study of persons initially exposed or later involved in clean-up operations, conducted 27 years after the accident (Thiess et al., 1982), an update and expansion of this study (Zober et al., 1990), and a morbidity follow up (Zober et al., 1994).

More recently, Ott and Zober (1996) examined another cohort of workers exposed to TCDD after the accident during reactor cleanup, maintenance, or

demolition. They studied cancer incidence and mortality up to 1992 for a group of 243 men and developed TCDD dose estimates based upon work activity information, blood TCDD determinations on a subset of the population, and estimates of TCDD elimination rates. Expected numbers of incident cancer cases and cancer deaths were obtained from German sources by five-year age and calendar intervals. Analysis included proportional hazard modeling to include estimated TCDD dose, diagnosis of chloracne, smoking history, body mass index (BMI), time since first exposure, and potential confounders including exposure to asbestos and aromatic amines.

International Register of Workers Exposed to Phenoxy Herbicides (IARC)

To avoid problems of small studies with insufficient power to detect increased cancer risks, IARC created this multinational registry of workers exposed to phenoxy herbicides, chlorophenols, and their contaminants (Saracci et al., 1991). The IARC register included information on mortality and exposures of 18,390 workers—16,863 men and 1,527 women. *Update 1996* describes the individual national cohorts included in this multinational registry.

In a study covering ten countries, cancer mortality from soft-tissue sarcoma and malignant lymphoma was evaluated on the entire cohort (Kogevinas et al., 1992). Two nested case-control studies were also undertaken to evaluate the relationship between soft-tissue sarcoma and non-Hodgkin's lymphoma (Kogevinas et al., 1995). A cohort study of cancer incidence and mortality was conducted among 701 women occupationally exposed to chlorophenoxy herbicides, chlorophenols, and dioxins from seven countries (Kogevinas et al., 1993). *VAO* and *Update 1996* highlight these studies.

In an update and expansion, Kogevinas et al. (1997) assembled national studies from 12 countries using the same core protocol jointly developed by study participants and coordinated by IARC. The expanded study consisted of 26,615 male and female workers engaged in the production or application of phenoxy herbicides and was composed of (1) the Saracci et al. (1991) cohort, (2) the German cohorts of Becher et al. (1996), and (3) the NIOSH cohorts of Fingerhut et al. (1991).

Of the total study population, 21,863 (20,851 men and 1,012 women) were classified as exposed to phenoxy herbicides or chlorophenols based on individual job records and company exposure questionnaires; 4,160 were unexposed; and 592 were classified as "unknown exposure." Most workers were classified as exposed if they had ever worked in production or spraying of phenoxy herbicides or chlorophenols (for four cohorts, a minimum employment period of 1 to 12 months was specified). The period of follow-up also varied between cohorts; overall, it extended from 1939 to 1992 (488,482 person years at report). Overall, 4.4 percent (970 workers) were lost to follow-up. Exposure information varied between cohorts, but in general, exposures were reconstructed from job records.

The exposed workers were aggregated into five groups: main production, maintenance, other exposed jobs, unspecified tasks, and sprayers. Based on these categories and information on production processes and the composition of the materials used, the exposed workers were further classified into three categories: (1) exposed to TCDD or higher chlorinated dioxins; (2) unexposed to the same; and (3) unknown exposure to the same. Analysis was performed by calculating SMRs and 95 percent confidence intervals (95% CI), using the World Health Organization (WHO) mortality data bank to calculate national mortality rates by sex, age (five-year intervals), and calendar period (five years). Within-cohort analysis was also performed using Poisson regression adjusting for time since first exposure, duration of exposure, and employment status.

A number of these individual cohorts were evaluated apart from the IARC coordinated efforts. These cohorts included Danish production workers studied by Lynge (1985, 1993); the British production workers of Coggon et al. (1986, 1991); the Dutch production workers of Bueno de Mesquita et al. (1993); and the German production workers of Manz et al. (1991). *VAO* and *Update 1996* discuss these studies in more detail.

More recently, Becher et al. (1996) assessed cancer mortality among German workers in four phenoxy herbicide- and chlorophenol-producing facilities. The population included workers who had a least one month of employment, resulting in a cohort consisting of 2,479 male workers. The cohort was assembled from four plants, and the analysis was conducted on the total cohort divided into four subcohorts corresponding to each plant considered separately. The period of follow-up varied between plants, and 100 workers were lost to follow-up. The nature of the chemicals produced varied substantially between plants and over time; some facilities synthesized and formulated a wide range of phenoxy herbicides and chlorophenols (subcohorts III and IV); others produced primarily 2,4,5-T and/or TCP (subcohorts I and II). Manz et al. (1991) previously reported on subcohort I. SMRs and 95% CI were calculated using West German mortality rates by five-year age and calendar intervals. Cox regression was performed to evaluate the effect of smoking in the one subcohort where smoking information was available. Each subcohort was analyzed separately because the exposure pattern was judged to be characteristic of each facility. Based on production information and limited blood dioxin measurements, subcohorts I and II are supposed to have higher TCDD exposures than subcohorts III and IV.

Flesch-Janys et al. (1995) described cancer and circulatory system mortality among 1,189 male workers in a chemical plant in Hamburg, Germany. Workers had been exposed in varying degrees to herbicides contaminated with PCDD/F. The authors developed a quantitative estimate of polychlorinated dibenzodioxin (PCDD) and polychlorinated dibenzofuran (PCDF) exposure for the entire cohort derived from blood and adipose tissue levels measured in a subgroup of 190 workers. An unexposed cohort of gas workers served as an external reference group.

Other Chemical Plants

Other studies have reviewed health outcomes among chemical workers in the United Kingdom exposed to TCDD as a result of an industrial accident in 1968 (Jennings et al., 1988; May, 1982, 1983); production workers in the former USSR involved in the production of 2,4-D (Bashirov, 1969); factory workers in Prague, Czechoslovakia, who exhibited symptoms of TCDD intoxication 10 years after occupational exposure to 2,4,5-T (Pazderova-Vejlupkova et al., 1981); 2,4-D and 2,4,5-T productions workers in the U.S. (Poland et al., 1971); and white male workers employed at a chemical plant manufacturing flavors and fragrances (Thomas, 1987). *VAO* details these studies.

More recently, Tonn et al. (1996) examined the long-term immune system effects of TCDD in 11 industrial workers involved in production and maintenance operations at a German chemical factory producing 2,4,5-T. Members of this group worked at the factory for several years between 1966 and 1976. In 1989 or 1992, the research team took blood samples from these workers and from 10 aged-matched healthy male volunteers with no known exposure to TCDD. The exposed cohort had TCDD body burdens at least ten times higher than the general population. A number of immune system parameters, including lymphocyte subsets and lympoproliferative responses were characterized.

Agricultural and Forestry Workers

Cohort Studies of Agricultural Workers

VAO and *Update 1996* details a number of cohort studies examining health effects among those involved in agricultural activity, including proportionate mortality among Iowa farmers (Burmeister, 1981), cancer mortality among Danish and Italian farmers (Ronco et al., 1992), cancer incidence among farmers licensed to spray pesticides in the southern Piedmont area of Italy (Corrao et al., 1989), sperm abnormalities among Argentinian farmers (Lerda and Rizzi, 1991), and cancer among Danish gardeners (Hansen et al., 1992). A set of Canadian studies called the Mortality Study of Canadian Male Farm Operators, evaluated the risk to farmers of general mortality and specific health outcomes including NHL (Wigle et al., 1990; Morrison et al., 1994), prostate cancer (Morrison et al., 1992), brain cancer (Morrison et al., 1993), multiple myeloma (Semenciw et al., 1993), leukemia (Semenciw et al., 1994), and asthma (Senthilselvan et al., 1992). Based on data from the Swedish Cancer Environment Register (which links population census data, including occupation, with the Swedish Cancer Registry), cohorts studies evaluated cancer mortality and farmwork (Wiklund, 1983), STS and malignant lymphoma among agricultural and forestry workers (Wiklund and Holm, 1986; Wiklund et al., 1988a), the risk of NHL, HD, and multiple myeloma in relation to numerous occupational activities (Eriksson et al., 1992), and brain, lymphatic, and hematopoietic cancers in Irish agricultural workers

(Dean, 1994). In the United States, a large-scale proportionate mortality study was performed using data on more than 100,000 male and female farmers from 23 states (Blair et al., 1993).

More recently, Gambini and colleagues (1997) investigated cancer mortality among a cohort of rice growers in the Novara Province of northern Italy. Using a set of registered farm owners, they evaluated 1,493 males who worked on farms from 1957 to 1992. The cause of death was identified for 958 subjects and compared with the expected numbers calculated from national rates for five-year period and age group. No direct exposure information was available, so employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides used during the study period.

Kristensen et al. (1997) investigated birth defects among the offspring of Norwegian farmers by linking several Norwegian national registries. Farm holders born after 1924 were identified from the computerized files of national agricultural censuses held in 1969, 1979, 1989, and horticultural censuses in 1974 and 1985. Linkages with the Central Population Register and Medical Birth Registry identified a total of 192,417 births in 1967–1991 to farm holders. A comparison group consisted of 61,351 births to mothers residing in agricultural municipalities but determined not to be farm holders. Birth defects were identified from the Medical Birth Registry, a national registry of all births of 16 completed weeks gestation with up to three birth defects recorded. In addition, data were available on potential confounding factors including maternal age, birth order, parental consanguinity, geographic location, and maternal chronic diseases. Exposure information for each farm was obtained from the agricultural censuses. Exposure variables used in the analysis were based on the type of farming (animal husbandry, grain farming, and orchard or greenhouse farming) and use indicators (amount of money spent on pesticides, tractor pesticide spraying equipment, and amount of phosphorus and nitrogen in fertilizers). Exposure information was derived from the census closest to the time of birth. The sensitive period for exposure was considered to be three months before the estimated date of conception.

Faustini and colleagues (1996) carried out a study of 10 farmers who mixed and applied commercial formulations containing the chlorophenoxy herbicides 2,4-D and MCPA during March 1994. Researchers collected blood samples one week before herbicide exposure, and 1–12 and 50–70 days after exposure. A number of immune system components and functions were assessed, including lymphocyte count, natural killer cell-mediated cytotoxicity, and lymphoproliferative response. The farmers served as their own controls.

Cohort Studies of Forestry Workers

Studies have been conducted among forestry workers potentially exposed to herbicides used in Vietnam. These studies include a cohort mortality study among

men employed at a Canadian public utility (Green, 1987, 1991) and a briefly outlined Dutch study of forestry workers exposed to 2,4,5-T which compared the prevalence of acne and liver dysfunction (van Houdt et al., 1983). *VAO* describes these studies in greater detail.

Cohort Studies of Herbicide/Pesticide Appliers

A number of cohort studies have assessed health outcomes among herbicide and pesticide appliers including cancer mortality among Swedish railroad workers (Axelson and Sundell, 1974; Axelson et al., 1980), mortality among pesticide appliers in Florida (Blair et al., 1983), general and cancer mortality and morbidity measured prospectively among Finnish male 2,4-D and 2,4,5-T appliers (Riihimaki et al., 1982, 1983; Asp et al., 1994), and reproductive outcomes among male chemical appliers in New Zealand (Smith et al., 1981, 1982). Other studies examined the risk of cancer including STS, HD, and NHL among pesticide and herbicide appliers in Sweden (Wiklund et al., 1987, 1988b, 1989a,b), general and cancer mortality among Dutch male herbicide appliers (Swaen et al., 1992), cancer mortality among Minnesota highway maintenance workers (Bender et al., 1989), and lung cancer morbidity in male agricultural plant protection workers in the former German Democratic Republic (Barthel, 1981). Some of these studies include agricultural and forestry worker cohorts.

More recently, Garry et al. (1994) conducted a cross-sectional study of 1,000 pesticide appliers in Minnesota to evaluate health outcomes associated with pesticide use. Study participants were selected from a current list of licensed pesticide appliers obtained from the state Department of Agriculture. All persons certified and/or recertified within the past five years were eligible to participate in the study. One thousand pesticide appliers were chosen by random selection and contacted by telephone. Seven hundred and nineteen individuals who chose to participate in the study received a questionnaire in the mail regarding general health, occupation, pesticide use, and use of protective gear. *Update 1996* describes this study in more detail.

Using this base population of 719 licensed pesticide appliers, Garry et al. (1996a) obtained blood samples from 23 fumigant appliers, 18 insecticide appliers, and 20 herbicide appliers; 33 subjects who were not involved in applying pesticides were used as controls and frequency-matched on age and smoking status. Although blood samples were collected at various times in the year, only the samples obtained during the traditional pesticide application season were evaluated.

Garry et al. (1996b) further conducted a series of analyses using data on birth defects among the offspring of these male pesticide appliers in Minnesota. Information on private state-licensed pesticide appliers registered with the Minnesota Department of Agriculture in 1991 ($N = 34,772$) were linked with live birth data for the state of Minnesota (1989–1992). Birth defect data were contained in these

birth files. Analyses of the relationship between birth defect rates and county-specific agricultural data were also performed. Pesticide data for units or clusters of Minnesota counties with similar geologic features and crops served to provide use data for 12 herbicides (including 2,4-D). An additional analysis was conducted to evaluate specific pesticide use. Based on pounds of active ingredient per county, low- and high-use categories were defined for the 12 specific pesticides, and comparisons were made of the birth defect rates within each region.

Hertzman and colleagues (1997) conducted a large retrospective cohort study of British Columbia sawmill workers potentially exposed chlorophenate wood preservatives. The researchers selected 23,829 cases from 11 sawmills that used chlorophenates, and 2,658 controls from 3 other sawmills. Study participants worked in these sawmills for at least one year or, for those who worked only intermittantly, 260 days between 1950 and 1985. These data were linked to the British Columbia Death File and the British Columbia Cancer Incidence File. Researchers conducted a second link to the Canadian Mortality Database and also to data provided by Statistics Canada. Based on a protocol from a previous exposure study (Hertzman et al., 1988; Teschke et al., 1989), a retrospective exposure assessment was conducted and combined with worker estimates of frequency and duration of exposure, to yield a quantitative measure of exposure. Standardized mortality and incidence ratio analysis were conducted between cases and control, with the male population of British Columbia serving a external controls. Cancer latency was assessed using 5-, 15-, and 20-year time interval categories. A separate standardized rate ratio analysis was conducted for NHL.

As part of the larger cohort study of Hertzman et al. (1997), Dimich-Ward et al. (1996) conducted a nested case-control analysis of birth defects among offspring of fathers employed in these British Columbia sawmills. The cohort included 9,512 fathers who had worked at least one year in sawmills where chlorophenate wood preservatives had been used. Chlorophenates are known to be contaminated by dioxin. Births (1952–1988) to these men were identified by linkage with the British Columbia (BC) live and stillbirth records. Further linkage with the BC Health Surveillance Registry identified cases of birth defects. The registry system is population based and uses multiple sources of identification. A case-control analysis was conducted, matching five controls (non-defect births) per case on year of birth and gender. Covariates included mother's and father's age. Exposure to chlorophenates for specific time periods was assessed by a team of industrial hygienists based on job title. Continuous estimates of cumulative hours of chlorophenate exposure were calculated for time windows relative to conception and pregnancy. Estimates of maximal exposure were determined for the most exposed job in each time period.

Heacock et al. (1998) further evaluated fertility in the sawmill worker cohort in British Columbia. The worker cohort was linked with provincial marriage and birth files. The person-year contributions and live births of workers less than 55 years of age, who had worked for at least one year between 1950 and 1985 (N =

26,487), were included. The exposure of these workers to chlorophenates, possibly contaminated by dioxin, was estimated for each worker and an index of cumulative chlorophenate exposure duration was developed (<120, 120–1,999, 2,000–3,999, 4,000–9,999, and >10,000 hours). For the external analysis, exposure was defined as sawmills in which chlorophenates were used; for the internal analysis, the cumulative exposure index was used. Among those exposed to chlorophenates, 18,016 births were recorded, while 1,668 births were recorded for the comparison group. The internal analysis estimated the rate ratio to evaluate the effects of chlorophenate exposure and time since hire, adjusting for age and calendar period.

Zhong and Rafnsson (1996) examined cancer risk among pesticide users in Iceland. Based on data provided by the Icelandic Cancer Registry, the researchers followed a cohort of 2,449 people, including 1,860 men and 589 women, who had all come into contact with pesticides. Six subcohorts were formed based on previous contact with pesticides, including (1) specially licensed pesticide users, (2) students of the Icelandic Horticultural College, (3) members of the Icelandic Market Gardeners' Association Pension Fund, (4) members of the Horticulturist's Association, (5) members of the Association of Vegetable Farmers, and (6) vegetable producers of the Farmers' Association of Iceland. Only a group of 594 of the specially license pesticide users were assumed to have been heavily exposed to pesticides. While it is not known whether study participants were exposed to the herbicides used in Vietnam, data from the Icelandic Committee on Toxic Substances suggest that 2,4-D was used heavily in agricultural during 1976–1993. The data from the Icelandic Cancer Registry were linked to the National Registry and the Register of Deaths to ascertain vital statistics of study participants. Standardized incidence ratios were calculated based on observed and expected number of cancers for each subgroup.

Case-Control Studies

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 included studies of STS from data provided by the Department of Oncology, University Hospital, Umea (Hardell and Sandstrom, 1979); the Cancer Registry of five southern counties, where MCPA, 2,4-D, and phenoxypropionic acids are used in agricultural areas (Eriksson et al., 1979, 1981); the Regional Cancer Registry in Umea, Sweden (Hardell and Eriksson, 1988); the Regional Cancer Registry at the University Hospital in Linkoping in southeastern Sweden (Wingren et al., 1990); and the Regional Cancer Registry in Uppsala in central Sweden (Eriksson et al., 1990).

Based on these results, other researchers conducted cases-control studies of other health outcomes including HD, NHL, and other lymphomas from the north-

ern Sweden cancer registry in Umea (Hardell et al., 1980, 1981; Hardell and Bengtsson, 1983); HD and NHL from the Orebro Medical Center Hospital registry (Persson et al., 1989); NHL from the Lund University Hospital registry (Olsson and Brandt, 1988); HD and NHL from the Regional Cancer Registry at the University Hospital in Linkoping (Persson et al., 1993); and nasal and nasopharyngeal carcinomas (Hardell et al., 1982), and primary or unspecified liver cancer (Hardell et al., 1984) from the northern Swedish Cancer Registry. To address criticism regarding potential observer bias in some of these Swedish case-control series, Hardell (1981) conducted another case-control study on colon cancer. Later, Hardell et al. (1994) examined the relationship between occupational exposure to phenoxyacetic acids and chlorophenols and various parameters related to NHL, including histopathology, stage, and anatomical location, based on the NHL cases from a previous study (Hardell et al., 1981).

Prompted by the Swedish studies of STS and exposure to phenoxy herbicides, a set of case-control studies was undertaken in New Zealand to evaluate the risks of phenoxyherbicide and chlorophenol exposure and STS incidence and mortality (Smith et al., 1983, 1984; Smith and Pearce, 1986). Additional case-control studies and an expanded case series were conducted of phenoxy herbicide and chlorophenol exposure and the risks of malignant lymphoma, NHL, and multiple myeloma (Pearce et al., 1985, 1986a,b, 1987).

Geographic mortality patterns for white males indicated elevated leukemia mortality in the central part of the United States, which prompted a study of the leukemia mortality of Nebraska farmers (Blair and Thomas, 1979). Additional case-control studies were later conducted on leukemia in Nebraska (Blair and White, 1985); Iowa (Burmeister et al., 1982), based on the cohort study of Burmeister (1981); Iowa and Minnesota (Brown et al., 1990); and leukemia associated with NHL in eastern Nebraska (Zahm et al., 1990). *VAO* and *Update 1996* summarize these studies in greater detail.

Case-control studies have been conducted on other cancers, including NHL in Iowa and Minnesota (Cantor et al., 1992); multiple myeloma in a nation-wide American Cancer Society Cancer Prevention Study (Boffetta et al., 1989); cancers of the stomach, prostate, NHL, and multiple myeloma in Iowa (Burmeister et al., 1983); STS, HD, and NHL in Kansas (Hoar et al., 1986); multiple myeloma in Iowa (Brown et al., 1993); and NHL among white women in Nebraska. (Zahm et al., 1993).

Other researchers have conducted additional studies on NHL among white male residents of certain Wisconsin counties (Cantor, 1982); NHL and HD in Hancock County, Ohio, an area of reported heavy herbicide use (Dubrow et al., 1988); multiple myeloma in four Surveillance, Epidemiology, and End Results (SEER) areas from Detroit, Washington State, Atlanta, and Utah (Morris et al., 1986); and STS and NHL 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).

Numerous case-control studies have examined other health effects, including spontaneous abortions in Oregon and Washington (Carmelli et al., 1981); immunosuppression and subsequently decreased host resistance to infection among AIDS patients with Kaposi's sarcoma (Hardell et al., 1987); and mortality of U.S. Department of Agriculture extension agents (Alavanja et al., 1988, 1989).

More recently, Blatter and colleagues (1997) recently conducted a multicenter case-control study of paternal occupation and risk of spina bifida in offspring. The study identified live-born cases of spina bifida by medical records review at seven hospitals and two rehabilitation centers in the Netherlands (1980–1992). Controls were children who were born healthy, but developed trauma capitis or meningitis during early childhood and were diagnosed at three of the hospitals where cases were identified ($N = 456$). Birth registries were used to identify another group of controls ($N = 1,894$). Case and control parents were initially mailed a questionnaire to collect data on occupational histories and potentially confounding factors. A follow-up telephone interview was conducted for fathers that had an occupation involving potential environmental exposures. This second interview included items on the frequency of tasks and exposures and the use of protective gear. Agricultural workers were included in the second interview. Estimation of exposure level was based on the self-reported information and the judgment of industrial hygienists. Exposure was analyzed for the time period from three months prior to the estimated date of conception to one month after. Response to the initial questionnaire included 77 percent of cases and 68 percent of controls. The final sample, including the second interview, totaled 222 cases and 764 controls. Data were collected on a number of potentially confounding factors including medication use, maternal diabetes, parity, family history of neural tube defects, and parental smoking and alcohol consumption. No analyses were presented on specific pesticides, especially herbicides of interest such as 2,4-D, probably because of the small numbers of exposed subjects.

Based on the work of Amadori et al. (1995), Nanni et al. (1996) conducted a population-based case-control study in northeastern Italy of occupational and chemical risk factors for lymphocytic leukemia and non-Hodgkin's lymphomas. Between 1987 and 1990, the population tumor registry of the Forli Province was consulted to identify all hematologically or histologically diagnosed cases of NHL and chronic lymphocytic leukemia (CLL) among 15–75 year olds ($N = 187$). Controls ($N = 977$) were selected randomly using a residents' list and were frequency matched by sex and five-year age interval. Study participants were mailed a questionnaire regarding occupational history, potential pesticide exposure and confounders. Exposure recall and a priori matrices of occupational status and exposure were used to define exposure. Unconditional logistic analysis was performed with adjustment for confounders.

Tatham et al. (1997) conducted a population-based case-control study of the occupational risk factors for subgroups of NHL patients using information contained in the CDC's Selected Cancers Study, a multicenter case-control study

(CDC, 1990a–d). In 1983, the CDC undertook the Selected Cancers Study to investigate the health effects of Vietnam military service and exposure to herbicides. Tumor registries covering three states and five large metropolitan areas were reviewed to identify all cases of several types of cancers diagnosed from December 1984 to November 1988. Controls were selected by random-digit dialing and were frequency matched for geographic area covered by the tumor registry and five-year date-of-birth intervals. A second control group consisted of deceased individuals from the same registry area, who were pair matched to deceased cases by date of birth, race, and time interval between death and proxy interview. Researchers interviewed study participants, collecting information on medical history, occupation, contact with chemicals, personal characteristics and habits, and military service in Vietnam (CDC, 1990a–d). *VAO* describes the study design in more detail.

Based on these controls and a subset of cases diagnosed with NHL, Tatham et al. (1997) conducted another study. In all, 1,048 cases and 1,659 controls were identified. Cases were categorized into three subgroups representing different histological types of NHL: (1) small-cell diffuse lymphomas, (2) follicular lymphomas, and (3) large-cell diffuse lymphomas. Comparisons were made between these three subgroups and controls for chemical exposure and occupation using conditional logistic regression.

Schulte et al. (1996) conducted a proportionate mortality study of neurodegenerative diseases and occupational risk factors. The researchers collected 130,420 death certificates for the years 1982–1991 from 27 states in the National Occupational Mortality Surveillance System. They coded occupation or industry of employment according to Bureau of Census standards. Subsequently, a certified industrial hygienist and senior epidemiologist grouped these occupational codes based on common exposures and tasks. Age-standardized proportionate mortality ratios (PMRs) were calculated for the each occupational group for four neurodegenerative diseases: (1) presenile dementia, (2) Alzheimer's disease, (3) Parkinson's disease, and (4) motor neuron disease.

Liou et al. (1997) conducted a case-control study of occupational and environmental risk factors and Parkinson's disease (PD) in Taiwan. The researchers recruited 120 PD patients from the Movement Disorder Clinic of National Taiwan University Hospital from 1993 to 1995 along with 240 controls matched on age and sex. Interviewers obtained data on demographic and residential history and potential exposure to occupational and environmental agents. Although the structured interview included questions about pesticide or herbicide use, no specific information about phenoxy herbicide exposure was obtained. Subjects were asked about exposure to the herbicide paraquat. Researchers calculated chi square and odds ratios for matched subjects. In addition, conditional logistic regression was employed in the multivariate analysis.

Seidler et al. (1996) conducted a case-control study of Parkinson's disease and various rural factors, including exposure to herbicides and wood preserva-

tives. Researchers recruited 380 PD patients from nine German neurologic clinics, along with 379 neighborhood and 376 regional controls. Trained interviewers collected data on demographic, residential, and occupational variables and on exposures to numerous chemical agents. Subjects were questioned about frequency and length of herbicide use and contact with wood preservatives. Researchers used a job exposure matrix to more objectively assess exposure to these chemicals. They controlled educational status and smoking through the use of conditional logistic regression.

VAO and *Update 1996* describe a number of other studies that look at various health outcomes and associated exposures to phenoxyherbicides or surrogate measures of exposure. The examined health outcomes include including ovarian cancer in the Piedmont region of Italy (Donna et al., 1984); brain gliomas in two hospitals in Milan, Italy (Musicco et al., 1988); STS and other cancers from the 15 regional cancer registries that constitute the National Cancer Register in England (Balarajan and Acheson, 1984); STS and malignant lymphomas in the Victorian Cancer Registry of Australia (Smith and Christophers, 1992); lymphoid cancer in Milan, Italy (LaVecchia et al., 1989); STS among rice weeders in northern Italy (Vineis et al., 1986); primary lung cancer among pesticide users in Saskatchewan (McDuffie et al., 1990); renal cell carcinoma from the Denmark Cancer Registry (Mellemgaard et al., 1994); Parkinson's disease in relation to occupational risk factors in Canada (Semchuk et al., 1993); and birth defects among agricultural workers in Finland (Nurminen et al., 1994).

Paper and Pulp Workers

VAO describes studies of workers potentially exposed to TCDD at paper and pulp mills and various health outcomes, including general mortality of workers at five mills in Washington, Oregon, and California (Robinson et al., 1986); cancer incidence among male Finnish paper mill workers (Jappinen and Pukkala, 1991); respiratory health in a New Hampshire mill (Henneberger et al., 1989); and cause-specific mortality among white males employed in plants identified by the United Paperworkers International Union (Solet et al., 1989).

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.

Seveso

One of the largest industrial accidents involving environmental exposures to TCDD occurred in Seveso, Italy, 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: 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., 1989a,b).

Several cohort studies based on these exposure categories have been conducted. These studies are reviewed extensively in *VAO* and *Update 1996* and summarized here. Caramaschi et al. (1981) presented the distribution of chloracne among Seveso children, while Mocarelli et al. (1986) tested the children for laboratory levels of several chemicals in the blood and urine based on previous chloracne. In a follow-up to these studies, dermatologic findings and laboratory tests were conducted among a group of the children with chloracne compared to controls (Assennato et al., 1989a).

Other studies looked at specific health effects associated with TCDD exposure among Seveso residents, including chloracne, birth defects, spontaneous abortions, crude birth and death rates (Bisanti et al., 1980), chloracne and peripheral nervous system conditions (Barbieri et al., 1988), hepatic enzyme associated conditions (Ideo et al., 1982, 1985), abnormal birth outcomes (Mastroiacovo et al., 1988), cytogenetic abnormalities in maternal and fetal tissues (Tenchini et al., 1983), neurological disorders (Boeri et al., 1978; Filippini et al., 1981), and cancer incidence (Pesatori et al., 1992, 1993; Bertazzi et al., 1993). A two-year prospective controlled study was conducted of workers potentially exposed to TCDD during cleanup of the most highly contaminated areas following the accident (Assennato et al., 1989b).

Mocarelli et al. (1996) recently evaluated the sex ratio among offspring who were born in zone A of Seveso from 1977–1984 (74 births) and from 1985–1994 (124 births). Stored serum samples were used to determine the TCDD levels in 13 families in which both parents were from zone A to further examine the relationship with sex ratio.

The Seveso residents have had long-term follow-up of their health outcomes, especially cancer. For example, Bertazzi et al. (1989a,b, 1992) conducted ten-year mortality follow-up studies among adults and children age 1 to 19 at the time of the accident.

More recently, Bertazzi et al. (1997) evaluated the Seveso population after 15 years of follow-up through the end of 1991. Study subjects were assigned to one of the exposure zones previously described: 45,373 cases (805 in zone A; 5,943 in zone B; and 38,625 in zone R) and 232,747 controls were identified. Poisson regression was employed to compare age-adjusted rates in each exposure and control zone. Additional analyses were conducted using a surrogate of duration of exposure and time since first exposure within each exposure group.

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 number of studies were conducted to evaluate health effects from the potential exposure (Hoffman et al., 1986; Stehr et al., 1986; Stehr-Green et al., 1987; Webb et al., 1987; Evans et al., 1988; Stockbauer et al., 1988). *VAO* discusses these studies in greater detail.

Vietnam

Vietnamese researchers have conducted studies of the native population exposed to the spraying that occurred during the Vietnam conflict. In a review paper, Constable and Hatch (1985) have summarized the unpublished results of these studies. The review article included nine reports that focus primarily on reproductive outcomes (Can et al., 1983a,b; Huong and Phuong, 1983; Khoa, 1983; Lang et al., 1983a,b; Nguyen, 1983; Phuong and Huong, 1983; Trung and Chien, 1983). Vietnamese researchers later published results of four additional studies conducted in Vietnam, two focusing on reproductive abnormalities (Phuong et al., 1989a,b), one on mortality (Dai et al., 1990), and one on hepatocellular carcinoma (Cordier et al., 1993). *VAO* and *Update 1996* discuss these studies in more thorough detail.

Other Environmental Studies

VAO and *Update 1996* reported on numerous studies focusing on reproductive outcomes of potential environmental exposure in Oregon (U.S. EPA, 1979); Arkansas (Nelson et al., 1979); Iowa and Michigan (Gordon and Shy, 1981); New Brunswick, Canada (White et al., 1988); Skaraborg, Sweden (Jansson and Voog, 1989); and Northland, New Zealand (Hanify et al., 1981).

Numerous studies have focused on other outcomes due to environmental exposure, including STS and connective tissue cancers in Midland County, Michigan (Michigan Department of Public Health, 1983); NHL in Yorkshire, England (Cartwright et al., 1988); cancer in Finland (Lampi et al., 1992); and lymphomas and STS in Italy (Vineis et al., 1991). Additional studies were conducted on neuropsychological effects in Germany (Peper et al., 1993); young-onset Parkinson's disease in Oregon and Washington (Butterfield et al., 1993); and adverse health effects following an electrical transformer fire in Binghamton, New York (Fitzgerald et al., 1989).

More recently, Gallagher et al. (1996) conducted a community case-control study of skin cancer in Alberta, Canada. Using pathological reports obtained

from the Alberta Cancer Registry, researchers identified and interviewed 226 male patients diagnosed during 1983–1984 with primary basal cell carcinoma (BCC) and 180 male patients with primary squamous cell carcinoma (SCC). By randomly sampling the Alberta Health Care Insurance Plan patient files, 406 age-matched controls were identified and interviewed. Interview questions included self-reported duration, intensity, and source of exposure to numerous chemical agents. To better characterize total lifetime exposure, the duration of exposure was weighted by its source (direct job, workplace environment, hobby, or home) and intensity (duration per week: <1 hour, 1–4 hours, 5–19 hours, or ≥20 hours). Exposed subjects were dichotomized into low and high exposure levels based on this total lifetime exposure. Adjustments were made for age, skin and hair color, mother's ethnic origin, and sunlight exposure in 10 years prior to diagnosis by use of conditional logistic regression.

Based on a longitudinal cohort study of residents of Tecumseh, Michigan, Waterhouse et al. (1996) conducted a nested case-control study of NHL, HD, and CLL in a rural Michigan community. Researchers identified 7,016 study participants who had been involved in earlier rounds of the longitudinal cohort study, and sent health surveillance questionnaires to them or their living relatives. Cause of death was determined for 99 percent of the individuals. Researchers collected death certificates and those indicating cancer as the cause of death were placed into several categories (definite, probably, suspect, unconfirmed, and misclassified cases) based on medical confirmation of tumor type. Comparisons were made between the observed incidence of cancer in these categories and that expected based on a referent population in the Connecticut tumor registry. A nested case-control study was then conducted based on risk factors obtained in the longitudinal study, including smoking history, family cancer history, and occupational and environmental exposure to numerous chemicals. Four sets of controls were matched to each case ($N = 42$ males and 32 females) with leukemia or lymphoma by sex and year of birth. Exposure to pesticides and herbicides was evaluated by geographic coding of acreage sprayed in 1978 or 1982–1987 by Michigan county.

Masala et al. (1996) conducted a large multicenter case-control study of HD ($N = 421$), NHL ($N = 1822$), multiple myelomas ($N = 325$) and acute myeloid leukemias ($N = 263$) in various regions of Italy. Cases were ascertained from data provided by cancer registries, hospitals in the regions studied, and referral hospitals. Cases were categorized based on area of residence, including the heavily industrialized regions in the north of Italy, rural areas, and mixed rural and urban settings. Based on these data, annual incidence rates were calculated according to five-year age groups. Comparison were made across area of residence.

Wolf and Karmaus (1995) reported on a cross-sectional study of the effects of inhalative exposure to TCDD and related compounds in wood preservatives on cell-mediated immunity in German day care center employees. The study population consisted of 221 exposed persons and an unexposed control group of 189 persons who worked at neighboring day-care centers not treated with wood pre-

servatives. Research staff conducted physical exams, administered clinical tests on cell-mediated immunity, and interviewed study participants about occupational exposure. Measurements of the indoor air concentrations of the wood preservatives and the contaminant, dioxin were taken. Researchers also took into account other sources of exposure to dioxin and wood preservatives.

A number of studies evaluated TCDD exposure among fishermen It is assumed that diet constitutes the primary exposure route in these cases. Svensson et al. (1995) assessed mortality and cancer incidence in two cohorts of Swedish fishermen. One group (2,896 men) resided on the east coast of Sweden and consumed fish from the Baltic Sea. These fatty fish (particularly salmon and herring) are reported to contain elevated levels of PCBs, PCDDs, and PCDFs. The other group of fishermen (8,477) resided on the west coast of Sweden and were presumed to have a higher intake of lean (and less contaminated) fish, including cod and flat fish. This distinction of exposure by place of residence is reportedly confirmed by the finding that blood levels of dioxin-like compounds were two times higher among east coast than west coast fishermen; however, no supporting data are provided relating to this point.

Lovik and colleagues (1996) conducted a cohort study of hobby fishermen in the Frierfjord in southeastern Norway. The researchers recruited 24 fishermen with possible dietary exposure to a number of halogenated aromatic hydrocarbons, such as PCDD and PCF, through crab consumption. The locally caught crabs are known to contain high levels of these organochlorine compounds. Ten control subjects were randomly selected from the Population Registry. All study participants were asked detailed questions about health status, potential occupational and environmental exposure to PCDDs, PCDFs, and PCBs, and fish and crab consumption. The researchers also collected blood samples, measuring levels of the organochlorine compounds and various biological parameters.

Based on the previous study of Dutch infants (Koopman-Esseboom et al., 1994), Weisglas-Kuperus et al. (1995) explored the immunological effects of pre- and postnatal PCB or TCDD exposure in 207 Dutch infants from birth to 18 months of age. Mother-infant pairs were selected from the Rotterdam area and 102 infants were exclusively bottle-fed, while the remaining 105 were breast-fed for at least 6 weeks. Postnatal dioxin and PCB exposure for breastfed infants was estimated from the total toxic equivalent level of each compound in human milk multiplied by the weeks of breastfeeding. Prenatal exposure to the compounds was estimated for all infants based on PCB-plasma levels for individual mother-infant pairs. Blood samples were collected on a subgroup of infants and immunological tests conducted. Mothers were questioned about health status and history.

VIETNAM VETERANS STUDIES

Studies of Vietnam veterans who were potentially exposed to herbicides, including Agent Orange, have been conducted in the United States at the national

and state levels, as well as in Australia. Exposure measures in these studies have been done on a variety of levels, and evaluations of health outcomes have been made using a variety of different comparison or control groups. This section is organized primarily by the sponsors of the research, because this format is more conducive to methodologic presentation of the articles. In these studies, exposure measures fall along a crude scale from individual levels for Ranch Hands, as reflected in serum dioxin measurements, to use of service in Vietnam as a surrogate for TCDD exposure in some state studies.

It should also be noted that comparison groups for the veteran cohort studies vary to include 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 military at the time of the conflict but did not serve in Vietnam; non-Vietnam veterans who served in other wars or conflicts such as the Korean War 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 in Vietnam were volunteers from the Air Force who participated in Operation Ranch Hand. 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 Ranch Hands (AFHS, 1982). *VAO* and *Update 1996* discuss the cohort in more detail.

A retrospective matched cohort study design was implemented to examine morbidity and mortality, with follow-up scheduled to continue until 2002. National Personnel Records Center and U.S. Air Force Human Resources Laboratory records were searched and cross-referenced to ascertain completely all Ranch Hand personnel (AFHS, 1982; Michalek et al., 1990). A total of 1,269 participants were originally identified (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 used to identify the Ranch Hand population. Controls were matched on age, type of job (using Air Force specialty code), and race (white or not white). The rationale for matching on these variables was to control for the clinical aging process, educational 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 and 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 random sample of half of each subject's control set are 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" is selected; controls who die are not replaced.

The baseline exam occurred in 1982, and future exams are scheduled until 2002. Morbidity is ascertained through questionnaire and physical examination, which emphasize 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, the DVA's Death Beneficiary Identification and Record Location System (BIRLS), and the Internal Revenue Service's data base of active social security numbers. Death certificates were obtained from the appropriate health departments (Michalek et al., 1990). Eighty-four percent of the 1,148 eligible Ranch Hands (N = 952), 76 percent of the original comparison group (N = 912), and 65 percent of the 567 replacement comparisons (N = 369) invited to the 1992 follow-up chose to participate in the examination and questionnaire (AFHS, 1995). The methods used to assess mortality and morbidity were identical to the methods described previously for the 1982 and 1987 examinations.

Ranch Hands were divided into three categories on the basis of their potential exposures:

1. *Low potential:* This group included pilots, copilots, and navigators. Exposure was primarily through preflight checks and during actual spraying.
2. *Moderate potential:* This group included crew chiefs, aircraft mechanics, and support personnel. Exposure could occur by contact during dedrumming and aircraft loading operations, on-site repair of aircraft, and repair of spray equipment.
3. *High potential:* This group included spray console operators and flight engineers.

Results have been published for the baseline morbidity (AFHS, 1984a) and baseline mortality studies (AFHS, 1983); first (1984b), second (1987), and third (1992) follow-up examinations (AFHS, 1987, 1990, 1995); and reproductive outcomes study (AFHS, 1992; Wolfe et al., 1995). 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 (455 Ranch Hands; AFHS, 1995). Serum dioxin analysis of the 1987 follow-up examinations was published in 1991 (AFHS, 1991b). Continued follow-up and results will be forthcoming.

In an interim technical report, the Air Force Health Study (AFHS, 1996; Michalek et al., 1998b) updated the cause-specific mortality among 1,261 Ranch Hand personnel compared to 19,080 controls through the end of 1993. Study design followed that of the previous Ranch Hand studies. The study team reported few other details.

A recent Ranch Hand publication addressed the relationship between serum dioxin and reproductive hormones (Henriksen et al., 1996). The Air Force investigators measured serum testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testicular abnormality in clinic visits by Ranch Hand and comparison participants in 1982, 1985, 1987, and 1992. In the baseline year of 1982, a total of 1,045 Ranch Hands and 1,224 comparisons participated in the study. Serum dioxin was measured in 1987, testicular volume in 1992, and sperm count and percentage of abnormal sperm in 1982. The semen data was collected from 474 Ranch Hands and 532 comparisons. Potential confounding factors adjusted for in the analysis included age, race, personality type, diabetes, current alcohol consumption, current cigarette smoking, and percentage of body fat. Sperm count and sperm abnormality were adjusted only for age and exposure to industrial chemicals.

Henriksen and colleagues (1997) analyzed the Ranch Hand data to address the relationship between TCDD and diabetes mellitus and glucose and insulin levels. For this analysis, a total of 989 Ranch Hands and 1,276 comparisons were clinically examined. Blood samples were collected and medical records were reviewed to determine diabetes status, severity and time-to-diabetes onset. Serum insulin and glucose levels were calculated from blood samples taken in 1992. Exposure to TCDD was classified on the basis of original exposure calculated from serum (lipid-adjusted) dioxin levels determined in 1987 or 1992. At follow-up (1992), the mean age of the comparison group was 53.5 years (±7.6), and the mean ages of the exposed groups were 54.6 ± 7.2, 54.9 ± 7.6, and 50.9 ± 7.4 years by increasing exposure category.

A recent Ranch Hand publication reported results for the analysis of dioxin levels in relation to infant death (Michalek et al., 1998a). Infant death was ascertained from medical records, vital statistics, and autopsy records. Cause of death was coded based on record review. The analysis included a total of 2,082 children (859 children of Ranch Hands and 1,223 children of comparisons) conceived before the father's service in Southeast Asia. The 859 Ranch Hands children were stratified into four exposure categories including children of comparison veterans (current dioxin levels less than 10 ppt), "background" Ranch Hand children ($\leq$10 ppt), "low" Ranch Hand children (>10 and $\leq$79 ppt), and "high" Ranch Hand children (> 79 ppt). The cutpoint of 79 ppt is the median initial dioxin level based on the extrapolated current levels (in 1987 or 1992). The stratified analyses

adjusted for father's race, mother's smoking and alcohol consumption during pregnancy, parental age, and father's military occupation. The investigators also examined the distribution of post-SEA infant deaths in the low and high categories by quintile of initial dioxin. Another analysis was conducted excluding infants whose mothers had medical conditions such as hypertension during pregnancy, abruptio placentae, placenta previa, and Ranch Hand incompatibility.

Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC) 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). *VAO* and *Update 1996* describe these studies in more detail. The first of these was a case-control interview study of birth defects among offspring of fathers serving in Vietnam (Erickson et al., 1984a,b).

To examine the concerns about Agent Orange more directly, the CDC conducted the Agent Orange Validation Study 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 (CDC, 1989a). Using the exposure estimates from this study, the CDC subsequently conducted the Vietnam Experience Study (VES), a historical cohort study of the health experience of Vietnam veterans (CDC, 1989b). The study was divided into three parts: (1) physical health; (2) reproductive outcomes and child health; and (3) psychosocial characteristics (CDC, 1987, 1988a–c, 1989b).

Using data from the VES, 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). An additional study (O'Brien et al., 1991) combined the mortality and interview data to identify all veterans with NHL. To evaluate whether self-reported assessment of exposure to herbicides influences the reporting of adverse health outcomes, the CDC designed a study using VES subjects (Decoufle et al., 1992).

Finally, the CDC undertook the Selected Cancers Study (CDC, 1990a) to investigate the effects of military service in Vietnam and exposure to herbicides on the health of American veterans. Outcomes studied were NHL (CDC, 1990b); STS and other sarcomas (CDC, 1990c); and HD, nasal, nasopharyngeal, and primary liver cancers (CDC, 1990d).

Department of Veterans Affairs

The DVA has conducted numerous cohort and case-control studies, which *VAO* and *Update 1996* discuss in greater detail. One of the first of these was a

proportionate mortality study conducted by Breslin et al. (1988). 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. A list of 186,000 Vietnam era veterans who served in the Army or Marine Corps 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. Cause of death was ascertained for 51,421 men, including 24,235 who served in Vietnam. Based on this proportionate mortality study (Breslin et al., 1988), Burt et al. (1987) conducted a nested case-control study of NHL with controls selected from among the cardiovascular disease mortality deaths. Later, Bullman et al. (1990) examined whether Army I Corps Vietnam veterans had cancer mortality experiences similar to other Army Vietnam era veterans, based on the study design of Breslin et al. (1988).

Watanabe et al. (1991) conducted an additional study using this Vietnam veteran mortality experience (Breslin et al., 1988) compared with three different referent groups and with additional follow-up through 1984. The final study group of 62,068 veterans included 50,743 from the earlier mortality study of Breslin et al. (1988).

Watanabe and Kang (1996) conducted a third follow-up proportionate mortality study using the 75,617 veterans from Breslin et al. (1988) and the 15,038 veterans from Watanabe et al. (1991). Using the original study design, a random sample of 11,851 veterans was selected from a BIRLS-generated file of 59,259 veterans who died between July 1, 1984, and June 30, 1988. The three groups were combined to yield a final sample of 102,506 veterans. Excluding those who served in Southeast Asia but not in Vietnam, the researchers collected detailed demographic and military records and ascertained the cause of death for 70, 630 of these veterans, including 33,833 who served in Vietnam and 36,797 era veterans. 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 different branches of the military. Three separate comparison groups were identified: (1) branch-specific Vietnam era veterans; (2) all Vietnam era veterans combined; and (3) for external comparisons, the U.S. male population.

The DVA also examined the morbidity and mortality experience of a subgroup of Vietnam veterans potentially exposed to high levels of herbicides from certain U.S. Army Chemical Corps units (Thomas and Kang, 1990). *VAO* discusses the study in greater detail.

In an extension of Thomas and Kang (1990), Dalager and Kang (1997) recently compared veterans of the Chemical Corps specialties, including 2,872 Vietnam veterans and 2,737 non-Vietnam veterans. All study subjects served at least 18 months active duty between 1965 and 1973, and vital status ascertainment was complete for both groups. Direct exposure information on the two cohorts was not available, and the presumption that Vietnam veterans had potentially higher levels of dioxin exposure because of their duties (which involved

Agent Orange and other dioxin-contaminated herbicides) than non-Vietnam veterans has not been verified. The effects of race, military rank, duration of service, and age at entry to follow-up were adjusted using proportional hazards modeling.

A recent DVA cohort study (Watanabe and Kang, 1995) examined post-service mortality among 10,716 Marine Vietnam veterans compared to 9,346 Vietnam era Marines who did not actually serve in Vietnam. The researchers first linked files of a sample of all active-duty (1967–1969) Marines to military records from the National Personnel Records Centers and then checked the vital status of these Marines using BIRLS. BIRLS identified 701 cases and 562 controls as deceased between 1973-1991. Subsequently, the researchers located death certificates, and evaluated and coded the cause of death for each Marine. Comparisons of cause-specific mortality were made between cases and controls using relative frequency of death along with a proportional hazards multivariate model. Comparisons were also made to mortality rates of U.S. males by adjusting for age, race, and calendar year period. Finally, follow-up categories of less than 16 years and 16 years or more were created and used to compare cancer latency.

The DVA has also evaluated specific disease and health outcomes, including case-control studies of STS (Kang et al., 1986, 1987), NHL (Dalager et al., 1991), and testicular cancer (Bullman et al., 1994), as well as a co-twin study of self-reported physical health in a series of Vietnam era monozygotic twins (Eisen et al., 1991). Mortality among women Vietnam veterans was assessed by Thomas et al. (1991) and Dalager et al. (1995a). *VAO* and *Update 1996* provide more detail.

Using the study design of Dalager et al. (1991), a case-control study assessed the risk of Vietnam service for the development of Hodgkin's disease (Dalager et al., 1995b). A review of the DVA Patient Treatment File (PTF) from 1969 to 1985 identified all malignant lymphomas among male Vietnam era veterans. A pathologist reviewed pathology reports from VA medical centers that had 10 or more lymphoma cases and identified 770 cases of HD. Using the PTF, 1,540 controls were selected from the same Vietnam-era veteran population as the cases, matched by hospital, discharge year of hospitalization for HD, and birth date. Researchers linked these data with military personnel records provided by the National Personnel Records Center. After exclusion of some individuals due to potential bias, 283 cases and 404 controls were identified. Surrogate measures of Agent Orange exposure were specified for cases based on military branch, duration of service, region of military assignment in Vietnam, and occupational specialty. A multiple logistic regression model was employed for comparisons.

Mahan et al. (1997) conducted a case-control study of lung cancer among veterans. Using the VA's Patient Treatment File (PTF), 329 Vietnam era veterans with a diagnosis of lung cancer made between 1983–1990 were identified. Variables abstracted from the military record include education, race, branch of service, Military Occupational Specialty Code, rank, and units served within Vietnam. Two groups of controls were randomly selected from the PTF file of (1) men hospitalized for a reason other than cancer ($N = 269$), and (2) patients with

colon cancer ($N = 111$). The researchers characterized the veterans' exposure to Agent Orange by assessing information on the location of each individual ground troop veteran's unit in relation to an area sprayed and the time elapsed since that area was sprayed.

Other outcomes including posttraumatic stress disorder (True et al., 1988; Bullman et al., 1991), suicide, and motor vehicle accidents (Farberow et al., 1990) among Vietnam veterans have also been examined by the DVA. *VAO* discusses these studies in greater detail. In many of these studies, exposure to Agent Orange is not discussed, but exposure to "combat" is evaluated as the risk factor of interest.

More recently, Bullman and Kang (1996) assessed the risk of cause-specific mortality among 34,534 veterans with nonlethal (combat and noncombat) wounds sustained during the Vietnam war. This study did not evaluate or control for chemical exposures.

McKinney et al. (1997) compared the smoking behavior of veterans and nonveterans using the 1987 National Medical Expenditure Survey (NMES). The NMES is designed to explore trends in health service utilization. Based on a stratified area probability design, 15,000 households were asked to participate in the five rounds of NMES interviews. Self-reported smoking status or history and service status or history were compared using covariance and chi squared analysis and adjusting for age, ethnicity, and sex. The study did not evaluate or control for any chemical exposures.

American Legion

The American Legion conducted a cohort study of the health and well-being of Vietnam veterans who belonged to the American Legion, a voluntary veterans service organization. A series of studies examining physical health and reproductive outcomes, social–behavioral consequences, and PTSD was conducted on 2,858 veterans who had served in Southeast Asia and 3,933 who served elsewhere (Snow et al., 1988; Stellman et al., 1988a–c).

State Studies

Several states have conducted studies of Vietnam veterans. Most of these studies remain unpublished in the scientific literature. *VAO* and *Update 1996* review studies from Hawaii (Rellahan, 1985); Iowa (Wendt, 1985); Maine (Deprez et al., 1991); Massachusetts (Kogan and Clapp, 1985, 1988; Levy, 1988; Clapp et al., 1991); Michigan (Visintainer et al., 1995); New Jersey (Kahn et al., 1988; Fielder and Gochfeld, 1992; Kahn et al., 1992a–c); New Mexico (Pollei et al., 1986); New York (Greenwald et al., 1984; Lawrence et al., 1985); Pennsylvania (Goun and Kuller, 1986); Texas (Newell, 1984); West Virginia (Holmes et al., 1986); and Wisconsin (Anderson et al., 1986a,b).

Recently, Clapp (1997) updated the Massachusetts veterans cancer surveillance study reported six years earlier (Clapp et al., 1991). In the first study, detailed in *VAO*, the researchers identified cases of selected cancers from the Massachusetts Cancer Registry between 1982 and 1988 (Clapp et al., 1991). Data were linked to status as Vietnam era veterans or Vietnam bonus recipients. Controls for each cancer site analysis included veterans with other cancers, excluding STS, NHL, and kidney cancer. The study identified 727 male Vietnam era veterans and 214 Vietnam veterans. Study participants were males between the ages of 30 and 59 at the time of cancer diagnosis, and Vietnam service served as the exposure of interest.

For the update, Clapp (1997) conducted an additional records linkage covering the years 1988 to 1993. During this six-year period, researchers identified 245 and 999 cases of cancer in Vietnam veterans and Vietnam era veterans, respectively. Age-adjusted odds ratios for selected cancers were calculated and used in the comparison.

Other U.S. Vietnam Veteran Studies

Additional studies have been conducted to examine a number of health outcomes including spontaneous abortion (Aschengrau and Monson, 1989), late adverse pregnancy outcomes (Aschengrau and Monson, 1990) in spouses of veterans, and PTSD among monozygotic twins who served during the Vietnam era (Goldberg et al., 1990). After a published study indicating a potential association with testicular cancer in dogs that served in Vietnam (Hayes et al., 1990), Tarone et al. (1991) conducted a case-control study of testicular cancer in male veterans. *VAO* summarizes these studies.

Australia

The Australian government has also commissioned studies to investigate the health risks of Australian veterans. Studies of birth anomalies (Donovan et al., 1983, 1984; Evatt, 1985); mortality (Commonwealth Institute of Health, 1984a–c; Evatt, 1985; Fett et al., 1987a,b; Forcier et al., 1987); deaths from all causes (Fett et al., 1987b); and cause-specific mortality (Fett et al., 1987a) have been conducted. An independent study in Tasmania evaluated numerous reproductive and childhood health problems for association with paternal Vietnam service (Field and Kerr, 1988). *VAO* describes these Australian studies.

More recently, the Australian Department of Veterans' Affairs conducted a mortality study of more than 59,000 male Australian veterans who served in Vietnam (Crane et al., 1997a). Based on data provided by the Australian Department of Defense and civilian agencies, researchers created a nominal list of all members of the Army, Navy, and Air Force and some civilian personnel who served on land or in Vietnamese waters for at least one day during the period of

the Vietnam war—59,036 in all. In addition, 484 females were identified. Vital statistics, including cause of death, collected from Department of Defense records, Department of Veterans' Affairs records, the National Death Index, Electoral Commission rolls, and the Health Insurance Medicare data base were matched to the nominal list. Of the 59,036 male veterans on the nominal list, 6.5 percent (3,840) died between the end of their service and December 31, 1994; 90.4 percent (53,391) were alive. Vital status of the remaining 3.1 percent (1,805) was unknown. There were no direct measures or indirect estimates of veterans' exposure to herbicides or other chemical agents, and the authors suggest that any variations in mortality found in the study would "probably need to be attributed to service in Vietnam rather than exposure to particular agents." Cause-specific standardized mortality ratios were calculated and compared to death rates for the Australian male population.

A second cohort study of Australian veterans compared mortality for the period 1982–1994 for 18,949 national servicemen who had served in Vietnam (veterans) with that of 24,646 national servicemen who had not served in Vietnam (nonveterans) (Crane et al., 1997b).

O'Toole and colleagues (1996a–c) describe the results of a simple random sample of Australian Army Vietnam veterans on self-reported health status. Data were obtained on 641 veterans from the Australian Bureau of Statistics Health Interview Survey 1989–1990, and illness rates were compared to the age- and sex-matched Australian population. The researchers also adjusted these illness rates for the effects of nonresponse bias based on the 950 veterans who were initially eligible for participation as cases.

Other Vietnam Veterans Studies

A team of Vietnamese scientists recently examined 25 Vietnamese veterans who served 5–10 years in a "dioxin-sprayed zone" (Chinh et al., 1996). Few other details of the cohort were provided. The researchers administered tests to detect antinuclear antibodies in the veterans and 63 age-matched controls. Additionally, tests to detect sperm autoantibodies were conducted on the veterans and 36 male controls.

REFERENCES

Air Force Health Study (AFHS). 1982. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides: Study Protocol, Initial Report. Brooks AFB, TX: USAF School of Aerospace Medicine. SAM-TR-82-44. 189 pp.

Air Force Health Study (AFHS). 1983. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Baseline Mortality Study Results. Brooks AFB, TX: USAF School of Aerospace Medicine. NTIS AD-A130 793.

Air Force Health Study (AFHS). 1984a. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Baseline Morbidity Study Results. Brooks AFB, TX: USAF School of Aerospace Medicine. NTIS AD-A138 340. 362 pp.

Air Force Health Study (AFHS). 1984b. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update: 1984. Brooks AFB, TX: USAF School of Aerospace Medicine.

Air Force Health Study (AFHS). 1985. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update: 1985. Brooks AFB, TX: USAF School of Aerospace Medicine.

Air Force Health Study (AFHS). 1986. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update: 1986. Brooks AFB, TX: USAF School of Aerospace Medicine. USAFSAM-TR-86-43. 12 pp.

Air Force Health Study (AFHS). 1987. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. First Followup Examination Results. 2 vols. Brooks AFB, TX: USAF School of Aerospace Medicine. USAFSAM-TR-87-27. 629 pp.

Air Force Health Study (AFHS). 1989. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update: 1989. Brooks AFB, TX: USAF School of Aerospace Medicine. USAFSAM-TR-89-9. 35 pp.

Air Force Health Study (AFHS). 1990. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. 2 vols. Brooks AFB, TX: USAF School of Aerospace Medicine. USAFSAM-TR-90-2.

Air Force Health Study (AFHS). 1991a. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update: 1991. Brooks AFB, TX: Armstrong Laboratory. AL-TR-1991-0132. 33 pp.

Air Force Health Study (AFHS). 1991b. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Serum Dioxin Analysis of 1987 Examination Results. Brooks AFB, TX: USAF School of Aerospace Medicine. 9 vols.

Air Force Health Study (AHFS). 1992. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Reproductive Outcomes. Brooks AFB, TX: Armstrong Laboratory. AL-TR-1992-0090. 602 pp.

Air Force Health Study (AFHS). 1995. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. 1992 Followup Examination Results. 10 vols. Brooks AFB, TX: Epidemiologic Research Division. Armstrong Laboratory.

Air Force Health Study (AFHS). 1996. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update 1996. Brooks AFB, TX: Epidemiologic Research Division. Armstrong Laboratory. AL/AO-TR-1996-0068. 31 pp.

Alavanja MC, Blair A, Merkle S, Teske J, Eaton B. 1988. Mortality among agricultural extension agents. American Journal of Industrial Medicine 14:167–176.

Alavanja MC, Merkle S, Teske J, Eaton B, Reed B. 1989. Mortality among forest and soil conservationists. Archives of Environmental Health 44:94–101.

Alderfer R, Sweeney M, Fingerhut M, Hornung R, Wille K, Fidler A. 1992. Measures of depressed mood in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Chemosphere 25:247–250.

Amadori D, Nanni O, Falcini F, Saragoni A, Tison V, Callea A, Scarpi E, Ricci M, Riva N, Buiatti E. 1995. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on job titles. Occupational and Environmental Medicine 52(6):374–379.

Anderson HA, Hanrahan LP, Jensen M, Laurin D, Yick W-Y, Wiegman P. 1986a. Wisconsin Vietnam Veteran Mortality Study: Proportionate Mortality Ratio Study Results. Madison: Wisconsin Division of Health.

Anderson HA, Hanrahan LP, Jensen M, Laurin D, Yick W-Y, Wiegman P. 1986b. Wisconsin Vietnam Veteran Mortality Study: Final Report. Madison: Wisconsin Division of Health.

Aschengrau A, Monson RR. 1989. Paternal military service in Vietnam and risk of spontaneous abortion. Journal of Occupational Medicine 31:618–623.

Aschengrau A, Monson RR. 1990. Paternal military service in Vietnam and the risk of late adverse pregnancy outcomes. American Journal of Public Health 80:1218–1224.

Asp S, Riihimaki V, Hernberg S, Pukkala E. 1994. Mortality and cancer morbidity of Finnish chlorophenoxy herbicide applicators: an 18-year prospective follow-up. American Journal of Industrial Medicine 26:243–253.

Assennato G, Cervino D, Emmett E, Longo G, Merlo F. 1989a. Follow-up of subjects who developed chloracne following TCDD exposure at Seveso. American Journal of Industrial Medicine 16:119–125.

Assennato G, Cannatelli P, Emmett E, Ghezzi I, Merlo F. 1989b. Medical monitoring of dioxin clean-up workers. American Industrial Hygiene Association Journal 50:586–592.

Axelson O, Sundell L. 1974. Herbicide exposure, mortality and tumor incidence. An epidemiological investigation on Swedish railroad workers. Scandinavian Journal of Work, Environment, and Health 11:21–28.

Axelson O, Sundell L, Andersson K, Edling C, Hogstedt C, Kling H. 1980. Herbicide exposure and tumor mortality: an updated epidemiologic investigation on Swedish railroad workers. Scandinavian Journal of Work, Environment, and Health 6:73–79.

Balarajan R, Acheson ED. 1984. Soft tissue sarcomas in agriculture and forestry workers. Journal of Epidemiology and Community Health 38:113–116.

Barbieri S, Pirovano C, Scarlato G, Tarchini P, Zappa A, Maranzana M. 1988. Long-term effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the peripheral nervous system. Clinical and neurophysiological controlled study on subjects with chloracne from the Seveso area. Neuroepidemiology 7:29–37.

Barthel E. 1981. Increased risk of lung cancer in pesticide-exposed male agricultural workers. Journal of Toxicology and Environmental Health 8:1027–1040.

Bashirov AA. 1969. The health of workers involved in the production of amine and butyl 2,4-D herbicides. Vrachebnoye Delo 10:92–95.

Becher H, Flesch-Janys D, Kauppinen T, Kogevinas M, Steindorf K, Manz A, Wahrendorf J. 1996. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. Cancer Causes and Control 7(3):312–321.

Bender AP, Parker DL, Johnson RA, Scharber WK, Williams AN, Marbury MC, Mandel JS. 1989. Minnesota highway maintenance worker study: cancer mortality. American Journal of Industrial Medicine 15:545–556.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989a. Mortality in an area contaminated by TCDD following an industrial incident. Medicina Del Lavoro 80:316–329.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989b. Ten-year mortality study of the population involved in the Seveso incident in 1976. American Journal of Epidemiology 129:1187–1200.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Consonni D, Tironi A, Landi MT. 1992. Mortality of a young population after accidental exposure to 2,3,7,8-tetrachlorodibenzodioxin. International Journal of Epidemiology 21:118–123.

Bertazzi A, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C. 1993. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin [see comments]. Epidemiology 4:398–406.

Bertazzi PA, Zochetti C, Guercilena S, Consonni D, Tironi A, Landi MT, Pesatori AC. 1997. Dioxin exposure and cancer risk: a 15-year mortality study after the "Seveso Accident." Epidemiology 8(6):646–652.

Bisanti L, Bonetti F, Caramaschi F, Del Corno G, Favaretti C, Giambelluca SE, Marni E, Montesarchio E, Puccinelli V, Remotti G, Volpato C, Zambrelli E, Fara GM. 1980. Experiences from the accident of Seveso. Acta Morphologica Academiae Scientarum Hungaricae 28:139–157.

Blair A, Thomas TL. 1979. Leukemia among Nebraska farmers: a death certificate study. American Journal of Epidemiology 110:264–273.

Blair A, White DW. 1985. Leukemia cell types and agricultural practices in Nebraska. Archives of Environmental Health 40:211–214.

Blair A, Grauman DJ, Lubin JH, Fraumeni JF Jr. 1983. Lung cancer and other causes of death among licensed pesticide applicators. Journal of the National Cancer Institute 71:31–37.

Blair A, Mustafa D, Heineman EF. 1993. Cancer and other causes of death among male and female farmers from twenty-three states. American Journal of Industrial Medicine 23:729–742.

Blatter BM, Hermens R, Bakker M, Roeleveld N, Verbeek AL, Zielhuis GA. 1997. Paternal occupational exposure around conception and spina bifida in offspring. American Journal of Industrial Medicine 32(3):283–291.

Bloemen LJ, Mandel JS, Bond GG, Pollock AF, Vitek RP, Cook RR. 1993. An update of mortality among chemical workers potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. Journal of Occupational Medicine 35:1208–1212.

Boeri R, Bordo B, Crenna P, Filippini G, Massetto M, Zecchini A. 1978. Preliminary results of a neurological investigation of the population exposed to TCDD in the Seveso region. Rivista di Patologia Nervosa e Mentale 99:111–128.

Boffetta P, Stellman SD, Garfinkel L. 1989. A case-control study of multiple myeloma nested in the American Cancer Society prospective study. International Journal of Cancer 43:554–559.

Bond GG, Ott MG, Brenner FE, Cook RR. 1983. Medical and morbidity surveillance findings among employees potentially exposed to TCDD. British Journal of Industrial Medicine 40:318–324.

Bond GG, Cook RR, Brenner FE, McLaren EA. 1987. Evaluation of mortality patterns among chemical workers with chloracne. Chemosphere 16:2117–2121.

Bond GG, Wetterstroem NH, Roush GJ, McLaren EA, Lipps TE, Cook RR. 1988. Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. British Journal of Industrial Medicine 45:98–105.

Bond GG, McLaren EA, Brenner FE, Cook RR. 1989a. Incidence of chloracne among chemical workers potentially exposed to chlorinated dioxins. Journal of Occupational Medicine 31:771–774.

Bond GG, McLaren EA, Lipps TE, Cook RR. 1989b. Update of mortality among chemical workers with potential exposure to the higher chlorinated dioxins. Journal of Occupational Medicine 31:121–123.

Boyle C, Decoufle P, Delaney RJ, DeStefano F, Flock ML, Hunter MI, Joesoef MR, Karon JM, Kirk ML, Layde PM, McGee DL, Moyer LA, Pollock DA, Rhodes P, Scally MJ, Worth RM. 1987. Postservice Mortality Among Vietnam Veterans. Atlanta: Centers for Disease Control. CEH 86-0076. 143 pp.

Breslin P, Kang H, Lee Y, Burt V, Shepard BM. 1988. Proportionate mortality study of U.S. Army and U.S. Marine Corps veterans of the Vietnam War. Journal of Occupational Medicine 30:412–419.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Research 50:6585–6591.

Brown LM, Burmeister LF, Everett GD, Blair A. 1993. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes Control 4:153–156.

Bueno de Mesquita HB, Doornbos G, van der Kuip DA, Kogevinas M, Winkelmann R. 1993. Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in the Netherlands. American Journal of Industrial Medicine 23:289–300.

Bullman TA, Kang HK. 1996. The risk of suicide among wounded Vietnam veterans. American Journal of Public Health 86(5):662–667.

Bullman TA, Kang HK, Watanabe KK. 1990. Proportionate mortality among U.S. Army Vietnam veterans who served in Military Region I. American Journal of Epidemiology 132:670–674.

Bullman TA, Kang H, Thomas TL. 1991. Posttraumatic stress disorder among Vietnam veterans on the Agent Orange Registry: a case-control analysis. Annals of Epidemiology 1:505–512.

Bullman TA, Watanabe KK, Kang HK. 1994. Risk of testicular cancer associated with surrogate measures of Agent Orange exposure among Vietnam veterans on the Agent Orange Registry. Annals of Epidemiology 4:11–16.

Burmeister LF, Van Lier SF, Isacson P. 1982. Leukemia and farm practices in Iowa. American Journal of Epidemiology 115:720–728.

Burmeister LF, Everett GD, Van Lier SF, Isacson P. 1983. Selected cancer mortality and farm practices in Iowa. American Journal of Epidemiology 118:72–77.

Burmeister LF. 1981. Cancer mortality in Iowa farmers: 1971–1978. Journal of the National Cancer Institute 66:461–464.

Burt VL, Breslin PP, Kang HK, Lee Y. 1987. Non-Hodgkin's lymphoma in Vietnam veterans. Washington: Department of Medicine and Surgery, Veterans Administration.

Butterfield PG, Valanis BG, Spencer PS, Lindeman CA, Nutt JG. 1993. Environmental antecedents of young-onset Parkinson's disease. Neurology 43:1150–1158.

Calvert GM, Sweeney MH, Morris JA, Fingerhut MA, Hornung RW, Halperin WE. 1991. Evaluation of chronic bronchitis, chronic obstructive pulmonary disease, and ventilatory function among workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. American Review of Respiratory Disease 144:1302–1306.

Calvert GM, Hornung RW, Sweeney MH, Fingerhut MA, Halperin WE. 1992. Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. Journal of the American Medical Association 267:2209–2214.

Calvert GM, Sweeney MH, Fingerhut MA, Hornung RW, Halperin WE. 1994. Evaluation of porphyria cutanea tarda in U.S. workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. American Journal of Industrial Medicine 25:559–571.

Can N, Xiem NT, Tong NK, Duong DB. 1983a. A case-control survey of congenital defects in My Van District, Hai Hung Province. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Can N, Xiem NT, Tong NK, Duong DB. 1983b. An epidemiologic survey of pregnancies in Viet Nam. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, Schuman L, Dick FR. 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Research 52:2447–2455.

Cantor KP. 1982. Farming and mortality from non-Hodgkin's lymphoma: a case-control study. International Journal of Cancer 29:239–247.

Caramaschi F, Del Corno G, Favaretti C, Giambelluca SE, Montesarchio E, Fara GM. 1981. Chloracne following environmental contamination by TCDD in Seveso, Italy. International Journal of Epidemiology 10:135–143.

Carmelli D, Hofherr L, Tomsic J, Morgan RW. 1981. A Case-Control Study of the Relationship Between Exposure to 2,4-D and Spontaneous Abortions in Humans. SRI International. Prepared for the National Forest Products Association and the U.S. Department of Agriculture, Forest Service.

Cartwright RA, McKinney PA, O'Brien C, Richards IDG, Roberts B, Lauder I, Darwin CM, Bernard SM, Bird CC. 1988. Non-Hodgkin's lymphoma: case-control epidemiological study in Yorkshire. Leukemia Research 12:81–88.

Centers for Disease Control (CDC). 1987. Postservice mortality among Vietnam veterans. Journal of the American Medical Association 257:790–795.

Centers for Disease Control (CDC). 1988a. Health status of Vietnam veterans. I. Psychosocial characteristics. Journal of the American Medical Association 259:2701–2707.

Centers for Disease Control (CDC). 1988b. Health status of Vietnam veterans. II. Physical health. Journal of the American Medical Association 259:2708–2714.

Centers for Disease Control (CDC). 1988c. Health status of Vietnam veterans. III. Reproductive outcomes and child health. Journal of the American Medical Association 259:2715–2717.

Centers for Disease Control (CDC). 1989a. Comparison of Serum Levels of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin with Indirect Estimates of Agent Orange Exposure among Vietnam Veterans: Final Report. Atlanta: U.S. Department of Health and Human Services.

Centers for Disease Control (CDC). 1989b. Health Status of Vietnam Veterans: Vietnam Experience Study. Vols. I–V, Supplements A–C. Atlanta: U.S. Department of Health and Human Services.

Centers for Disease Control (CDC). 1990a. The Association of Selected Cancers with Service in the U.S. Military in Vietnam: Final Report. Atlanta: U.S. Department of Health and Human Services.

Centers for Disease Control (CDC). 1990b. The association of selected cancers with service in the U.S. military in Vietnam. I. Non-Hodgkin's lymphoma. Archives of Internal Medicine 150:2473–2483.

Centers for Disease Control (CDC). 1990c. The association of selected cancers with service in the U.S. military in Vietnam. II. Soft-tissue and other sarcomas. Archives of Internal Medicine 150:2485–2492.

Centers for Disease Control (CDC). 1990d. The association of selected cancers with service in the U.S. military in Vietnam. III. Hodgkin's disease, nasal cancer, nasopharyngeal cancer, and primary liver cancer. Archives of Internal Medicine 150:2495–2505.

Chinh TT, Phi PT, Thuy NT. 1996. Sperm auto-antibodies and anti-nuclear antigen antibodies in chronic dioxin-exposed veterans. Chemosphere 32(3):525–530.

Clapp RW, Cupples LA, Colton T, Ozonoff DM. 1991. Cancer surveillance of veterans in Massachusetts, 1982–1988. International Journal of Epidemiology 20:7–12.

Clapp RW. 1997. Update of cancer surveillance of veterans in Massachusetts, USA. International Journal of Epidemiology 26(3):679–681.

Coggon D, Pannett B, Winter PD, Acheson ED, Bonsall J. 1986. Mortality of workers exposed to 2-methyl-4-chlorophenoxyacetic acid. Scandinavian Journal of Work, Environment, and Health 12:448–454.

Coggon D, Pannett B, Winter P. 1991. Mortality and incidence of cancer at four factories making phenoxy herbicides. British Journal of Industrial Medicine 48:173–178.

Collins JJ, Strauss ME, Levinskas GJ, Conner PR. 1993. The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a trichlorophenol process accident. Epidemiology 4:7–13.

Commonwealth Institute of Health. Australian Veterans Health Studies. 1984a. Mortality Report. Part I. A Retrospective Cohort Study of Mortality Among Australian National Servicemen of the Vietnam Conflict Era, and An Executive Summary of the Mortality Report. Canberra: Australian Government Publishing Service.

Commonwealth Institute of Health. Australian Veterans Health Studies. 1984b. The Mortality Report. Part II. Factors Influencing Mortality Rates of Australian National Servicemen of the Vietnam Conflict Era. Canberra: Australian Government Publishing Service.

Commonwealth Institute of Health. Australian Veterans Health Studies. 1984c. The Mortality Report. Part III. The Relationship Between Aspects of Vietnam Service and Subsequent Mortality Among Australian National Servicemen of the Vietnam Conflict Era. Canberra: Australian Government Publishing Service.

Constable JD, Hatch MC. 1985. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250.

Cook RR, Townsend JC, Ott MG, Silverstein LG. 1980. Mortality experience of employees exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Journal of Occupational Medicine 22:530–532.

Cook RR, Bond GG, Olson RA. 1986. Evaluation of the mortality experience of workers exposed to the chlorinated dioxins. Chemosphere 15:1769–1776.

Cook RR, Bond GG, Olson RA, Ott MG. 1987. Update of the mortality experience of workers exposed to chlorinated dioxins. Chemosphere 16:2111–2116.

Cordier S, Le TB, Verger P, Bard D, Le CD, Larouze B, Dazza MC, Hoang TQ, Abenhaim L. 1993. Viral infections and chemical exposures as risk factors for hepatocellular carcinoma in Vietnam. International Journal of Cancer 55:196–201.

Corrao G, Caller M, Carle F, Russo R, Bosia S, Piccioni P. 1989. Cancer risk in a cohort of licensed pesticide users. Scandinavian Journal of Work, Environment, and Health 15:203–209.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997a. Mortality of Vietnam veterans: the veteran cohort study. A report of the 1996 retrospective cohort study of Australian Vietnam veterans. Canberra: Department of Veterans' Affairs.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997b. Mortality of national service Vietnam veterans: A report of the 1996 retrospective cohort study of Australian Vietnam veterans. Canberra: Department of Veterans' Affairs.

Dai LC, Phuong NTN, Thom LH, Thuy TT, Van NTT, Cam LH, Chi HTK, Thuy LB. 1990. A comparison of infant mortality rates between two Vietnamese villages sprayed by defoliants in wartime and one unsprayed village. Chemosphere 20:1005–1012.

Dalager NA, Kang HK, Burt VL, Weatherbee L. 1991. Non-Hodgkin's lymphoma among Vietnam veterans. Journal of Occupational Medicine 33:774–779.

Dalager NA, Kang HK, Thomas TL. 1995a. Cancer mortality patterns among women who served in the military: the Vietnam experience. Journal of Occupational and Environmental Medicine 37:298–305.

Dalager NA, Kang HK, Burt VL, Weatherbee L. 1995b. Hodgkin's disease and Vietnam service. Annals of Epidemiology 5(5):400–406.

Dalager NA, Kang HK. 1997. Mortality among Army Chemical Corps Vietnam veterans. American Journal of Industrial Medicine 31(6):719–726.

Dean G. 1994. Deaths from primary brain cancers, lymphatic and haematopoietic cancers in agricultural workers in the Republic of Ireland. Journal of Epidemiology and Community Health 48:364–368.

Decoufle P, Holmgreen P, Boyle CA, Stroup NE. 1992. Self-reported health status of Vietnam veterans in relation to perceived exposure to herbicides and combat. American Journal of Epidemiology 135:312–323.

Deprez RD, Carvette ME, Agger MS. 1991. The Health and Medical Status of Maine Veterans: A Report to the Bureau of Veterans Services, Commission of Vietnam and Atomic Veterans. Portland, ME: Public Health Resource Group.

Dimich-Ward H, Hertzman C, Teschke K, Hershler R, Marion SA, Ostry A, Kelly S. 1996. Reproductive effects of paternal exposure to chlorophenate wood preservatives in the sawmill industry. Scandinavian Journal of Work, Environment and Health 22(4):267–273.

Donna A, Betta P-G, Robutti F, Crosignani P, Berrino F, Bellingeri D. 1984. Ovarian mesothelial tumors and herbicides: a case-control study. Carcinogenesis 5:941–942.

Donovan JW, Adena MA, Rose G, Battistutta D. 1983. Case-Control Study of Congenital Anomalies and Vietnam Service: Birth Defects Study. Report to the Minister for Veterans' Affairs. Canberra: Australian Government Publishing Service.

Donovan JW, MacLennan R, Adena M. 1984. Vietnam service and the risk of congenital anomalies: a case-control study. Medical Journal of Australia 140:394–397.

Dubrow R, Paulson JO, Indian RW. 1988. Farming and malignant lymphoma in Hancock County, Ohio. British Journal of Industrial Medicine 45:25–28.

Egeland GM, Sweeney MH, Fingerhut MA, Wille KK, Schnorr TM, Halperin WE. 1994. Total serum testosterone and gonadotropins in workers exposed to dioxin. American Journal of Epidemiology 139:272–281.

Eisen S, Goldberg J, True WR, Henderson WG. 1991. A co-twin control study of the effects of the Vietnam War on the self-reported physical health of veterans. American Journal of Epidemiology 134:49–58.

Erickson JD, Mulinare J, McClain PW, Fitch TG, James LM, McClearn AB, Adams MJ Jr. 1984a. Vietnam Veterans' Risks for Fathering Babies with Birth Defects. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control.

Erickson JD, Mulinare J, McClain PW, Fitch TG, James LM, McClearn AB, Adams MJ Jr. 1984b. Vietnam veterans' risks for fathering babies with birth defects. Journal of the American Medical Association 252:903–912.

Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. 1979. Case-control study on malignant mesenchymal tumor of the soft tissue and exposure to chemical substances. Lakartidningen 76:3872–3875. [In Swedish]

Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. 1981. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. British Journal of Industrial Medicine 38:27–33.

Eriksson M, Hardell L, Adami HO. 1990. Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. Journal of the National Cancer Institute 82:486–490.

Eriksson M, Hardell L, Malker H, Weiner J. 1992. Malignant lymphoproliferative diseases in occupations with potential exposure to phenoxyacetic acids or dioxins: a register-based study. American Journal of Industrial Medicine 22:305–312.

Evans RG, Webb KB, Knutsen AP, Roodman ST, Roberts DW, Bagby JR, Garrett WA Jr, Andrews JS Jr. 1988. A medical follow-up of the health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Archives of Environmental Health 43:273–278.

Evatt P. 1985. Royal Commission on the Use and Effect of Chemical Agents on Australian Personnel in Vietnam, Final Report. Canberra: Australian Government Publishing Service. 9 vols.

Farberow NL, Kang H, Bullman T. 1990. Combat experience and postservice psychosocial status as predictors of suicide in Vietnam veterans. Journal of Nervous and Mental Disease 178:32–37.

Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F. 1996. Immunological changes among farmers exposed to phenoxy herbicides: Preliminary observations. Occupational and Environmental Medicine 53(9):583–585.

Fett MJ, Adena MA, Cobbin DM, Dunn M. 1987a. Mortality among Australian conscripts of the Vietnam conflict era. I. Death from all causes. American Journal of Epidemiology 126: 869–877.

Fett MJ, Nairn JR, Cobbin DM, Adena MA. 1987b. Mortality among Australian conscripts of the Vietnam conflict era. II. Causes of death. American Journal of Epidemiology 125:878–884.

Field B, Kerr C. 1988. Reproductive behaviour and consistent patterns of abnormality in offspring of Vietnam veterans. Journal of Medical Genetics 25:819–826.

Fielder N, Gochfeld M. 1992. Neurobehavioral Correlates of Herbicide Exposure in Vietnam Veterans. New Jersey Agent Orange Commission.

Filippini G, Bordo B, Crenna P, Massetto N, Musicco M, Boeri R. 1981. Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-tetrachlorodibenzodioxin. Scandinavian Journal of Work, Environment, and Health 7:257–262.

Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. 1991. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New England Journal of Medicine 324:212–218.

Fitzgerald EF, Weinstein AL, Youngblood LG, Standfast SJ, Melius JM. 1989. Health effects three years after potential exposure to the toxic contaminants of an electrical transformer fire. Archives of Environmental Health 44:214–221.

Flesch-Janys D, Berger J, Gurn P, Manz A, Nagel S, Waltsgott H, Dwyer. 1995. Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. American Journal of Epidemiology 142(11):1165–1175.

Forcier L, Hudson HM, Cobbin DM, Jones MP, Adena MA, Fett MJ. 1987. Mortality of Australian veterans of the Vietnam conflict and the period and location of their Vietnam service. Military Medicine 152:9–15.

Gallagher RP, Bajdik CD, Fincham S, Hill GB, Keefe AR, Coldman A, McLean DI. 1996. Chemical exposures, medical history, and risk of squamous and basal cell carcinoma of the skin. Cancer Epidemiology, Biomarkers and Prevention 5(6):419–424.

Gambini GF, Mantovani C, Pira E, Piolatto PG, Negri E. 1997. Cancer mortality among rice growers in Novara Province, Northern Italy. American Journal of Industrial Medicine 31(4):435–441.

Garry VF, Kelly JT, Sprafka JM, Edwards S, Griffith J. 1994. Survey of health and use characterization of pesticide appliers in Minnesota. Archives of Environmental Health 49:337–343.

Garry VF, Tarone RE, Long L, Griffith J, Kelly JT, Burroughs B. 1996a. Pesticide appliers with mixed pesticide exposure: G-banded analysis and possible relationship to non-Hodgkin's lymphoma. Cancer Epidemiology, Biomarkers and Prevention 5(1):11–16.

Garry VF, Schreinemachers D, Harkins ME, and Griffith J. 1996b. Pesticide appliers, biocides, and birth defects in rural Minnesota. Environmental Health Perspectives 104(4):394–399.

Goldberg J, True WR, Eisen SA, Henderson WG. 1990. A twin study of the effects of the Vietnam war on posttraumatic stress disorder. Journal of the American Medical Association 263: 1227–1232.

Gordon JE, Shy CM. 1981. Agricultural chemical use and congenital cleft lip and/or palate. Archives of Environmental Health 36:213–221.

Goun BD, Kuller LH. 1986. Final Report: A Case-Control Mortality Study on the Association of Soft Tissue Sarcomas, Non-Hodgkin's Lymphomas, and Other Selected Cancers and Vietnam Military Service in Pennsylvania Males. Pittsburgh, PA: University of Pittsburgh.

Green LM. 1987. Suicide and exposure to phenoxy acid herbicides. Scandinavian Journal of Work, Environment, and Health 13:460.

Green LM. 1991. A cohort mortality study of forestry workers exposed to phenoxy acid herbicides. British Journal of Industrial Medicine 48:234–238.

Greenwald P, Kovasznay B, Collins DN, Therriault G. 1984. Sarcomas of soft tissues after Vietnam service. Journal of the National Cancer Institute 73:1107–1109.

Halperin W, Kalow W, Sweeney MH, Tang BK, Fingerhut M, Timpkins B, Wille K, 1995. Induction of P-450 in workers exposed to dioxin. Occupational and Environmental Medicine 52(2): 86–91.

Hanify JA, Metcalf P, Nobbs CL, Worsley KJ. 1981. Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science 212:349–351.

Hansen ES, Hasle H, Lander F. 1992. A cohort study on cancer incidence among Danish gardeners. American Journal of Industrial Medicine 21:651–660.

Hardell L, Bengtsson NO. 1983. Epidemiological study of socioeconomic factors and clinical findings in Hodgkin's disease, and reanalysis of previous data regarding chemical exposure. British Journal of Cancer 48:217–225.

Hardell L, Eriksson M. 1988. The association between soft tissue sarcomas and exposure to phenoxyacetic acids: a new case-referent study. Cancer 62:652–656.

Hardell L, Sandstrom A. 1979. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. British Journal of Cancer 39:711–717.

Hardell L, Eriksson M, Lenner P. 1980. Malignant lymphoma and exposure to chemical substances, especially organic solvents, chlorophenols and phenoxy acids. Lakartidningen 77:208–210.

Hardell L, Eriksson M, Lenner P, Lundgren E. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. British Journal of Cancer 43:169–176.

Hardell L, Johansson B, Axelson O. 1982. Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid or chlorophenol exposure. American Journal of Industrial Medicine 3:247–257.

Hardell L, Bengtsson NO, Jonsson U, Eriksson S, Larsson LG. 1984. Aetiological aspects on primary liver cancer with special regard to alcohol, organic solvents and acute intermittent porphyria: an epidemiological investigation. British Journal of Cancer 50:389–397.

Hardell L, Moss A, Osmond D, Volberding P. 1987. Exposure to hair dyes and polychlorinated dibenzo-*p*-dioxins in AIDS patients with Kaposi sarcoma: an epidemiological investigation. Cancer Detection and Prevention Supplement 1:567–570.

Hardell L, Eriksson M, Degerman A. 1994. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. Cancer Research 54:2386–2389.

Hardell L. 1977. Malignant mesenchymal tumors and exposure to phenoxy acids: a clinical observation. Lakartidningen 74:2753–2754. [In Swedish]

Hardell L. 1979. Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet 1(8106):55–56.

Hardell L. 1981. Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scandinavian Journal of Work, Environment, and Health 7:119–130.

Hayes HM, Tarone RE, Casey HW, Huxsoll DL. 1990. Excess of seminomas observed in Vietnam service U.S. military working dogs. Journal of the National Cancer Institute 82:1042–1046.

Heacock H, Hogg R, Marion SA, Hershler R, Teschke K, Dimich-Ward H, Demers P, Kelly S, Ostry A, Hertzman C. 1998. Fertility among a cohort of male sawmill workers exposed to chlorophenate fungicides. Epidemiology 9(1):56–60.

Henneberger PK, Ferris BG Jr, Monson RR. 1989. Mortality among pulp and paper workers in Berlin, New Hampshire. British Journal of Industrial Medicine 46:658–664.

Henriksen GL, Michalek JE, Swaby JA, Rahe AJ. 1996. Serum dioxin , testosterone, and gonadotropins in veterans of Operation Ranch Hand. Epidemiology 7(4):352–357.

Henriksen GL, Ketchum NS, Michalek JE, Swaby JA. 1997. Serum dioxin and diabetes mellitus in veterans of operation ranchhand. Epidemiology 8(3):252–258.

Hertzman C, Teschke K, Dimich-Ward H, Ostry A. 1988. Validity and reliability of a method for retrospective evaluation of chlorophenate exposure in the lumber industry. American Journal of Industrial Medicine 14(6):703–713.

Hertzman C, Teschke K, Ostry A, Hershler R, Dimich-Ward H, Kelly S, Spinelli JJ, Gallagher RP, McBride M, Marion SA. 1997. Mortality and cancer incidence among sawmill workers exposed to chlorophenate wood preservatives. American Journal of Public Health 87(1): 71–79.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. Journal of the American Medical Association 256:1141–1147.

Hoffman RE, Stehr-Green PA, Webb KB, Evans RG, Knutsen AP, Schramm WF, Staake JL, Gibson BB, Steinberg KK. 1986. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Journal of the American Medical Association 255:2031–2038.

Holmes AP, Bailey C, Baron RC, Bosanac E, Brough J, Conroy C, Haddy L. 1986. West Virginia Department of Health Vietnam-Era Veterans Mortality Study, Preliminary Report. Charleston: West Virginia Health Department.

Huong LD, Phuong NTN. 1983. The state of abnormal pregnancies and congenital malformations at the Gyneco-Obstetrical Hospital of Ho Chi Minh City (formerly Tu Du Hospital). Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Ideo G, Bellati G, Bellobuono A, Mocarelli P, Marocchi A, Brambilla P. 1982. Increased urinary *d*-glucaric acid excretion by children living in an area polluted with tetrachlorodibenzo-paradioxin (TCDD). Clinica Chimica Acta 120:273–283.

Ideo G, Bellati G, Bellobuono A, Bissanti L. 1985. Urinary *d*-glucaric acid excretion in the Seveso area, polluted by tetrachlorodibenzo-*p*-dioxin (TCDD): five years of experience. Environmental Health Perspectives 60:151–157.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine (IOM). 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Jansson B, Voog L. 1989. Dioxin from Swedish municipal incinerators and the occurrence of cleft lip and palate malformations. International Journal of Environmental Studies 34:99–104.

Jappinen P, Pukkala E. 1991. Cancer incidence among pulp and paper workers exposed to organic chlorinated compounds formed during chlorine pulp bleaching. Scandinavian Journal of Work, Environment, and Health 17:356–359.

Jennings AM, Wild G, Ward JD, Ward AM. 1988. Immunological abnormalities 17 years after accidental exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. British Journal of Industrial Medicine 45:701–704.

Kahn PC, Gochfeld M, Nygren M, Hansson M, Rappe C, Velez H, Ghent-Guenther T, Wilson WP. 1988. Dioxins and dibenzofurans in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls. Journal of the American Medical Association 259:1661–1667.

Kahn PC, Gochfeld M, Lewis WW. 1992a. Dibenzodioxin and Dibenzofuran Congener Levels in Four Groups of Vietnam Veterans Who Did Not Handle Agent Orange. New Jersey Agent Orange Commission.

Kahn PC, Gochfeld M, Lewis WW. 1992b. Immune Status and Herbicide Exposure in the New Jersey Pointman I Project. New Jersey Agent Orange Commission.

Kahn PC, Gochfeld M, Lewis WW. 1992c. Semen Analysis in Vietnam Veterans with Respect to Presumed Herbicide Exposure. New Jersey Agent Orange Commission.

Kang HK, Weatherbee L, Breslin PP, Lee Y, Shepard BM. 1986. Soft tissue sarcomas and military service in Vietnam: a case comparison group analysis of hospital patients. Journal of Occupational Medicine 28:1215–1218.

Kang HK, Enzinger FM, Breslin P, Feil M, Lee Y, Shepard B. 1987. Soft tissue sarcoma and military service in Vietnam: a case-control study. Journal of the National Cancer Institute 79:693–699 (published erratum appears in Journal of the National Cancer Institute 1987, 79:1173).

Khoa ND. 1983. Some biologic parameters collected on the groups of people in an area affected by chemicals. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Kogan MD, Clapp RW. 1985. Mortality Among Vietnam Veterans in Massachusetts, 1972–1983. Massachusetts Office of the Commissioner of Veterans Services, Agent Orange Program.

Kogan MD, Clapp RW. 1988. Soft tissue sarcoma mortality among Vietnam veterans in Massachusetts, 1972 to 1983. International Journal of Epidemiology 17:39–43.

Kogevinas M, Saracci R, Bertazzi PA, Bueno de Mesquita BH, Coggon D, Green LM, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Osman J, Pearce N, Winkelmann R. 1992. Cancer mortality from soft-tissue sarcoma and malignant lymphomas in an international cohort of workers exposed to chlorophenoxy herbicides and chlorophenols. Chemosphere 25:1071–1076.

Kogevinas M, Saracci R, Winkelmann R, Johnson ES, Bertazzi PA, Bueno de Mesquita BH, Kauppinen T, Littorin M, Lynge E, Neuberger M. 1993. Cancer incidence and mortality in women occupationally exposed to chlorophenoxy herbicides, chlorophenols, and dioxins. Cancer Causes Control 4:547–553.

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Bas B, Coggon D, Green L, Johnson E, Littorin M, Lynge E, Marlow DA, Mathews JD, Neuberger M, Benn T, Pannett B, Pearce N, Saracci R. 1995. Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols and dioxins: two nested case-control studies. Epidemiology 6:396–402.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno de Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Pearce N, Saracci R. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. American Journal of Epidemiology 145(12):1061–1075.

Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, van der Paauw CG, Tuinstra LGMT, Boersma ER, Sauer PJJ. 1994. PCB, dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 28:1721–1732.

Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L. 1997. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 8(5):537–544.

Lampi P, Hakulinen T, Luostarinen T, Pukkala E, Teppo L. 1992. Cancer incidence following chlorophenol exposure in a community in southern Finland. Archives of Environmental Health 47:167–175.

Lang TD, Tung TT, Van DD. 1983a. Mutagenic effects on the first generation after exposure to "Orange Agent." Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Lang TD, Van DD, Dwyer JH, Flamenbuam C, Dwyer KM, Fantini D. 1983b. Self-reports of exposure to herbicides and health problems: a preliminary analysis of survey data from the families of 432 veterans in northern Vietnam. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

LaVecchia C, Negri E, D'Avanzo B, Franceschi S. 1989. Occupation and lymphoid neoplasms. British Journal of Cancer 60:385–388.

Lawrence CE, Reilly AA, Quickenton P, Greenwald P, Page WF, Kuntz AJ. 1985. Mortality patterns of New York State Vietnam veterans. American Journal of Public Health 75:277–279.

Lerda D, Rizzi R. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). Mutation Research 262:47–50.

Levy CJ. 1988. Agent Orange exposure and posttraumatic stress disorder. Journal of Nervous and Mental Disorders 176:242–245.

Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, Chen RC. 1997. Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. Neurology 48(6):1583–1588.

Lovik M, Johansen HR, Gaarder PI, Becher G, Aaberge IS, Gdynia W, Alexander J. 1996. Halogenated organic compounds and the human immune system: preliminary report on a study in hobby fishermen. Archives of Toxicology Supplement 18:15–20.

Lynge E. 1985. A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. British Journal of Cancer 52:259–270.

Lynge E. 1993. Cancer in phenoxy herbicide manufacturing workers in Denmark, 1947–87—an update. Cancer Causes and Control 4:261–272.

Mahan CM, Bullman TA, Kang HK, Selvin S. 1997. A case-control study of lung cancer among Vietnam veterans. Journal of Occupationa and Environmental Medicine 39(8):740–747.

Manz A, Berger J, Dwyer JH, Flesch-Janys D, Nagel S, Waltsgott H. 1991. Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 338:959–964.

Masala G, Di Lollo S, Picoco C, Crosignani P, Demicheli V, Fontana A, Funto I, Miligi L, Nanni O, Papucci A, Ramazzotti V, Rodella S, Stagnaro E, Tumino R, Vigano C, Vindigni C, Seniori Costantini A, Vineis P. 1996. Incidence rates of leukemias, lymphomas and myelomas in Italy: geographic distribution and NHL histotypes. International Journal of Cancer 68(2):156–159.

Mastroiacovo P, Spagnolo A, Marni E, Meazza L, Bertollini R, Segni G, Borgna-Pignatti C. 1988. Birth defects in the Seveso area after TCDD contamination. Journal of the American Medical Association 259:1668–1672 (published erratum appears in the Journal of the American Medical Association 1988, 260:792).

May G. 1982. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. British Journal of Industrial Medicine 39:128–135.

May G. 1983. TCDD: a study of subjects 10 and 14 years after exposure. Chemosphere 12:771–778.

McDuffie HH, Klaassen DJ, Dosman JA. 1990. Is pesticide use related to the risk of primary lung cancer in Saskatchewan? Journal of Occupational Medicine 32:996–1002.

McKinney WP, McIntire DD, Carmody TJ, Joseph A. 1997. Comparing the smoking behavior of veterans and nonveterans. Public Health Reports 112(3):212–217.

Mellemgaard A, Engholm G, McLaughlin JK, Olsen JH. 1994. Occupational risk factors for renal-cell carcinoma in Denmark. Scandinavian Journal of Work, Environment, and Health 20:160–165.

Michalek JE, Wolfe WH, Miner JC. 1990. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. II. Mortality. Journal of the American Medical Association 264:1832–1836.

Michalek JE, Rahe AJ, Boyle CA. 1998a. Paternal dioxin, preterm birth, intrauterine growth retardation, and infant death. Epidemiology 9(2):161–167.

Michalek JE, Ketchum NS, Akhtar FZ. 1998b. Post-service mortality of Air Force veterans occupationally exposed to herbicides in Vietnam: 15 year follow-up. American Journal of Epidemiology 148(8):786–792.

Michigan Department of Public Health. 1983. Evaluation of Soft and Connective Tissue Cancer Mortality Rates for Midland and Other Selected Michigan Counties. Michigan Department of Public Health.

Mocarelli P, Marocchi A, Brambilla P, Gerthoux P, Young DS, Mantel N. 1986. Clinical laboratory manifestations of exposure to dioxin in children. A six-year study of the effects of an environmental disaster near Seveso, Italy. Journal of the American Medical Association 256:2687–2695.

Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr, Needham LL. 1996. Change in sex ratio with exposure to dioxin. Lancet 348(9024):409.

Morris PD, Koepsell TD, Daling JR, Taylor JW, Lyon JL, Swanson GM, Child M, Weiss NS. 1986. Toxic substance exposure and multiple myeloma: a case-control study. Journal of the National Cancer Institute 76:987–994.

Morrison H, Semenciw RM, Morison D, Magwood S, Mao Y. 1992. Brain cancer and farming in western Canada. Neuroepidemiology 11: 267–276.

Morrison H, Savitz D, Semenciw RM, Hulka B, Mao Y, Morison D, Wigle D. 1993. Farming and prostate cancer mortality. American Journal of Epidemiology 137:270–280.

Morrison HI, Semenciw RM, Wilkins K, Mao Y, Wigle DT. 1994. Non-Hodgkin's lymphoma and agricultural practices in the prairie provinces of Canada. Scandinavian Journal of Work, Environment, and Health 20:42–47.

Moses M, Lilis R, Crow KD, Thornton J, Fischbein A, Anderson HA, Selikoff IJ. 1984. Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. American Journal of Industrial Medicine 5:161–182.

Musicco M, Sant M, Molinari S, Filippini G, Gatta G, Berrino F. 1988. A case-control study of brain gliomas and occupational exposure to chemical carcinogens: the risks to farmers. American Journal of Epidemiology 128:778–785.

Nanni O, Amadori D, Lugaresi C, Falcini F, Scarpi E, Saragoni A, Buiatti E. 1996. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on a priori exposure matrices. Occupational and Environmental Medicine 53(10):652–657.

Needham LL, Gerthoux PM, Patterson DG Jr, Brambilla P, Turner WE, Beretta C, Pirkle JL, Colombo L, Sampson EJ, Tramacere PL, Signorini S, Meazza L, Carreri V, Jackson RJ, Mocarelli P. 1997. Serum dioxin levels in Seveso, Italy, population in 1976. Teratogenesis, Carcinogenesis, and Mutagenesis 17(4–5):225–240.

Nelson CJ, Holson JF, Green HG, Gaylor DW. 1979. Retrospective study of the relationship between agricultural use of 2,4,5-T and cleft palate occurrence in Arkansas. Teratology 19:377–383.

Newell GR. 1984. Development and Preliminary Results of Pilot Clinical Studies. Report of the Agent Orange Advisory Committee to the Texas Department of Health. University of Texas System Cancer Center.

Nguyen HD. 1983. Pregnancies at the Polyclinic of Tay Ninh Province. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Nurminen T, Rantala K, Kurppa K, Holmberg PC. 1994. Agricultural work during pregnancy and selected structural malformations in Finland. Epidemiology 1:23–30.

O'Toole BI, Marshall RP, Grayson DA, Schureck RJ, Dobson M, Ffrench M, Pulvertaft B, Meldrum L, Bolton J, Vennard J. 1996a. The Australian Vietnam Veterans Health Study: I. Study design and response bias. International Journal of Epidemiology 25(2):307–318.

O'Toole BI, Marshall RP, Grayson DA, Schureck RJ, Dobson M, Ffrench M, Pulvertaft B, Meldrum L, Bolton J, Vennard J. 1996b. The Australian Vietnam Veterans Health Study: II. Self-reported health of veterans compared with the Australian population. International Journal of Epidemiology 25(2):319–330.

O'Toole BI, Marshall RP, Grayson DA, Schureck RJ, Dobson M, Ffrench M, Pulvertaft B, Meldrum L, Bolton J, Vennard J. 1996c. The Australian Vietnam Veterans Health Study: III. Psychological health of Australian Vietnam veterans and its relationship to combat. International Journal of Epidemiology 25(2):331–340.

O'Brien TR, Decoufle P, Boyle CA. 1991. Non-Hodgkin's lymphoma in a cohort of Vietnam veterans. American Journal of Public Health 81:758–760.

Olsson H, Brandt L. 1988. Risk of non-Hodgkin's lymphoma among men occupationally exposed to organic solvents. Scandinavian Journal of Work, Environment, and Health 14:246–251.

Ott MG, Zober A. 1996. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occuptional and Environmental Medicine 53(9):606–612.

Ott MG, Holder BB, Olson RD. 1980. A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. Journal of Occupational Medicine 22:47–50.

Ott MG, Olson RA, Cook RR, Bond GG. 1987. Cohort mortality study of chemical workers with potential exposure to the higher chlorinated dioxins. Journal of Occupational Medicine 29:422–429.

Pazderova-Vejlupkova J, Lukas E, Nemcova M, Pickova J, Jirasek L. 1981. The development and prognosis of chronic intoxication by tetrachlorodibenzo-*p*-dioxin in men. Archives of Environmental Health 36:5–11.

Pearce NE, Smith AH, Fisher DO. 1985. Malignant lymphoma and multiple myeloma linked with agricultural occupations in a New Zealand cancer registry-based study. American Journal of Epidemiology 121:225–237.

Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA. 1986a. Case-control study of multiple myeloma and farming. British Journal of Cancer 54:493–500.

Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA. 1986b. Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment: a case-control study. British Journal of Industrial Medicine 43:75–83.

Pearce NE, Sheppard RA, Smith AH, Teague CA. 1987. Non-Hodgkin's lymphoma and farming: an expanded case-control study. International Journal of Cancer 39:155–161.

Peper M, Klett M, Frentzel-Beyme R, Heller WD. 1993. Neuropsychological effects of chronic exposure to environmental dioxins and furans. Environmental Research 60:124–135.

Persson B, Dahlander A-M, Fredriksson M, Brage HN, Ohlson C-G, Axelson O. 1989. Malignant lymphomas and occupational exposures. British Journal of Industrial Medicine 46:516–520.

Persson B, Fredriksson M, Olsen K, Boeryd B, Axelson O. 1993. Some occupational exposures as risk factors for malignant lymphomas. Cancer 72:1773–1778.

Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C, Bertazzi PA. 1992. Cancer morbidity in the Seveso area, 1976–1986. Chemosphere 25:209–212.

Pesatori AC, Consonni D, Tironi A, Zocchetti C, Fini A, Bertazzi PA. 1993. Cancer in a young population in a dioxin-contaminated area. International Journal of Epidemiology 22:1010–1013.

Phuong NTN, Huong LTD. 1983. The effects of toxic chemicals on the pregnancy of the women living at two localities in the South of Vietnam. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

Phuong NTN, Thuy TT, Phuong PK. 1989a. An estimate of differences among women giving birth to deformed babies and among those with hydatidiform mole seen at the Ob-Gyn hospital of Ho Chi Minh City in the south of Vietnam. Chemosphere 18:801–803.

Phuong NTN, Thuy TT, Phuong PK. 1989b. An estimate of reproductive abnormalities in women inhabiting herbicide sprayed and non-herbicide sprayed areas in the south of Vietnam, 1952–1981. Chemosphere 18:843–846.

Pirkle JL, Wolfe WH, Patterson DG, Needham LL, Michalek JE, Miner JC, Peterson MR, Phillips DL. 1989. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam veterans of Operation Ranch Hand. Journal of Toxicology and Environmental Health 27:165–171.

Poland AP, Smith D, Metter G, Possick P. 1971. A health survey of workers in a 2,4-D and 2,4,5-T plant with special attention to chloracne, porphyria cutanea tarda, and psychologic parameters. Archives of Environmental Health 22:316–327.

Pollei S, Mettler FA Jr, Kelsey CA, Walters MR, White RE. 1986. Follow-up chest radiographs in Vietnam veterans: are they useful? Radiology 161:101–102.

Ramlow JM, Spadacene NW, Hoag SR, Stafford BA, Cartmill JB, Lerner PJ. 1996. Mortality in a cohort of pentachlorophenol manufacturing workers, 1940–1989. American Journal of Industrial Medicine 30(2):180–194.

Rellahan WL. 1985. Aspects of the Health of Hawaii's Vietnam-Era Veterans. Honolulu: Hawaii State Department of Health, Research and Statistics Office.

Riihimaki V, Asp S, Hernberg S. 1982. Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland: first report of an ongoing prospective cohort study. Scandinavian Journal of Work, Environment, and Health 8:37–42.

Riihimaki V, Asp S, Pukkala E, Hernberg S. 1983. Mortality and cancer morbidity among chlorinated phenoxyacid applicators in Finland. Chemosphere 12:779–784.

Robinson CF, Waxweiler RJ, Fowler DP. 1986. Mortality among production workers in pulp and paper mills. Scandinavian Journal of Work, Environment, and Health 12:552–560.

Ronco G, Costa G, Lynge E. 1992. Cancer risk among Danish and Italian farmers. British Journal of Industrial Medicine 49:220–225.

Saracci R, Kogevinas M, Bertazzi PA, Bueno de Mesquita BH, Coggon D, Green LM, Kauppinen T, L'Abbe KA, Littorin M, Lynge E, Mathews JD, Neuberger M, Osman J, Pearce N, Winkelmann R. 1991. Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. Lancet 338:1027–1032.

Schulte PA, Burnett CA, Boeniger MF, Johnson J. 1996. Neurodegenerative diseases: occupational occurrence and potential risk factors, 1982 through 1991. American Journal of Public Health 86(9):1281–1288.

Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, Oertel WH, Ulm G, Schneider E. 1996. Possible environmental, occupational, and other etiologic factors for Parkinson's disease: A case-control study in Germany. Neurology 46(5):1275–1284.

Semchuk KM, Love EJ, Lee RG. 1993. Parkinson's disease: a test of the multifactorial etiologic hypothesis. Neurology 43:1173–1180.

Semenciw RM, Morrison HI, Riedel D, Wilkins K, Ritter L, Mao Y. 1993. Multiple myeloma mortality and agricultural practices in the prairie provinces of Canada. Journal of Occupational Medicine 35:557–561.

Semenciw RM, Morrison HI, Morison D, Mao Y. 1994. Leukemia mortality and farming in the prairie provinces of Canada. Canadian Journal of Public Health 85:208–211.

Senthilselvan A, McDuffie HH, Dosman JA. 1992. Association of asthma with use of pesticides: results of a cross-sectional survey of farmers. American Review of Respiratory Disease 146:884–887.

Smith AH, Pearce NE. 1986. Update on soft tissue sarcoma and phenoxyherbicides in New Zealand. Chemosphere 15:1795–1798.

Smith AH, Matheson DP, Fisher DO, Chapman CJ. 1981. Preliminary report of reproductive outcomes among pesticide applicators using 2,4,5-T. New Zealand Medical Journal 93:177–179.

Smith AH, Fisher DO, Pearce N, Chapman CJ. 1982. Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Archives of Environmental Health 37:197–200.

Smith AH, Fisher DO, Giles HJ, Pearce N. 1983. The New Zealand soft tissue sarcoma case-control study: interview findings concerning phenoxyacetic acid exposure. Chemosphere 12:565–571.

Smith AH, Pearce NE, Fisher DO, Giles HJ, Teague CA, Howard JK. 1984. Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. Journal of the National Cancer Institute 73:1111–1117.

Smith JG, Christophers AJ. 1992. Phenoxy herbicides and chlorophenols: a case-control study on soft tissue sarcoma and malignant lymphoma. British Journal of Cancer 65:442–448.

Snow BR, Stellman JM, Stellman SD, Sommer, JF. 1988. Post-traumatic stress disorder among American Legionnaires in relation to combat experience in Vietnam: associated and contributing factors. Environmental Research 47:175–192.

Sobel W, Bond GG, Skowronski BJ, Brownson PJ, Cook RR. 1987. A soft tissue sarcoma case-control study in a large multi-chemical manufacturing facility. Chemosphere 16:2095–2099.

Solet D, Zoloth SR, Sullivan C, Jewett J, Michaels DM. 1989. Patterns of mortality in pulp and paper workers. Journal of Occupational Medicine 31:627–630.

Stehr PA, Stein G, Webb K, Schramm W, Gedney WB, Donnell HD, Ayres S, Falk H, Sampson E, Smith SJ. 1986. A pilot epidemiologic study of possible health effects associated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin contaminations in Missouri. Archives of Environmental Health 41:16–22.

Stehr-Green P, Hoffman R, Webb K, Evans RG, Knusten A, Schramm W, Staake J, Gibson B, Steinberg K. 1987. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Chemosphere 16:2089–2094.

Stellman SD, Stellman JM, Sommer JF Jr. 1988a. Combat and herbicide exposures in Vietnam among a sample of American Legionnaires. Environmental Research 47:112–128.

Stellman SD, Stellman JM, Sommer JF Jr. 1988b. Health and reproductive outcomes among American Legionnaires in relation to combat and herbicide exposure in Vietnam. Environmental Research 47:150–174.

Stellman JM, Stellman SD, Sommer JF. 1988c. Social and behavioral consequences of the Vietnam experience among American Legionnaires. Environmental Research 47:129–149.

Stockbauer JW, Hoffman RE, Schramm WF, Edmonds LD. 1988. Reproductive outcomes of mothers with potential exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. American Journal of Epidemiology 128:410–419.

Suskind RR, Hertzberg VS. 1984. Human health effects of 2,4,5-T and its toxic contaminants. Journal of the American Medical Association 251:2372–2380.

Svensson BG, Mikoczy Z, Stromberg U, Hagmar L. 1995. Mortality and cancer incidence among swedish fishermen with a high dietary intake of persistent organochlorine compounds. Scandinavian Journal of Work, Environment and Health 21(2):106–115.

Swaen GMH, van Vliet C, Slangen JJM, Sturmans F. 1992. Cancer mortality among licensed herbicide applicators. Scandinavian Journal of Work, Environment, and Health 18:201–204.

Sweeney MH, Fingerhut MA, Connally LB, Halperin WE, Moody PL, Marlow DA. 1989. Progress of the NIOSH cross-sectional study of workers occupationally exposed to chemicals contaminated with 2,3,7,8-TCDD. Chemosphere 19:973–977.

Sweeney MH, Fingerhut MA, Patterson DG, Connally LB, Piacitelli L, Morris JA, Greife AL. 1990. Comparison of serum levels of 2,3,7,8-TCDD in TCP production workers and in an unexposed comparison group. Chemosphere 20(7–9): 993–1000.

Sweeney MH, Fingerhut MA, Arezzo JC, Hornung RW, Connally LB. 1993. Peripheral neuropathy after occupational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). American Journal of Industrial Medicine 23:845–858.

Sweeney MH, Calvert G, Egeland GA, Fingerhut MA, Halperin WE, Piacitelli. 1996. Review and update of the results of the NIOSH medical study of workers exposed to chemicals contaminated with 2,3,7,8-tetrachlorodibenzodioxin. Presented at the symposium, Dioxin Exposure and Human Health—An Update, June 17, Berlin, Germany.

Sweeney MH, Calvert GM, Egeland GA, Fingerhut MA, Halperin WE, Piacitelli LA. 1997. Review and update of the results of the NIOSH medical study of workers exposed to chemicals contaminated with 2,3,7,8-tetrachlorodibenzodioxin. Teratogenesis, Carcinogenesis, and Mutagenesis 17(4–5):241–247.

Tarone RE, Hayes HM, Hoover RN, Rosenthal JF, Brown LM, Pottern LM, Javadpour N, O'Connell KJ, Stutzman RE. 1991. Service in Vietnam and risk of testicular cancer. Journal of the National Cancer Institute 83:1497–1499.

Tatham L, Tolbert P, Kjeldsberg C. 1997. Occupational risk factors for subgroups of non-Hodgkin's lymphoma. Epidemiology 8(5):551–558.

Tenchini ML, Crimaudo C, Pacchetti G, Mottura A, Agosti S, De Carli L. 1983. A comparative cytogenetic study on cases of induced abortions in TCDD-exposed and nonexposed women. Environmental Mutagenesis 5:73–85.

Teschke K, Hertzman C, Dimich-Ward H, Ostry A, Blair J, Hershler R. 1989. A comparison of exposure estimates by worker raters and industrial hygienists. Scandinavian Journal of Work, Environment and Health 15(6):424–429.

Thiess AM, Frentzel-Beyme R, Link R. 1982. Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF AG on November 17, 1953. American Journal of Industrial Medicine 3:179–189.

Thomas TL, Kang HK. 1990. Mortality and morbidity among Army Chemical Corps Vietnam veterans: a preliminary report. American Journal of Industrial Medicine 18:665–673.

Thomas TL, Kang H, Dalager N. 1991. Mortality among women Vietnam veterans, 1973–1987. American Journal of Epidemiology 134:973–980.

Thomas TL. 1987. Mortality among flavour and fragrance chemical plant workers in the United States. British Journal of Industrial Medicine 44:733–737.

Tonn T, Esser C, Schneider EM, Steinmann-Steiner-Haldenstatt W, Gleichmann E. 1996. Persistence of decreased T-helper cell function in industrial workers 20 years after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environmental Health Perspectives 104(4):422–426.

Townsend JC, Bodner KM, Van Peenen PFD, Olson RD, Cook RR. 1982. Survey of reproductive events of wives of employees exposed to chlorinated dioxins. American Journal of Epidemiology 115:695–713.

True WR, Goldberg J, Eisen SA. 1988. Stress symptomatology among Vietnam veterans. Analysis of the Veterans Administration Survey of Veterans II. American Journal of Epidemiology 128:85–92.

Trung CB, Chien NT. 1983. Spontaneous abortions and birth defects in area exposed to toxic chemical sprays in Giong Trom District. Summarized in: Constable JD, Hatch MC. Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis, Carcinogenesis, and Mutagenesis 5:231–250, 1985.

U.S. Environmental Protection Agency (EPA). 1979. Report of Assessment of a Field Investigation of Six-Year Spontaneous Abortion Rates in Three Oregon Areas in Relation to Forest 2,4,5-T Spray Practices. EPA, Epidemiologic Studies Program, Human Effects Monitoring Branch.

van Houdt JJ, Fransman LG, Strik JJ. 1983. Epidemiological case-control study in personnel exposed to 2,4,5-T. Chemosphere 12:575.

Vineis P, Terracini B, Ciccone G, Cignetti A, Colombo E, Donna A, Maffi L, Pisa R, Ricci P, Zanini E, Comba P. 1986. Phenoxy herbicides and soft-tissue sarcomas in female rice weeders. A population-based case-referent study. Scandinavian Journal of Work, Environment, and Health 13:9–17.

Vineis P, Faggiano F, Tedeschi M, Ciccone G. 1991. Incidence rates of lymphomas and soft-tissue sarcomas and environmental measurements of phenoxy herbicides. Journal of the National Cancer Institute 83:362–363.

Visintainer PF, Barone M, McGee H, Peterson EL. 1995. Proportionate mortality study of Vietnam-era veterans of Michigan. Journal of Occupational and Environmental Medicine 37(4): 423–428.

Watanabe KK, Kang HK. 1995. Military service in Vietnam and the risk of death from trauma and selected cancers. Annals of Epidemiology 5(5):407–412.

Watanabe KK, Kang HK. 1996. Mortality patterns among Vietnam veterans: a 24-year retrospective analysis. Journal of Occupational and Environmental Medicine 38(3):272–278.

Watanabe KK, Kang HK, Thomas TL. 1991. Mortality among Vietnam veterans: with methodological considerations. Journal of Occupational Medicine 33:780–785.

Waterhouse D, Carman WJ, Schottenfeld D, Gridley G, McLean S. 1996. Cancer incidence in the rural community of Tecumseh, Michigan: A pattern of increased lymphopoietic neoplasms. Cancer 77(4):763–770.

Webb K, Evans RG, Stehr P, Ayres SM. 1987. Pilot study on health effects of environmental 2,3,7,8-TCDD in Missouri. American Journal of Industrial Medicine 11:685–691.

Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A, Hooijkaas H, Sauer PJ. 1995. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatric Research 38(3):404–410.

Wendt AS. 1985. Iowa Agent Orange Survey of Vietnam Veterans. Iowa State Department of Health.

White FMM, Cohen FG, Sherman G, McCurdy R. 1988. Chemicals, birth defects and stillbirths in New Brunswick: associations with agricultural activity. Canadian Medical Association Journal 138:117–124.

Wigle DT, Semenciw RB, Wilkins K, Riedel D, Ritter L, Morrison HI, Mao Y. 1990. Mortality study of Canadian male farm operators: non-Hodgkin's lymphoma mortality and agricultural practices in Saskatchewan. Journal of the National Cancer Institute 82:575–582.

Wiklund K, Holm L-E. 1986. Soft tissue sarcoma risk in Swedish agricultural and forestry workers. Journal of the National Cancer Institute 76:229–234.

Wiklund K, Dich J, Holm L-E. 1987. Risk of malignant lymphoma in Swedish pesticide appliers. British Journal of Cancer 56:505–508.

Wiklund K, Lindefors BM, Holm L-E. 1988a. Risk of malignant lymphoma in Swedish agricultural and forestry workers. British Journal of Industrial Medicine 45:19–24.

Wiklund K, Dich J, Holm L-E. 1988b. Soft tissue sarcoma risk in Swedish licensed pesticide applicators. Journal of Occupational Medicine 30:801–804.

Wiklund K, Dich J, Holm L-E, Eklund G. 1989a. Risk of cancer in pesticide applicators in Swedish agriculture. British Journal of Industrial Medicine 46:809–814.

Wiklund K, Dich J, Holm L-E. 1989b. Risk of soft tissue sarcoma, Hodgkin's disease and non-Hodgkin lymphoma among Swedish licensed pesticide applicators. Chemosphere 18:395–400.

Wiklund K. 1983. Swedish agricultural workers: a group with a decreased risk of cancer. Cancer 51:566–568.

Wingren G, Fredrikson M, Brage HN, Nordenskjold B, Axelson O. 1990. Soft tissue sarcoma and occupational exposures. Cancer 66:806–811.

Wolf N, Karmaus W. 1995. Effects of inhalative exposure to dioxins in wood preservatives on cell-mediated immunity in day-care center teachers. Environmental Research 68(2):96–105.

Wolfe WH, Michalek JE, Miner JC, Rahe A, Silva J, Thomas WF, Grubbs WD, Lustik MB, Karrison TG, Roegner RH, Williams DE. 1990. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. I. Physical health. Journal of the American Medical Association 264:1824–1831.

Wolfe WH, Michalek JE, Miner JC, Rahe AJ, Moore CA, Needham LL, Patterson D.G. 1995. Paternal serum dioxin and reproductive outcomes among veterans of Operation Ranch Hand. Epidemiology 6(1):17–22.

Woods JS, Polissar L. 1989. Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in western Washington State. Chemosphere 18:401–406.

Woods JS, Polissar L, Severson RK, Heuser LS, Kulander BG. 1987. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxy herbicide and chlorinated phenol exposure in western Washington. Journal of the National Cancer Institute 78:899–910.

Zack JA, Gaffey WR. 1983. A mortality study of workers employed at the Monsanto company plant in Nitro, West Virginia. Environmental Science Research 26:575–591.

Zack JA, Suskind RR. 1980. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. Journal of Occupational Medicine 22:11–14.

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349–356.

Zahm SH, Weisenburger DD, Saal RC, Vaught JB, Babbitt PA, Blair A. 1993. The role of agricultural pesticide use in the development of non-Hodgkins lymphoma in women. Archives of Environmental Health 48:353–358.

Zhong Y, Rafnsson V. 1996. Cancer incidence among Icelandic pesticide users, International Journal of Epidemiology 25(6):1117–1124.

Zober A, Messerer P, Huber P. 1990. Thirty-four-year mortality follow-up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. International Archives of Occupational and Environmental Health 62:139–157.

Zober A, Ott MG, Messerer P. 1994. Morbidity follow-up study of BASF employees exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) after a 1953 chemical reactor incident. Occupational and Environmental Medicine 51:479–486.

7

Cancer

INTRODUCTION

Cancer is the second leading cause of death in the United States. Among males aged 45–64, the group that describes most Vietnam veterans, the risk of dying from cancer nearly equals the risk from heart disease, the overall leading cause of death in the United States (U.S. Census, 1997). Almost one-half of all men and slightly more than one in three women in the United States will develop an invasive cancer at some time in their lives; approximately one in five Americans will die from cancer (Ries, 1997).

In this chapter, the committee summarizes and reaches conclusions about the strength of the evidence in epidemiologic studies regarding associations between exposure to herbicides and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and each type of cancer under consideration in this report. The cancer types are, with minor exceptions, discussed in the order in which they are listed in the *International Classification of Diseases*, Ninth Edition (ICD·9). ICD·9 is a standardized means of classifying medical conditions used by physicians and researchers around the world. Appendix B lists ICD·9 codes for the major forms of cancer.

In assessing a possible relation between herbicide exposure and risk of cancer, one key issue is the level of exposure of those included in a study. As noted in Chapter 5, the detail and accuracy of exposure assessment varies widely among the studies reviewed by the committee. A small number of studies use a biomarker of exposure, for example, the presence of dioxin in serum or tissues; some develop an index of exposure from employment or activity records; and others use a surrogate measure of exposure, such as being present when herbicides were

used. Inaccurate assessment of exposure can obscure the presence or absence of exposure–disease associations and thus make it less likely that a true risk will be identified. A second key issue for herbicide exposure and cancer risks is latency, the effect of timing of exposure on subsequent risk of disease. Chapter 8 addresses this issue in detail.

The outcomes reviewed in this chapter follow a common format. Each section begins by providing some background information about the cancer under discussion, including data concerning its incidence in the general U.S. population. A brief summary of the scientific evidence described in the first two Agent Orange reports—*Veterans and Agent Orange* (1994; hereafter referred to as *VAO*), and *Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996*)—is then presented, followed by a discussion of the most recent scientific literature, and a synthesis of the material reviewed. Where appropriate, reviews are separated by the type of exposure (occupational, environmental, Vietnam veteran) being addressed. Each section concludes with the committee's finding regarding the strength of the evidence in epidemiologic studies, biologic plausibility, and evidence regarding Vietnam veterans.

The Department of Veterans Affairs (DVA) asked the committee to specifically address the classification of chondrosarcomas of the skull as part of its work. This is done in the discussion of bone cancer below.

Expected Number of Cancer Cases Among Vietnam Veterans in the Absence of Any Increase in Risk Due to Herbicide Exposure

To provide some background for the consideration of cancer risks in Vietnam veterans, this chapter also reports information on cancer incidence in the general U.S. population. Incidence rates are reported for individuals between the ages of 45 and 59 because most Vietnam era veterans are in this age group. The data, which were collected as part of the Surveillance, Epidemiology, and End Results (SEER) Program of the National Center for Health Statistics (NCHS), are categorized by sex, age, and race because these factors can have a profound effect on the estimated level of risk. Prostate cancer incidence, for example, is 14 times higher in men age 55–59 than in 45–49 year olds and nearly twice as high in African Americans age 45–59 than in whites of this age group (NCI, 1998). The figures presented for each cancer are estimates for the entire U.S. population, not precise predictions for the Vietnam veteran cohort. It should be remembered that numerous factors may influence the incidence reported here—including personal behavior (e.g., smoking and diet), genetic predisposition, and other risk factors such as medical history. These factors may make a particular individual more or less likely than average to contract a given cancer. Incidence data are reported for all races and also separately for African Americans and whites. The data reported are for the years 1990–1994, the most recent available at the time this report was written.

Given the large uncertainties that remain about the magnitude of potential risk from exposure to herbicides in the occupational, environmental, and veteran 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 to that of individual Vietnam veterans, the committee cannot quantify the degree of risk likely to have been experienced by Vietnam veterans due to exposure to herbicides in Vietnam.

GASTROINTESTINAL TRACT TUMORS

Background

As a group, the category of gastrointestinal (GI) tract tumors includes some of the most common cancers in the United States and the world. The committee reviewed the data on stomach cancer (ICD·9 151.0–151.9), colon cancer (ICD·9 153.0–153.9), rectal cancer (ICD·9 154.0–154.1), and pancreatic cancer (ICD·9 157.0–157.9). According to American Cancer Society estimates, approximately 183,000 individuals will be diagnosed with these cancers in the United States in 1998 and some 99,000 individuals will die from them (ACS, 1998). Colon cancer accounts for about half of these diagnoses and deaths. The cases are divided approximately equally between men and women. Collectively, GI tract tumors are expected to account for 15 percent of new diagnoses and 18 percent of cancer deaths in 1998.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Selected Gastrointestinal Cancers

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
Stomach									
males	6	5	10	12	10	23	20	17	39
females	3	2	4	5	4	7	9	7	14
Colon									
males	18	16	27	37	35	49	69	68	92
females	16	14	21	28	24	52	15	49	77
Rectal									
males	9	9	15	15	15	20	21	20	23
females	7	6	11	7	7	6	12	12	14
Pancreatic									
males	5	5	10	12	11	23	21	20	40
females	4	3	7	8	8	10	14	13	25

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

The incidence of stomach, colon, rectal, and pancreatic cancers increases with age for individuals between 45 and 59 years of age. In general, incidence in males is higher than in females, and incidence in African Americans exceeds that

of whites. Risk factors besides age and race vary for these cancers, but always include family history of the same form of cancer, certain diseases of the affected organ, and dietary factors. Cigarette smoking is a risk factor for pancreatic cancer and may also increase the risk of stomach cancer (Miller et al., 1996). Infection with the bacterium *Helicobacter pylori* also increases the risk of stomach cancer.

Summary of *VAO* and *Update 1996*

Numerous studies were considered in *VAO* and *Update 1996* that examined one or more gastrointestinal tract cancers; no consistent associations between herbicide exposure and these cancers were found. These included studies of chemical production workers in the United States 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; Bloemen et al., 1993; Bueno de Mesquita et al., 1993; Collins et al., 1993; Kogevinas et al., 1993); agricultural workers (Burmeister, 1981; Hardell, 1981; Burmeister et al., 1983; Wiklund, 1983; Hoar et al., 1986; Alavanja et al., 1988, 1989; Wigle et al., 1990; Hansen et al., 1992; Ronco et al., 1992; Blair et al., 1993; Garry et al., 1994; Asp et al., 1994); pesticide appliers (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 population of Seveso, Italy (Bertazzi et al., 1989a,b; Pesatori et al., 1992; Bertazzi et al., 1993); others subjected to 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; CDC, 1988; Dalager et al., 1995a; Visintainer et al., 1995).

VAO and *Update 1996* found that, with rare exceptions, studies of GI cancers and herbicide exposure reported relative risks (RRs) close to 1.0, providing no evidence of any increased risk. They concluded that there is limited/suggestive evidence of no association between exposure to the herbicides (2,4-dichlorophenoxyacetic acid [2,4-D]; 2,4,5-trichlorophenoxyacetic acid [2,4,5-T] and its contaminant TCDD; cacodylic acid; and picloram) and GI cancers (stomach, pancreatic, rectal, and colon cancers). The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicide and herbicide components.

Update of the Scientific Literature

Occupational Studies

In an update and expansion of the International Agency for Research on Cancer (IARC) cohort study, Kogevinas et al. (1997) examined cancer mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. The workers for this cohort were assembled

from 12 countries, drawn from national studies that followed the same core protocol developed jointly by the participants and coordinated by IARC.

No excess risk of death from GI cancer of any site was observed among the group comprised of all workers exposed to any phenoxy herbicide or chlorophenol (stomach: standardized mortality ratio [SMR] = 0.9, 95 percent confidence interval [95% CI] 0.7–1.1, 72 deaths; colon: SMR = 1.1, CI 0.8–1.3, 86 deaths; rectum: SMR = 1.1, CI 0.8–1.4, 44 deaths; pancreas: SMR = 0.9, CI 0.7–1.2, 47 deaths). When this group was divided into those exposed and unexposed to TCDD or higher chlorinated dioxins, slight elevations were seen for certain cancers, but they were found in both the exposed and the unexposed groups, and none achieved statistical significance. More detailed analysis by exposure variables such as duration and time since first exposure was not conducted for GI cancers.

Although the study includes large numbers of workers who were likely to be exposed at levels substantially higher than general population exposures, the lack of information about actual exposures limits the investigator's ability to examine exposure–response relationships within the cohort. In addition, the inclusion of workers in the exposed group based on ever having worked in a job considered exposed makes it impossible to distinguish heavily exposed workers from those with very minor exposures.

Becher et al. (1996) examined cancer mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population included workers who had a least one month of employment, resulting in a cohort consisting of 2,479 male workers. The cohort was assembled from four plants; analysis was conducted on the total cohort, divided into four subcohorts that corresponded to each plant considered separately.

Based on production information and limited blood dioxin measurements, subcohorts I and II are supposed to have higher TCDD exposures than subcohorts III and IV. Of the four subcohorts, only group I had at least one observed or expected death at each of the GI cancer sites. Of these, stomach (SMR = 1.3, 95% CI 0.7–2.2, 12 cases) and rectum (SMR = 1.9, CI 0.7–4.0, 6 cases) were nonsignificantly elevated. The small numbers in the other three subcohorts resulted in few reportable results for GI cancers, and none of these demonstrated a significant excess risk.

Ott and Zober (1996) updated the experience of workers exposed to TCDD during the cleanup of a trichlorophenol (TCP) reactor, which exploded in 1953 at a BASF plant in Ludwigshafen, Germany. They studied cancer incidence and mortality up to 1992 for the group of 243 men and developed TCDD dose estimates based on work activity information, blood TCDD determinations on a subset of the population, and estimates of TCDD elimination rates. The analysis of cancer mortality showed a nonsignificant elevation of death for cancer of the digestive organs in the total cohort (SMR = 1.2, CI 0.6–2.1, 11 cases) with the lowest risk (SMR = 0.6) observed in the lowest-dose group (<0.1 μg/kg

body weight). The medium-dose group (0.1–0.99 μg/kg body weight) reported an SMR of 1.7 (CI 0.5–4.3), whereas the highest dose group (>1 μg/kg body weight) had an SMR of 1.5 (CI 0.5–3.4). Incident cancer cases showed a similar pattern, with a nonsignificant elevation of cancer of the digestive tract in the total cohort (standardized incidence ratio [SIR] = 1.1, CI 0.6–1.9, 12 cases). The lowest risk (SIR = 0.7) was observed in the lowest-dose group (<0.1 μg/kg body weight). The medium-dose group (0.1–0.99 μg/kg body weight) reported an SIR of 1.4 (CI 0.4–3.6), whereas the highest-dose group (>1 μg/kg body weight) showed an SIR of 1.2 (CI 0.4–2.9). Separate analysis for incidence of stomach cancer within this class revealed three cases overall (SIR = 1.0, CI 0.2–2.9), with no cases observed in the lowest-dose group, one case in the medium-dose group (SIR = 1.3, CI 0.0–7.0), and two cases in the highest-dose group (SIR = 1.7, CI 0.2–6.2).

Internal analysis of the cohort by proportional hazard analysis showed that TCDD dose was significantly associated with both death from cancer of the digestive tract (conditional regression ratio 1.5, CI 1.1–1.9) and incidence of digestive cancer (conditional risk ratio 1.4, CI 1.1–1.7). Review of these cases revealed that three had occurred among the four most highly exposed workers in the cohort and that one (stomach cancer) involved a worker with an estimated TCDD dose of 6.8 μg/kg and a second (pancreatic cancer), with an estimated dose of 6.1 μg/kg. However, information provided for the third case classified as a digestive cancer raises concerns. This case was a primary liver cancer with a dose estimate of 8.3 μg/kg. It is unclear why this case was not classified as a hepatobiliary cancer. Had it been, its exclusion from the digestive cancer analysis would probably have weakened the reported association in the proportional hazard analysis.

Ramlow et al. (1996) examined mortality in a cohort of workers exposed to pentachlorophenol (PCP), as part of a larger study of Dow chemical manufacturing workers exposed to the higher chlorinated dioxins. The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential polychlorinated dibenzodioxin (PCDD) exposure between 1937 and 1980.

In the study analysis, U.S. white male death rates (five-year age and calendar specific) and the non-PCP and PCDD male Dow Michigan employees for 1940 to 1989 were both used as reference values to calculate expected deaths. Four exposure groups were developed for TCDD (1 unit = very low, 1–1.9 units = low, 2–2.9 units = medium, 3 units = high). Calculation of SMRs with exposure lagged by 15 years using both the U.S. and the Dow referent populations found no significant excess mortality for digestive cancer (SMR = 0.9, CI 0.4–1.6, 10 deaths). The vast majority of deaths observed were among the unexposed group, leaving very few deaths distributed over the four categories of cumulative exposure (unexposed, 517 deaths from digestive cancer; very low exposure, 1 death; low exposure, 5 deaths; medium exposure, 4 deaths; high exposure, none).

For the hepta- or octa-chlorinated dibenzodioxin (H/OCDD) groups, 2 units was the low-exposure category; 2–2.9, units medium; and 3 or more units, high. SMRs for cancers of the digestive system (15-year lagged exposure) showed no significant excess compared to the U.S. and Dow referent populations. As for TCDD, a very small proportion of the population was considered to have any exposure, and very few cases were observed.

Gambini et al. (1997) investigated cancer mortality among a cohort of rice growers in northern Italy. Using a set of registered farm owners consisting of 1,493 males who worked on farms from 1957 to 1992, they examined the cause of death for 958 subjects and compared this with expected numbers calculated from national rates. No direct exposure information was available, so employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides employed during the study period. Cancer mortality was evaluated for three GI sites (stomach, pancreas, and intestines), and observed and expected deaths did not differ significantly for any of these sites in the overall cohort (stomach: SMR = 0.9, CI 0.7–1.3, 39 deaths; pancreas: SMR = 0.9, CI 0.4–1.9, 7 deaths; intestines: SMR = 1.1, CI 0.7–1.6, 27 deaths). Stratification by age at death and duration of exposure (employment as a farmer) did not change the finding of nonsignificant differences. Although the study population is small, it does describe the experience of a cohort with good follow-up (99 percent) and long latency (37 percent of deaths observed beyond the age of 80). It is limited by very crude exposure assessment, however, and the degree to which the study subjects were actually exposed to phenoxy herbicides cannot be established with any certainty.

Environmental Studies

Bertazzi et al. (1997) continued the follow-up of people environmentally exposed to TCDD in Seveso, Italy. The events that led to the exposure and the methods used to study this population have been described fully in the earlier reports. This report updates the population after 15 years follow-up. Death from cancer of the rectum was significantly elevated for men in zone B (SMR = 2.9, CI 1.2–5.9, 7 observed deaths). No other significant elevation of death from digestive cancer overall or at any GI sites was observed in men or women in any exposure zone. More detailed investigation of subjects in zone B showed that there were nonsignificant elevations of death from digestive cancers overall for women in the longest-latency (>10 years; overall digestive cancer: SMR = 1.5, CI 0.7–2.7, 10 deaths; stomach: SMR = 2.4, CI 0.8–5.7, 5 deaths) and length of stay (>10 years; overall digestive cancer: SMR = 1.6, CI 0.8–2.9, 9 deaths; stomach: SMR = 2.3, CI 0.6–6.0, 4 deaths) groups, whereas men showed significant excesses of rectal cancer in the group with the longest length of stay (SMR = 7.2, CI 1.9–18.4, 4 deaths) and the longest-latency group (SMR = 6.2, CI 1.7–15.9, 4 deaths).

Svensson et al. (1995) studied mortality and cancer incidence in two cohorts of Swedish fishermen. One group (2,896 men) resided on the east coast of Swe-

den and consumed fish from the Baltic Sea. These fatty fish (particularly salmon and herring) are reported to contain elevated levels of polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and PCDDs. The other group of fishermen (8,477) resided on the west coast of Sweden and was presumed to have a higher intake of lean (and less contaminated) fish, including cod and flat fish. This distinction in exposure by place of residence is reportedly confirmed by the finding that blood levels of "dioxin-like compounds" were two times higher among east coast than west coast fishermen; however, no data are provided to support this point. East coast fishermen were found to have nonsignificantly increased mortality from stomach cancer (SMR = 1.4, CI 0.8–2.2, 17 deaths) and increased incidence of stomach cancer (SIR = 1.6, CI 1.0–2.4, 24 cases) compared to Swedish national rates. No significant excess incidence or mortality was seen among west coast fishermen. When the two groups were compared directly, the east coast excess incidence of stomach cancer was 2.2 (incidence rate ratio [IRR], CI 1.3–3.5). East coast fishermen were found to have significantly decreased incidence and mortality of cancer of the colon, which was not observed in west coast fishermen. The degree to which the difference in stomach cancer between east and west coast fishermen can be attributed to organochlorine exposure is limited by the lack of any direct information on exposure, aside from the above (unreferenced) statement that blood levels of dioxin-like compounds were twice as high among east coast as west coast fishermen. The authors also report that east coast fishermen consume smoked fish twice as often as west coast fishermen, so the role of confounding exposures cannot be discounted.

Vietnam Veteran Studies

In a comparison of mortality between Army Chemical Corps Vietnam and non-Vietnam veterans, Dalager et al. (1997) reported a significant excess of death from digestive diseases, primarily cirrhosis, among Vietnam veterans. The study compared 2,872 Vietnam veterans with 2,737 non-Vietnam veterans (all of whom served in Chemical Corps specialties). All study subjects served at least 18 months' active duty between 1965 and 1973, and vital status ascertainment was complete for both groups. Nonsignificant decreases in deaths from digestive system cancer were observed in both groups when compared to general U.S. population rates. When Vietnam and non-Vietnam cohorts were compared directly, the crude rate ratio of GI cancer death was 4.8 (Vietnam versus non-Vietnam). The adjusted RR calculated by proportional hazards modeling to include the effect of race, military rank, duration of service, and age at entry to follow-up was 2.2 (CI 0.2–19.8). Direct exposure information on the two cohorts was not available, and the presumption that Vietnam veterans had potential for higher levels of dioxin exposure because of their duties involving Agent Orange and other dioxin-contaminated herbicides (compared to non-Vietnam Chemical Corps veterans) has not been verified.

The Australian study *Mortality of Vietnam Veterans: The Veteran Cohort Study* (Crane et al., 1997a) examined the mortality experience of male Australian Vietnam veterans from 1980 to 1994. This cohort consists of 59,036 male veterans, who were followed for a period ranging from 22 to 32 years. There were 2067 deaths recorded among this group from 1980 to 1994, and vital status was ascertained for 96.9 percent of the cohort. There was a statistically significant excess of death for all cancer (SMR = 1.2, CI 1.1–1.3) in the cohort, by comparison to the Australian white male population and by calculation of a standardized relative mortality ratio (SRMR), which is the ratio of the cause-specific SMR and the SMR for all other causes combined (SRMR = 1.2, CI 1.1–1.3). No excess mortality was observed from cancer at any of the four GI sites. Death from cancer of the colon (SMR = 1.2, CI 1.0–1.5), rectum (SMR = 0.6, CI 0.4–1.0), stomach (SMR = 1.1, CI 0.7–1.5), and pancreas (SMR = 1.4, CI 1.0–1.9) did not exceed expected numbers for all military Vietnam veterans, and when analyzed separately by branch of service, only Navy veterans were reported to have excess mortality for any GI site (colon cancer: SMR = 1.8, CI 1.0–2.8), which was not statistically significant. The study authors have described the strengths and limitations of this cohort study of Australian veterans, including virtually complete identification of the study population, a period of follow-up ranging from 22 to 32 years, and vital status ascertainment of 96.9 percent. Among the weaknesses of the study are the possibility of underascertainment of death and the uncertain quality of exposure assessment regarding a variety of risk factors, including smoking and alcohol consumption, as well as herbicide and dioxin exposure. The examination of mortality among Australian National Service Vietnam veterans (Crane et al., 1997b) reported similar findings for GI cancer.

Synthesis

With rare exceptions, studies on GI cancers and exposure to herbicide in production, in agricultural use, from environmental sources, and among veteran populations found RRs close to 1.0, providing no evidence of any increase in risk. A reported statistically significant positive association in the BASF cohort is rendered problematic by the inclusion of a liver cancer in the analysis. The positive association reported for rectal cancer in males in zone B of the Seveso cohort is not by itself compelling, and is not supported by findings in any other zone or for females in the cohort.

Conclusions

Strength of Evidence in Epidemiologic Studies

This report, like its predecessors, concludes that there is limited/suggestive evidence of no association between exposure to the herbicides (2,4-D, 2,4,5-T

(text continues on page 281)

TABLE 7-1 Selected Epidemiologic Studies—Stomach Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	42	0.9 (0.6–1.2)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	30	0.9 (0.6–1.3)
	Workers exposed to any phenoxy herbicide or chlorophenol	72	0.9 (0.7–1.1)
Becher et al., 1996	German chemical production workers		
	Plant I	12	1.3 (0.7–2.2)
	Plant II	0	
	Plant III	0	
	Plant IV	2	0.6 (0.1–2.3)
Gambini et al., 1997	Italian rice growers	39	0.9 (0.7–1.3)
Ott and Zober, 1996	BASF cleanup workers	3	1.0 (0.2–2.9)
	TCDD <0.1 μg/kg body wt	0	
	TCDD 0.1–0.99 μg/kg body wt	1	1.3 (0.0–7.0)
	TCDD >1 μg/kg body wt	2	1.7 (0.2–6.2)
Ramlow et al., 1996	Pentachlorophenol production workers		
	0 year latency	4	1.7 (0.4–4.3)
	15 year latency	3	1.8 (0.4–5.2)
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	White males	657	1.0 (1.0–1.1)
	Nonwhite females	23	1.9 (1.2–2.8)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers		NS
Collins et al., 1993	Monsanto 2,4-D production workers		NS
Kogevinas et al., 1993	Female herbicide spraying and production workers		NS
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farm workers	286	0.9
Swaen et al., 1992	Dutch herbicide applicators	1	0.5 (0–2.7)[c]
Fingerhut et al., 1991	NIOSH cohort	10	1.0 (0.5–1.9)
Manz et al., 1991	German production workers	12	1.2 (0.6–2.1)
Saracci et al., 1991	IARC cohort	40	0.9 (0.6–1.2)
Wigle et al., 1990	Canadian farmers	246	0.9 (0.8–1.0)
Zober et al., 1990	BASF production workers—basic cohort	3	3.0 (0.8–11.8)
Alavanja et al., 1989	USDA forest/soil conservationists	9	0.7 (0.3–1.3)
Henneberger et al., 1989	Paper and pulp workers	5	1.2 (0.4–2.8)
Solet et al., 1989	Paper and pulp workers	1	0.5 (0.1–3.0)
Alavanja et al., 1988	USDA agricultural extension agents	10	0.7 (0.4–1.4)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0.0–3.7)
Thomas, 1987	Flavor and fragrance chemical production workers		1.4
Coggon et al., 1986	British MCPA production workers	26	0.9 (0.6–1.3)
Robinson et al., 1986	Paper and pulp workers	17	1.2 (0.7–2.1)

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Lynge, 1985	Danish male production workers	12	1.3
Blair, 1983	Florida pesticide applicators	4	1.2
Burmeister et al., 1983	Iowa residents		
	Farming exposures		1.3 ($p < .05$)
Wiklund, 1983	Swedish agricultural workers	2,599	1.1 (1.0–1.2)[b]
Burmeister, 1981	Farmers in Iowa	338	1.1 ($p < .01$)
Axelson et al., 1980	Swedish railroad workers—total exposure	3	2.2
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	Males—zone A	0	
	Males—zone B	10	0.8 (0.4–1.5)
	Males—zone R	76	0.9 (0.7–1.1)
	Females—zone A	1	0.9 (0.0–5.3)
	Females—zone B	7	1.0 (0.4–2.1)
	Females—zone R	58	1.0 (0.8–1.3)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	17	1.4 (0.8–2.2)
	West coast	63	0.9 (0.7–1.2)
	Swedish fishermen incidence		
	East coast	24	1.6 (1.0–2.4)
	West coast	71	0.9 (0.7–1.2)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male residents—zone B	7	1.0 (0.5–2.1)
	Female residents—zone B	2	0.6 (0.2–2.5)
	Seveso male residents—zone R	45	0.9 (0.7–1.2)
	Female residents—zone R	25	1.0 (0.6–1.5)
Studies reviewed in *VAO*			
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)
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)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	32	1.1 (0.7–1.5)
Crane et al., 1997b	Australian national service veterans	4	1.7 (0.3–>10)
Studies reviewed in *VAO*			
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 7-2 Selected Epidemiologic Studies—Colon Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	52	1.0 (0.8–1.3)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	33	1.2 (0.8–1.6)
	Workers exposed to any phenoxy herbicide or chlorophenol	86	1.1 (0.8–1.3)
Becher et al., 1996	German chemical production workers		
	Plant I	2	0.4 (0.0–1.4)
	Plant II	0	
	Plant III	1	2.2(0–12)
	Plant IV	0	
Gambini et al., 1997	Italian rice growers	27	1.1 (0.7–1.6)
Ott and Zober, 1996	BASF cleanup workers	5	1.0 (0.3–2.3)[b]
	TCDD < 0.1 μg/kg body wt	2	1.1 (0.1–3.9)[b]
	TCDD 0.1–0.99 μg/kg body wt	2	1.4 (0.2–5.1)[b]
	TCDD > 1 μg/kg body wt	1	0.5 (0.0–3.0)[b]
Ramlow et al., 1996	Pentachlorophenol production workers		
	0 year latency	4	0.8 (0.2–2.1)
	15 year latency	4	1.0 (0.3–2.6)
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	White males	2,291	1.0 (0.9–1.0)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers		NS
Collins et al., 1993	Monsanto 2,4-D production workers		NS
Studies reviewed in *VAO*			
Swaen et al., 1992	Dutch herbicide applicators	4	2.6 (0.7–6.5)
Ronco et al., 1992	Danish male self-employed farm workers	277	0.7 ($p < .05$)
Fingerhut et al., 1991	NIOSH cohort	25	1.2 (0.8–1.8)
Manz et al., 1991	German production workers	8	0.9 (0.4–1.8)
Saracci et al., 1991	IARC cohort	41	1.1 (0.8–1.5)
Zober et al., 1990	BASF production workers—basic cohort	2	2.5 (0.4–14.1)[b]
Alavanja et al., 1989	USDA forest conservationists		1.4 (0.7–2.8)
	USDA soil conservationists		1.2 (0.7–2.0)
Henneberger et al., 1989	Paper and pulp workers	9	1.0 (0.5–2.0)
Solet et al., 1989	Paper and pulp workers	7	1.5 (0.6–3.0)
Alavanja et al., 1988	USDA agricultural extension agents		1.0 (0.7–1.5)
Bond et al., 1988	Dow 2,4-D production workers	4	2.1 (0.6–5.4)
Thomas, 1987	Flavor and fragrance chemical production workers		0.6
Coggon et al., 1986	British MCPA production workers	19	1.0 (0.6–1.6)
Hoar et al., 1986	Kansas residents		
	No herbicide use		1.6 (0.8–3.6)
	Herbicide use		1.5 (0.6–4.0)

TABLE 7-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Robinson et al., 1986	Paper and pulp workers	7	0.4 (0.2–0.9)
Lynge, 1985	Danish male production workers	10	1.0
Blair, 1983	Florida pesticide applicators	5	0.8
Wiklund, 1983	Swedish agricultural workers	1,332	0.8 (0.7–0.8)[c]
Thiess et al., 1982	BASF production workers		0.4
Burmeister, 1981	Farmers in Iowa	1,064	1.0 (NS)
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			
New studies			
Bertazzi et al., 1997	Seveso male residents		
	zone A	0	
	zone B	5	0.8 (0.3–2.0)
	zone R	34	0.8 (0.6–1.1)
	Seveso female residents		
	zone A	2	2.6 (0.3–9.4)
	zone B	3	0.6 (0.1–1.8)
	zone R	33	0.8 (0.6–1.1)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	4	0.1 (0.0–0.7)
	West coast	58	1.0 (0.8–1.3)
	Swedish fishermen incidence		
	East coast	5	0.4 (0.1–0.9)
	West coast	82	0.9 (0.8–1.2)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male residents—zone B	2	0.5 (0.1–2.0)
	Female residents—zone B	2	0.6 (0.1–2.3)
	Seveso male residents—zone R	32	1.1 (0.8–1.6)
	Female residents—zone R	23	0.8 (0.5–1.3)
Studies reviewed in *VAO*			
Lampi et al., 1992	Finnish community exposed to chlorophenol contamination	9	1.1 (0.7–1.8)
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)
VIETNAM VETERANS			
New studies			
Crane et al., 1997a	Australian military veterans	78	1.2 (1.0–1.5)
Crane et al., 1997b	Australian national service veterans	6	0.6 (0.2–1.5)
Studies reviewed in *Update 1996*			
Dalager et al., 1995a	Women Vietnam veterans		2.8 (0.8–10.2)
	Nurses		5.7 (1.2–27.0)

TABLE 7-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Studies reviewed in *VAO*			
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.

TABLE 7-3 Selected Epidemiologic Studies—Rectal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	29	1.3 (0.9–1.9)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	14	0.7 (0.4–1.2)
	Workers exposed to any phenoxy herbicide or chlorophenol	44	1.1 (0.8–1.4)
Becher et al., 1996	German chemical production workers		
	Plant I	6	1.8 (0.7–4.0)
	Plant II	0	
	Plant III	0	
	Plant IV	1	0.9 (0.0–4.9)
Ramlow et al., 1996	Pentachlorophenol production workers		
	0 year latency	0	
	15 year latency	0	
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	White males	367	1.0 (0.9–1.1)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers		NS
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farmers	309	0.8 ($p < .05$)
Fingerhut et al., 1991	NIOSH cohort	5	0.9 (0.3–2.1)
Saracci et al., 1991	IARC cohort	24	1.1 (0.7–1.6)
Alavanja et al., 1989	USDA forest/soil conservationists	9	1.0 (0.5–1.9)
Henneberger et al., 1989	Paper and pulp workers	1	0.4 (0.0–2.1)

TABLE 7-3 Selected Epidemiologic Studies—Rectal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Alavanja et al., 1988	USDA agricultural extension agents	5	0.6 (0.2–1.3)
Bond et al., 1988	Dow 2,4-D production workers	1	1.7 (0.0–9.3)
Thomas, 1987	Flavor and fragrance chemical production workers		2.5
Coggon et al., 1986	British MCPA chemical workers	8	0.6 (0.3–1.2)
Lynge, 1985	Danish male production workers	14	1.5
Blair, 1983	Florida pesticide applicators	2	1.0
Wiklund, 1983	Swedish agricultural workers	1,083	0.9 (0.9–1.0)[b]
ENVIRONMENTAL			
New studies			
Bertazzi et al., 1997	Seveso male residents		
	zone A	0	
	zone B	7	2.9 (1.2–5.9)
	zone R	19	1.1 (0.7–1.8)
	Seveso female residents		
	zone A	0	
	zone B	2	1.3 (0.1–4.5)
	zone R	12	0.9 (0.5–1.6)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	4	0.7 (0.2–1.9)
	West coast	31	1.0 (0.7–1.5)
	Swedish fishermen incidence		
	East coast	9	0.9 (0.4–1.6)
	West coast	59	1.1 (0.8–1.4)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male residents—zone B	3	1.4 (0.4–4.4)
	Female residents—zone B	2	1.3 (0.3–5.4)
	Seveso male residents—zone R	17	1.1 (0.7–1.9)
	Female residents—zone R	7	0.6 (0.3–1.3)
Studies reviewed in *VAO*			
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)
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)
VIETNAM VETERANS			
New studies			
Crane et al., 1997a	Australian military veterans	16	0.6 (0.4–1.0)
Crane et al., 1997b	Australian national service veterans	3	0.7
Studies reviewed in *VAO*			
Anderson et al., 1986a	Wisconsin Vietnam veterans	1	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	1	—

[a] Given when available.
[b] 99% CI.

TABLE 7-4 Selected Epidemiologic Studies—Pancreatic Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	30	1.0 (0.7–1.4)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	16	0.9 (0.5–1.4)
	Workers exposed to any phenoxy herbicide or chlorophenol	47	0.9 (0.7–1.2)
Becher et al., 1996	German chemical production workers		
	Plant I	2	0.6 (0.1–2.3)
	Plant II	0	
	Plant III	0	
	Plant IV	2	1.7 (0.2–6.1)
Gambini et al., 1996	Italian rice growers	7	0.9 (0.4–1.9)
Ramlow et al., 1996	Pentachlorophenol production workers		
	0 year latency	2	0.7 (0.1–2.7)
	15 year latency	2	0.9 (0.1–3.3)
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	White males	1,133	1.1 (1.1–1.2)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers		NS
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish self-employed male farm workers	137	0.6 ($p < .05$)
Swaen et al., 1992	Dutch herbicide applicators	3	2.2 (0.4–6.4)
Fingerhut et al., 1991	NIOSH cohort	10	0.8 (0.4–1.6)
Saracci et al., 1991	NIOSH cohort	26	1.1 (0.7–1.6)
Alavanja et al., 1989	USDA forest conservationists		1.2 (0.4–3.4)
	USDA soil conservationists		1.1 (0.5–2.2)
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)
Alavanja et al., 1988	USDA agricultural extension agents	21	1.3 (0.8–1.9)
Thomas, 1987	Flavor and fragrance chemical production workers		1.4
Coggon et al., 1986	British MCPA production workers	9	0.7 (0.3–1.4)
Robinson et al., 1986	Paper and pulp workers	4	0.3 (0.1–1.1)
Lynge, 1985	Danish male production workers	3	0.6
Blair, 1983	Florida pesticide applicators	4	1.0
Wiklund, 1983	Swedish agricultural workers	777	0.8 (0.8–0.9)[b]
Burmeister, 1981	Farmers in Iowa	416	1.1
ENVIRONMENTAL			
New studies			
Bertazzi et al., 1997	Seveso residents		
	Males—zone A	1	1.9 (0.0–10.5)
	Males—zone B	2	0.6 (0.1–2.0)

TABLE 7-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
	Males—zone R	20	0.8 (0.5–1.2)
	Females—zone A	0	
	Females—zone B	1	0.5 (0.0–3.1)
	Females—zone R	11	0.7 (0.4–1.3)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	5	0.7 (0.2–1.6)
	West coast	33	0.8 (0.6–1.2)
	Swedish fishermen incidence		
	East coast	4	0.6 (0.2–1.6)
	West coast	37	1.0 (0.7–1.4)
Studies reviewed in *VAO*			
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)
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)
VIETNAM VETERANS			
New studies			
Crane et al., 1997a	Australian military veterans	38	1.4 (1.0–1.9)
Crane et al., 1997b	Australian national service veterans	6	1.5
Studies reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans		
Studies reviewed in *VAO*			
Thomas et al., 1991	Women Vietnam veterans	5	2.7 (0.9–6.2)
Breslin et al., 1988	Army Vietnam veterans	82	0.9 (0.6–1.2)
	Marine Vietnam veterans	18	1.6 (0.5–5.8)
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

and its contaminant TCDD, cacodylic acid, and picloram) and gastrointestinal cancers (stomach, pancreatic, rectal, and colon cancers). The evidence regarding association was drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

Although a possible association between these exposures and cancer at gastrointestinal sites is considered plausible given the current knowledge of ways in which dioxin and herbicides affect human systems, the literature reviewed for

this update does not support a change from the previous conclusion of limited/suggestive evidence of no association. A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and gastrointestinal cancers is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

HEPATOBILIARY CANCERS

Background

This category includes cancers of the liver (ICD·9 155.0,155.2) and intrahepatic bile duct (ICD·9 155.1). According to American Cancer Society estimates, 9,300 men and 4,600 women will be diagnosed with liver cancer in the United States in 1998; 7,900 men and 5,100 women will die from the disease (ACS, 1998). Liver cancer is expected to account for about 1 percent of new diagnoses and 2 percent of cancer deaths in the United States in 1998. This disparity may be due to misclassification of metastatic cancers as primary liver cancer, leading to overreporting of deaths due to liver cancer (Percy et al., 1990). In developing countries, especially sub-Saharan Africa and Southeast Asia, liver cancers are common and are among the leading causes of death. The known risk factors for liver cancer include chronic infection with hepatitis B or hepatitis C virus and exposure to the carcinogens aflatoxin and vinyl chloride. In the general population, the incidence of liver and intrahepatic bile duct cancer increases slightly with age, and remains greater for men than women and greater for African Americans than whites throughout ages 45–59 years.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Liver and Intrahepatic Bile Duct Cancers

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	5	3	11	8	5	15	12	9	19
females	2	1	3	2	1	4	4	3	4

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Occupational Studies

Among studies of occupational groups, mortality studies by Lynge (1985), Collins et al. (1993), Fingerhut et al. (1991), and Saracci et al. (1991) found no elevation of risk. In studies of agricultural or forestry workers, Wiklund (1983), Hardell et al. (1984), Ronco et al. (1992), Asp et al. (1994), and Blair et al. (1993) observed no evidence of increased liver cancer. In a study of mortality among

pulp and paper workers, Solet et al. (1989) observed a nonsignificant excess of deaths from liver cancer, based on two deaths.

Environmental Studies

A follow-up of the population involved in the Seveso incident (Bertazzi et al., 1989b and Bertazzi et al., 1993) reported a statistically significant excess in liver cancer mortality for female residents of zone B (RR = 3.3, CI 1.3–8.1). Nonsignificant excesses and deficits in the risk of death due to liver cancer were observed among males in the three exposure zones and among females living in the other two zones. Additional liver cancer incidence data show similar results (Pesatori et al., 1992). Data from U.S. populations living in contaminated areas do not add any useful information. Residents of the Quail Run trailer park in Times Beach, Missouri, the site of a major release of TCDD-contaminated oil, were free from diagnosed liver cancer, whereas 1.5 cases were expected (Hoffman et al., 1986; Stehr-Green et al., 1987).

A case-control study by Cordier et al. (1993) described the hepatocellular carcinoma risk among 152 North Vietnamese cases and 241 controls, in relation to viral infections and chemical exposures. The dominant risk factor was found to be positivity for hepatitis B surface antigen, which carried an odds ratio (OR) of 0.62 (95% CI 0.30–1.28). Use of organochlorine pesticides did not indicate any statistically significant trend, but military service in South Vietnam did. Those who served for more than 10 years in South Vietnam (N = 11) had an OR for hepatocellular carcinoma of 8.8 (CI 1.9–41). However, direct contact with aerial spraying resulted in a slight, nonsignificantly increased OR of 1.3.

Vietnam Veteran Studies

Studies of liver cancer among Vietnam veterans have not found a significant excess of mortality from liver cancer; however, the studies are hampered by the small size. Studies include those of Wisconsin Vietnam veterans by Anderson et al. (1986a,b), and the mortality component of the Centers for Disease Control and Prevention's (CDC's) Vietnam Experience Study (Boyle et al., 1987). In a larger mortality study among U.S. Army and Marine Corps Vietnam veterans, Breslin et al. (1988) identified 34 liver cancer deaths among Army veterans; the proportional mortality ratio (PMR) was 1.0 (CI 0.8–1.4). The data from Marines are consistent with this result, although there were fewer deaths among the latter group.

The CDC's Selected Cancers Study (CDC, 1990) included a pathologic review of studies to confirm the diagnosis of 130 men with primary liver cancer. After adjusting for design and a range of established risk factors, the RR was 1.2 (CI 0.5–2.7). The risk for Vietnam veterans was slightly lower than for men who served elsewhere in the military.

Update of the Scientific Literature

Occupational Studies

Production Workers In an update and expansion of the IARC cohort study, Kogevinas et al. (1997) examined cancer mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. These workers were assembled from 12 countries, drawn from national studies that followed the same core protocol developed jointly by the participants and coordinated by IARC.

No excess risk of death from hepatobiliary cancer was observed among the group of all workers exposed to any phenoxy herbicide or chlorophenol. When this group was divided into those exposed and unexposed to TCDD or higher chlorinated dioxins, the TCDD-exposed group had a higher risk (SMR = 0.87, CI 0.45–1.52) than the unexposed group (SMR = 0.41, CI 0.09–1.22), and none achieved statistical significance. More detailed analysis by exposure variables such as duration and time since first exposure was not conducted for hepatobiliary cancers.

Although the study includes large numbers of workers likely to be exposed at levels substantially higher than the general population exposures, the lack of information about actual exposures limits the investigator's ability to examine exposure–response relationships within the cohort. In addition, the inclusion of workers in the exposed group based on ever having worked in a job considered exposed makes it impossible to distinguish heavily exposed workers from those with very minor exposures.

Becher et al. (1996) examined cancer mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population included workers who had a least one month of employment, resulting in a cohort consisting of 2,479 male workers. The cohort was assembled from four plants; analysis was conducted on the total cohort, divided into four subcohorts that corresponded to each plant considered separately.

Based on production information and limited blood dioxin measurements, subcohorts I and II are supposed to have higher TCDD exposures than subcohorts III and IV. Of the four subcohorts, only group IV had at least one observed death for hepatobiliary cancer. In this group, one death was observed (0.8 expected) for an SMR of 1.2 (CI 0–6.9).

Agricultural Workers Cancer mortality among a cohort of rice growers in northern Italy was investigated by Gambini et al. (1997). Using a set of registered farm owners consisting of 1,493 males who worked on farms from 1957 to 1992, they examined the cause of death for 958 subjects and compared this with expected numbers calculated from national rates. No direct exposure information was available, so employment on the farm was used as a surrogate for exposure to

the range of phenoxy herbicides employed during the study period. Mortality was evaluated for liver cancer, and observed and expected deaths did not differ significantly in the overall cohort (SMR = 1.3, CI 0.5–2.6). Stratification by age at death and duration of exposure (employment as a farmer) did not change the finding of nonsignificant differences. Although the study population is small, it does describe the experience of a cohort with good follow-up (99 percent) and long latency (37 percent of deaths observed beyond age 80). It is limited by a very crude exposure assessment, however, and the degree to which study subjects were actually exposed to phenoxy herbicides cannot be established with any certainty.

Environmental Studies

Bertazzi et al. (1997) continued the follow-up of the people environmentally exposed to TCDD in Seveso, Italy. The events that led to the exposure and the methods used to study this population have been fully described in earlier reports. This report updates the population after 15 years' follow-up. Death from liver cancer showed nonsignificant decreases in all three exposure groups except for women in zone B, who had a nonsignificant elevation (SMR = 1.3, CI 0.3–3.8, 3 cases). More detailed investigation of exposed subjects in zone B was not conducted for liver cancer.

Svensson et al. (1995) studied mortality and cancer incidence in two cohorts of Swedish fishermen. One group (2,896 men) resided on the east coast of Sweden and consumed fish from the Baltic Sea. These fatty fish (particularly salmon and herring) are reported to contain elevated levels of PCB, PCDD, and PCDF. The other group of fishermen (8,477) resided on the west coast of Sweden and were presumed to have a higher intake of lean (and less contaminated) fish, including cod and flat fish. This distinction in exposure by place of residence is reportedly confirmed by the study's finding that blood levels of dioxin-like compounds were two times higher among east coast than west coast fishermen; however, no data were provided to support this point. East and west coast fishermen were found to have nonsignificantly decreased mortality from liver cancer. East coast fishermen had a nonsignificantly increased incidence of liver cancer (SIR = 1.31, CI 0.48–2.85, 6 cases) compared to national Swedish rates. A nonsignificantly decreased incidence was seen among west coast fishermen.

Vietnam Veteran Studies

The Australian study *Mortality of Vietnam Veterans: The Veteran Cohort Study* (Crane et al., 1997a) examined the mortality experience of male Australian Vietnam veterans from 1980 to 1994. The cohort consists of 59,036 male veterans, who were followed from 22 to 32 years. There were 2,067 deaths recorded among this group from 1980 to 1994, and vital status was ascertained for 96.9

percent of the cohort. There was a statistically significant excess of death for all cancer (SMR = 1.21, CI 1.11–1.31) in this cohort compared to the Australian white male population and according to the SRMR (1.2, CI 1.1–1.3). No excess mortality was observed from cancer of the liver in the military population overall (SMR = 0.6, CI 0.3–1.2) or when analyzed separately by branch of service. The authors have described the strengths and limitations of the Australian veterans cohort study, including virtually complete identification of the study population, a period of follow-up ranging from 22 to 32 years, and vital status ascertainment of 96.9 percent. Among the weaknesses of the study are the possibility of under-ascertainment of death, and uncertain quality of exposure assessment regarding a variety of risk factors, including smoking and alcohol consumption, as well as herbicide and dioxin exposure.

The examination of mortality among Australian National Service Vietnam veterans (Crane et al., 1997b) reported similar findings for hepatobiliary cancer.

Synthesis

VAO and *Update 1996* found that there were relatively few occupational, environmental, or veteran studies of liver cancer (Table 7-5), and most of these are small in size and have not controlled for risk factors related to lifestyle. In summary, given the methodological difficulties associated with most of the few existing studies, the evidence regarding liver cancer was considered inadequate or insufficient to determine whether an association with herbicides exists. The earlier conclusion is unchanged in this report.

Conclusions

Strength of Evidence in Epidemiologic Studies

This committee finds no change in the conclusion of inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and hepatobiliary cancer.

The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components. Most of these studies are small in size and have not fully controlled for lifestyle-related risk factors.

Biologic Plausibility

Although a possible association between the exposures considered here and hepatobiliary cancer is considered plausible given the current knowledge of ways

TABLE 7-5 Selected Epidemiologic Studies—Hepatobiliary Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	12	0.9 (0.4–1.5)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	3	0.4 (0.1–1.2)
	Workers exposed to any phenoxy herbicide or chlorophenol	15	0.7 (0.4–1.2)
Gambini et al., 1996	Italian rice growers	7	1.3 (0.5–2.6)
Ott and Zober, 1996	BASF cleanup workers	2	2.1 (0.3–8.0)
	TCDD <0.1 μg/kg body wt	1	2.8 (0.1–15.5)
	TCDD 0.1–0.99 μg/kg body wt	0	
	TCDD >1 μg/kg body wt	1	2.8 (0.1–15.5)
Becher et al., 1996	German chemical production workers	1	1.2 (0.0–6.9)
Ramlow et al., 1996	Pentachlorophenol production workers		
	0 year latency	0	
	15 year latency	0	
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	2	0.6 (0.1–2.2)
Blair et al., 1993	U.S. farmers in 23 states	326	1.0 (0.9–1.1)
Collins et al., 1993	Monsanto 2,4-D production workers	2	1.4 (0.2–5.2)
Studies reviewed in *VAO*			
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
Fingerhut et al., 1991	NIOSH cohort	6	1.2 (0.4–2.5)
	20 years latency	1	0.6 (0.0–3.3)
Saracci et al., 1991	IARC cohort	4	0.4 (0.1–1.1)
Solet et al., 1989	Paper and pulp workers	2	2.0 (0.2–7.3)
Bond et al., 1988	Dow 2,4-D production workers		1.2
Lynge, 1985	Danish production workers	3	1.0
Hardell et al., 1984	Male residents of northern Sweden	102	1.8 (0.9–4.0)
Wiklund, 1983	Swedish agricultural workers	103	0.3 (0.3–0.4)[b]
Zack and Suskind, 1980	Monsanto production workers	0	—
ENVIRONMENTAL			
New studies			
Bertazzi et al., 1997	Seveso male residents		
	zone A	0	
	zone B	4	0.6 (0.2–1.4)
	zone R	35	0.7 (0.5–1.0)
	Seveso female residents		
	zone A	0	
	zone B	4	1.1 (0.3–2.9)
	zone R	25	0.8 0.5–1.3)

TABLE 7-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	1	0.5 (0.0–2.6)
	West coast	9	0.9 (0.4–1.7)
	Swedish fishermen incidence		
	East coast	6	1.3 (0.5–2.8)
	West coast	24	1.0 (0.6–1.5)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male residents—zone B	5	1.8 (0.7–4.4)
	Female residents—zone B	5	3.3 (1.3–8.1)
	Seveso male residents—zone R	11	0.5 (0.3–1.0)
	Female residents—zone R	12	0.9 (0.5–1.7)
Cordier et al., 1993	Military service in South Vietnam for ≥10 years after 1960	11	8.8 (1.9–41.0)
Studies reviewed in *VAO*			
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)
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)
Stehr et al., 1986	Missouri residents	0	—
Hoffman et al., 1986	Residents of Quail Run Mobile Home Park	0	—
VIETNAM VETERANS			
New studies			
Crane et al., 1997a	Australian military veterans	8	0.6 (0.3–1.2)
Crane et al., 1997b	Australian national service veterans	1	—
Studies reviewed in *VAO*			
CDC, 1990c	U.S. men born between 1921 and 1953	8	1.2 (0.5–2.7)
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	—

[a] Given when available.
[b] 99% CI.

in which dioxin and herbicides affect human systems, the literature reviewed for this update does not support a change from the previous conclusion of inadequate or insufficient evidence to determine whether an association exists. A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and hepatobiliary cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

NASAL/NASOPHARYNGEAL CANCER

Background

There are many types of nasal (ICD·9 160.0–160.9) and nasopharyngeal (ICD·9 147.0–147.9) cancer, although undifferentiated carcinoma, squamous

cell carcinoma and lymphomas account for the vast majority of maligancies. The epithelium of the nasal and nasopharyngeal cavities is partly squamous, partly columnar, and partly ciliated pseudostratified columnar. There are also serous and mucous glands and lymphoid aggregates in close association with the epithelium.

The American Cancer Society estimates that approximately 4,100 men and 1,200 women will be diagnosed with nasal, pleural, tracheal, and other respiratory system cancers in the United States in 1998 and that some 700 men and 500 women will die from the diseases (ACS, 1998). Roughly speaking, nasal and nasopharyngeal cancers account for between one-third and one-half of these totals. The American Cancer Society (1998) estimates suggest that approximately 6,500 men and 2,100 women will be diagnosed with cancers of the pharynx (including nasopharynx, tonsil, oropharynx, hypopharynx, and buccal cavity) and that 1,500 men and 600 women will die from them. Nasopharyngeal cancers make up approximately one in five of these cancers. The incidence rates reported below show that men are at a greater risk than women for these diseases and that incidence increases with age, although the very small number of cases indicates that care should be exercised in interpreting the numbers.

Nasopharyngeal cancer is relatively common in China and Southeast Asia. It is also more common in Chinese and Vietnamese Americans than in whites, African Americans, or other groups, suggesting that genetic factors may play a role in this disease (Miller et al., 1996). There is no similar association for nasal cancer. Reported risk factors for nasal cancer include occupational exposure to nickel and chromium compounds (Hayes, 1997), wood dust (Demers et al., 1995), and formaldehyde (Blair and Kazerouni, 1997). Studies of nasopharyngeal cancer have reported associations with the consumption of salt-preserved foods (Miller et al., 1996), cigarette smoking (Zhu et al., 1995), and Epstein-Barr virus (Mueller, 1995).

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Nasal and Nasopharyngeal Cancers

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
Nose, nasal cavity, and mid ear									
males	1.0	0.8	1.3	1.3	1.3	1.7	1.8	1.8	2.2
females	0.5	0.5	1.1	0.9	0.7	1.8	0.8	0.7	2.2
Nasopharynx									
males	1.0	0.3	1.6	2.3	1.3	2.6	2.8	1.7	3.2
females	0.5	0.3	0.3	0.6	0.4	[b]	0.9	0.6	1.7

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.
[b] Insufficient data to provide meaningful incidence rate.

Summary of *VAO* and *Update 1996*

Studies that specifically considered nasal and nasopharyngeal cancers were very limited. Occupational studies included phenoxy herbicide production workers and sprayers, herbicide applicators, agricultural workers, and paper and pulp workers. These studies had very few cases of nasal or nasopharyngeal cancers and the results were inconsistent. The sole environmental study was the Seveso follow-up, with no cases reported in the two exposed zones, A and B. Vietnam veteran studies found no significant associations for these cancers.

Update of the Scientific Literature

Occupational Studies

IARC (Kogevinas et al., 1997) has brought together almost all of the phenoxy herbicide production workers in 36 cohorts in 12 countries (Fingerhut et al., 1991; Sarraci et al., 1991; Manz et al., 1991; Flesch-Janys et al., 1995; Becher et al., 1996) for a joint analysis. This cohort contains 26,976 workers and was divided into those who were exposed to TCDD or higher chlorinated dioxins and those who were not so. The combined cohort study showed no effect of phenoxy herbicide exposure on oral cavity and pharyngeal cancers (26 cases coded ICD·9 140–149), RR = 1.1 (CI 0.7–1.6). None of the three deaths from cancer of the nose and nasal sinuses (ICD·9 160), RR = 1.6 (CI 0.3–4.7) were in the TCDD-exposed group.

Environmental Studies

In the earlier Seveso population study (Bertazzi et al., 1993) no cases of nasal or nasopharyngeal cancer were observed in zones A and B. Two cases were seen in zone R, with 2.8 expected. In the more recent follow-up of the Seveso population (Bertazzi et al., 1997) nasopharyngeal cancers are not specifically mentioned.

Vietnam Veteran Studies

The mortality experience of Australian Vietnam veterans was compared to that of the general public for 1964–1979 and 1980–1994 (Crane et al., 1997a). During 1964–1979, no cases of nasal or nasopharyngeal cancer deaths were found, whereas 0.8 was expected. For 1980–1994, there were two deaths due to nasal and two to nasopharyngeal cancers, whereas 1.7 and 3.9 were expected, respectively. A companion study comparing conscripted Australian veterans of Vietnam with military personnel who did not serve there reported an RR of 1.3 for nasopharyngeal cancer based on a single death in each of the populations between 1982 and 1994 (Crane et al., 1997b). There was one death due to nasal cancer in the comparison population and none in Vietnam veterans over the same period.

Synthesis

Nasal and nasopharyngeal cancers are relatively rare in many parts of the world and thus difficult to study epidemiologically. Scientific evidence on the association between herbicide exposure and nasopharyngeal cancer continues to be too sparse to make a definitive statement.

TABLE 7-6 Selected Epidemiologic Studies—Nasal/Nasopharyngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New studies			
Kogevinas et al., 1997	IARC cohort		
	Oral cavity and pharynx cancer (ICD·9 140–9)	26	1.1 (0.7–1.6)
	Nose and nasal sinuses cancer (ICD·9 160)	3	1.6 (0.3–4.7)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	1	0.5 (0.01–2.9)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish and Italian farm workers		0.6 (NS)
Saracci et al., 1991	IARC cohort	3	2.9 (0.6–8.5)
Coggon et al., 1986	British MCPA production workers	3	4.9 (1.0–14.4)
Robinson et al., 1986	Paper and pulp workers	0	—
Wiklund, 1983	Swedish agricultural workers	64	0.8 (0.6–1.2)
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)
ENVIRONMENTAL			
Studies reviewed in *VAO*			
Bertazzi et al., 1993	Residents in Seveso (zone R)	2	2.6 (0.5–13.3)
VIETNAM VETERANS			
New studies			
Crane et al., 1997a	Australian military veterans		
	Nasal cancer	2	1.2 (0.2–4.4)
	Nasopharyngeal cancer	2	0.5 (0.1–1.9)
Crane et al., 1997b	Australian national service veterans		
	Nasal cancer	0	0 (0.0–>10)
	Nasopharyngeal cancer	1	1.3 (0.0–>10)
Studies reviewed in *VAO*			
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 the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and nasal or nasopharyngeal cancer. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and nasal or nasopharyngeal cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

LARYNGEAL CANCER

Background

According to American Cancer Society estimates, 9,000 men and 2,100 women will be diagnosed with cancer of the larynx (ICD·9 161.0–161.9) in the United States in 1998, and 3,400 men and 900 women will die from the disease (ACS, 1998). These numbers represent approximately 1 percent of new cancer diagnoses and deaths. Cancer of the larynx is more common in men than women, with an overall ratio in the United States of about 5:1. Incidence also increases with age in the 45–59 age group.

Risk factors include tobacco and alcohol, which act individually and synergistically. Research suggests that gastroesophageal reflux, human papillomavirus, a weakened immune system, and occupational exposure to asbestos and certain chemicals and dusts may also increase incidence (ACS, 1998).

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Laryngeal Cancer

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	6	6	16	13	13	25	22	20	43
females	1	1	3	3	3	8	6	6	10

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

In nearly all studies analyzing respiratory cancers, the authors either group all of the different types of cancer in this broad group together (ICD·9 161–165, which include trachea, bronchus, lung, and larynx) or present data for the largest category within the group (ICD·9 162, which includes trachea, bronchus, and lung). However, in a few studies, data are separated to allow assessment of laryngeal cancer.

Of note are five studies of production workers in which data for laryngeal cancer (ICD·9 161) are presented separately (Coggon et al., 1986; Bond et al., 1988; Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991). Although the numbers are too small to draw strong conclusions, the consistency of a mild elevation in RR is suggestive of an association for laryngeal cancer. Pooling all but the Coggon data (Coggon et al., 1986, 1991) yields an OR of 1.8 (CI 1.0–3.2). As mentioned above, the potential confounders of an occupational risk for this cancer include tobacco and alcohol consumption. These studies did not directly control for smoking, although its magnitude in Manz et al. (1991) and Fingerhut et al. (1991) is not likely to be large. There is no information on alcohol consumption in any of the studies.

Other than these studies of production workers, only one study reported separate results for cancer of the larynx: a proportional cancer mortality ratio (PCMR) study was performed for farmers in 23 states, using occupational information from death certificates (Blair et al., 1993). Based on 162 deaths from laryngeal cancer in white male farmers, the PCMR was significantly decreased, at 0.7 (CI 0.6–0.8). This is consistent with a significant decrease in lung cancer in the same subgroup. The PCMR for laryngeal cancer in nonwhite male farmers was 1.1 (CI 0.8–1.5), based on 32 deaths. There were no deaths from this cancer in female farmers.

Update of the Scientific Literature

Occupational Studies

The IARC joint analysis (Kogevinas et al., 1997) of phenoxy herbicide production workers reported an SMR for laryngeal cancer of 1.6 (CI 1.0–2.5), based on 21 deaths among 21,863 workers in cohorts from 12 countries. Workers exposed to TCDD or higher chlorinated dioxins had an SMR of 1.7 (CI 1.0–2.8), based on 12 deaths among 13,831 workers.

Ramlow et al. (1996) examined the mortality of 770 workers potentially exposed to PCP between 1937 and 1980. Dioxin is an unintended by-product of PCP production. Exposure was estimated on the basis of job description information and industrial hygiene characterizations of the job environment. The SMR, calculated without any factoring for latency, was 2.9 (CI 0.3–10.3), based on two deaths.

Gambini et al. (1997) investigated cancer mortality in rice growers in northern Italy for 1957–1992. Employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides including 2,4-D and 2,4,5-T. An SMR of 0.9 (CI 0.4–1.9) was calculated, based on seven cases among 1,493 subjects.

Veterans Studies

Watanabe and Kang (1996) compare laryngeal cancer rates among Army and Marine Vietnam veterans with veterans who did not serve in Vietnam. Among Army veterans, the PMR for cancer of the larynx was 1.3 for Vietnam veterans and 0.9 for non-Vietnam veterans, based on 50 and 34 deaths, respectively. The corresponding numbers for Marines are 0.7 versus 1.4, based on four deaths in each group.

The Australian Vietnam veteran study (Crane et al., 1997a) found an SMR of 1.3 (0.7–2.3) based on 12 laryngeal cancer deaths. A second study examining the

TABLE 7-7 Selected Epidemiologic Studies—Laryngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers	7	0.9 (0.4–1.9)
Kogevinas et al., 1997	IARC cohort	21	1.6 (1.0–2.5)
	Workers exposed to TCDD (or higher chlorinated dioxins)	12	1.7 (1.0–2.8)
Ramlow et al., 1996	Pentachlorophenol production workers	2	2.9 (0.3–10.3)
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	White males	162	0.7 (0.6–0.8)
	Nonwhite males	32	1.1 (0.8–1.5)
Studies reviewed in *VAO*			
Fingerhut et al., 1991	NIOSH cohort		
	1 year exposure, 20 years latency	3	2.7 (0.6–7.8)
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)
Bond et al., 1988	Dow 2,4-D production workers	1	3.0 (0.4–16.8)
Coggon et al., 1986	British MCPA production workers	4	2.3 (0.5–4.5)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	12	1.3 (0.7–2.3)
Crane et al., 1997b	Australian national service veterans	0	0 (0–>10)
Watanabe and Kang, 1996	Army Vietnam veterans	50	1.3
	Marine Vietnam veterans	4	0.7

mortality experience of conscripted Australian veterans relative to military personnel who did not serve in the conflict reported no laryngeal cancer deaths among Vietnam veterans and one in the comparison population between 1982 and 1994 (Crane et al., 1997b).

Synthesis

Studies published since *Update 1996* continue to support the conclusion that there is limited/suggestive evidence of an association. The committee concluded 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 and types of exposure and because the most important risk factors for laryngeal cancers—cigarette smoking and alcohol consumption—were not fully controlled for or evaluated in the studies.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and laryngeal cancer. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and laryngeal cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

LUNG CANCER

Background

Lung cancer (carcinomas of the lung and bronchus, ICD·9 162.2–162.9) is the leading cause of cancer death in the United States. According to American Cancer Society estimates, 91,400 men and 80,100 women will be diagnosed with this cancer in the United States in 1998, and approximately 93,100 men and 67,000 women will die from the disease (ACS, 1998). These numbers represent roughly 14 percent of new cancer diagnoses and 28 percent of cancer deaths in 1998. The principal types of lung neoplasms are identified collectively as bronchogenic carcinoma (“bronchus” is the term used to describe either of the two

main branches of the trachea) or carcinoma of the lung. The lung is also a common site for the development of metastatic cancer.

Lung cancer incidence can vary greatly in the age groups that describe most Vietnam veterans. For men and women, the incidence of lung cancer increases rapidly beginning about age 40. Incidence in 50–54 year olds is double that of 45–49 year olds; and it doubles again for 55–59 year olds. The rate for African-American males is consistently higher than for females or white males.

The American Cancer Society estimates that more than 90 percent of lung cancers in males are the result of tobacco smoking (ACS, 1998). Tobacco smoke may include both tumor initiators and promoters. Among the other risk factors are occupational exposure to asbestos, chromium, nickel, aromatic hydrocarbons, and radioactive ores.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Lung and Bronchus Cancer

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	40	35	86	90	82	196	171	161	320
females	30	29	46	65	66	86	113	116	133

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Numerous studies were considered in the earlier reports that specifically evaluated lung cancer and its relationship to herbicides. These included both cohort and case-control studies, which were primarily occupational. The strongest data in support of a lung cancer association come from the phenoxy herbicide formulators (Fingerhut et al., 1991; Manz et al., 1991). In both studies, attempts were made to compare the exposed workers to comparable worker groups with assumed similar smoking patterns. The large studies of farmers that did not show an association were not particularly informative due to lower smoking rates among farmers compared to the general population. The environmentally exposed Seveso population had relatively few lung cancers but had too short a follow-up period to conclude anything about lung cancer and TCDD.

Most studies either considered respiratory cancers as a group (ICD·9 161–165) or specifically addressed cancer of the lung and bronchus.

Update of the Scientific Literature

Occupational Studies

IARC (Kogevinas et al., 1997) has brought together almost all of the phenoxy herbicide production workers in 36 cohorts in 12 countries (Fingerhut et al., 1991; Manz et al., 1991; Sarraci et al., 1991; Flesch-Janys et al., 1995; Becher et al., 1996) for a joint analysis. This cohort contains 26,976 workers and was divided into those

who were exposed to TCDD or higher chlorinated dioxins and those who were not exposed. There were 225 lung cancer deaths in the exposed group, and 148 lung cancer deaths among unexposed workers. Lung cancer deaths were slightly elevated for the entire worker cohort, with an SMR of 1.1 (CI 1.0–1.2). No relationship was observed between incidence and years since first exposure or duration of exposure. For the TCDD-exposed group, the SMR was 1.1 (CI 1.0–1.3), whereas the non-TCDD exposed group had an SMR of 1.0 (CI 0.9–1.2). Becher et al. (1996) separately report 47 deaths from lung cancer in workers from four phenoxy herbicide plants examined as part of the IARC cohort (SMR = 1.4, CI 1.1–1.9). No association was observed between mortality and time since first exposure.

Ott and Zober (1996) updated their research on 243 male workers exposed to TCDD during the cleanup of a TCP reactor that exploded in 1953 at a BASF plant in Ludwigshafen, Germany. Dose estimates for these individuals were developed from work activity information, blood TCDD determinations on a subset of the population, and estimates of TCDD elimination rates: 11 deaths from respiratory cancers were identified during 1953–1992, 7 of them among the 69 workers with the highest estimated TCDD dose (SMR = 2.4, CI 1.0–5.0). There was also a trend of increased incidence with increased estimated dose for 8 observed cases of lung or bronchus cancer in the highest-dose group (out of eleven total) between 1960 and 1993, yielding an SIR of 2.2 (CI 1.0–4.3).

Ramlow et al. (1996) examined the mortality of 770 workers potentially exposed to PCP between 1937 and 1980. Dioxin is an unintended by-product of PCP production. Exposure was estimated on the basis of job description information and industrial hygiene characterizations of the job environment. Sixteen lung cancers were observed in the cohort, twelve of them in the higher estimated exposure group (RR = 1.1, CI 0.7–2.0).

The Svensson et al. (1995) study of Swedish fishermen reported a nonsignificant decrease in lung and larynx cancer mortality relative to the general population (SMR = 0.8, CI 0.5–1.3, based on 16 cases) in a group hypothesized to have greater exposure to PCB, PCDD, and PCDF through consumption of contaminated fish. This same group had a nonsignificantly elevated incidence of lung cancer (SIR = 1.2, CI 0.8–1.8, based on 24 cases).

Gambini et al. (1997) investigated cancer mortality among a cohort of 1,493 rice growers in northern Italy during 1957–1992. Employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides including 2,4-D and 2,4,5-T. Researchers reported an SMR for lung cancer of 0.8 (CI 0.6–1.1) based on 46 cases. Lower lung cancer mortality has also been observed in studies of farmers in the United States (e.g., Blair et al., 1993).

Environmental Studies

Studies of the population exposed to TCDD in a 1976 industrial accident in Seveso, Italy, included estimates of lung cancer risk (Bertazzi et al., 1989b;

Pesatori et al., 1992; Bertazzi, 1993; Bertazzi et al., 1997). Fifteen years of follow-up for mortality and cancer incidence demonstrated an inconsistent pattern of lung cancer rates in the different exposed groups, as well as between males and females. People most heavily exposed (those living in zone A at the time of the accident, but subsequently evacuated permanently) were too few in number (four observed lung cancer deaths in 15 years) to provide information. In zone B, the lung cancer incidence in males was slightly elevated, based on 34 observed cases (RR = 1.2, CI 0.9–1.7), and there were two observed cases among women (expected number 3.5). The largest and least contaminated area, zone R, was not found to have elevated lung cancer incidence in either males (176 observed cases, RR = 0.9, CI 0.8–1.0), or females (29 observed cases, RR = 1.0, CI 0.7–1.5).

The Seveso studies followed the population for 15 years after the accident. If the release of TCDD did increase the risk for lung cancers, one might not expect to see a full impact on tumor incidence for some years to come. At least another five years is needed before the impact of the accident on cancer incidence can be meaningfully assessed.

Vietnam Veteran Studies

Watanabe has updated his work (Watanabe and Kang, 1995,1996) comparing lung cancer rates among Army and Marine Vietnam veterans with non-Vietnam veterans. Among Army veterans, the PMR for lung cancer was 1.1 for Vietnam veterans and 1.1 for non-Vietnam veterans based on 1,139 and 1,141 lung cancer deaths, respectively. The corresponding numbers for Marines are 1.2 and 0.9 based on 215 and 77 deaths, respectively. Twenty-two U.S. Army Chemical Corps units assigned to Vietnam between 1966 and 1971 have been followed for vital status. In a review of Chemical Corps units by Dalager and Kang (1997), 11 lung cancer deaths among Vietnam units were found, compared to 3 among units not in Vietnam, for an RR of 1.4 (CI 0.4–5.4). A case-control study of lung cancer mortality in Vietnam veterans (Mahan et al., 1997) found an increased OR of 1.4 (CI 1.0–1.9) based on 111 cases.

In a study (Crane et al., 1997a) of the mortality of all Australian Vietnam service personnel compared with the general population, 212 lung cancer deaths were found with an adjusted 164 expected for an SMR of 1.3 (CI 1.1–1.5). A second study (Crane et al., 1997b) compared cases among Vietnam veterans with those who did not serve in Vietnam. Lung cancer mortality showed an RR of 2.2 (CI 1.1–4.3) based on 27 deaths in the Vietnam group.

Synthesis

Since *Update 1996*, there have been several studies of respiratory cancer among occupationally exposed groups and Vietnam veterans. Newly published

studies of phenoxy herbicide production workers (Kogevinas et al., 1997) and workers exposed as a result of an industrial accident (Ott and Zober, 1996) show small but statistically significant excesses of lung cancer mortality. Results in both studies indicate higher estimated risk for individuals with higher estimated exposure. A third study addressing workers potentially exposed to dioxins including TCDD and other congeners (Ramlow et al., 1996) reported a RR indistinguishable from 1. A study of rice farmers in Italy found lower lung cancer incidence than observed in the general population, which is similar to results found in studies of U.S. farmers and may reflect lower smoking incidence in this occupational group.

Newer data from Seveso do not indicate any increase in lung cancer mortality in this environmentally exposed group, but an insufficient number of years have passed since exposure to draw conclusions about any effect the accidental exposure may have had.

Although increases in respiratory cancers were seen in new studies of Vietnam veterans, there is evidence that cigarette smoking was more prevalent among Vietnam veterans than among non-Vietnam veterans (McKinney et al., 1997). Also, veterans smoked at a higher rate than the general public both in the United States and Australia (O'Toole et al., 1996; McKinney et al., 1997). Current studies of lung cancer risk in veterans are of limited usefulness in evaluating the effect of herbicide exposure because it is not possible to identify the soldiers likely to have been exposed to herbicides and smoking information is not available. The studies do, however, show a consistent pattern of increased lung cancer mortality among those who served in Vietnam.

In summary, the most recently published studies continue to support the placement of lung and trachea cancer in the category "limited/suggestive evidence of an association." Several studies suggest a higher rate of these cancers in individuals with known exposure to phenoxy herbicides or dioxin, and there is some evidence of a dose–response relationship. Whereas smoking undoubtedly plays a role in these cancers, the consistency of the finding across several studies argues against the notion that it is the sole explanatory factor.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to herbicides and/or dioxin and cancers of the lung/bronchus and trachea. The primary evidence comes from studies of individuals occupationally exposed to phenoxy herbicides. Although studies show an increase in lung cancer among Vietnam veterans in the United States and Australia, the lack of information regarding herbicide exposure and cigarette smoking limits their usefulness.

TABLE 7-8 Selected Epidemiologic Studies—Lung/Bronchus Cancer

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers	45	0.8 (0.6–1.1)
Kogevinas et al., 1997	Phenoxy herbicides: 36 cohorts		
	Exposed to TCDD or higher PCDD	225	1.1 (1.0–1.3)
	Exposed to no or lower PCDD	148	1.0 (0.9–1.2)
Becher et al., 1996	German chemical production workers	47	1.4 (1.1–1.9)
Ott and Zober, 1996	BASF cleanup workers	6	3.1 (1.1–6.7)
Ramlow et al., 1996	Pentachlorophenol production workers	18	1.0 (0.6–1.5)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	37	1.0 (0.7–1.4)
Blair et al., 1993	U.S. farmers in 23 states		
	White males	6,473	0.9 (0.9–0.9)
	Nonwhite males	664	1.0 (0.9–1.1)
Bloemen et al., 1993	Dow 2,4-D production workers	9	0.8 (0.4–1.5)
Kogevinas et al., 1993	Female herbicide spraying and production workers	2	1.4 (0.2–4.9)
Lynge, 1993	Danish male production workers	13	1.6 (0.9–2.8)
Studies reviewed in *VAO*			
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers	9	1.7 (0.5–6.3)
Swaen et al., 1992	Herbicide applicators	12	1.1 (0.6–1.9)
Coggon et al., 1991	Phenoxy herbicide production workers	19	1.3 (0.8–2.1)
		14	1.2 (0.7–2.1)
Fingerhut et al., 1991	TCDD-exposed workers	89	1.1 (0.9–1.4)
	≥1 year exposure; ≥20 years latency	40	1.4 (1.0–1.9)
Green, 1991	Herbicide sprayers in Ontario	5	1.1 (0.4–2.5)
Manz et al., 1991	Phenoxy herbicide production workers	26	1.7 (1.1–2.4)
Saracci et al., 1991	Herbicide spraying and production workers	173	1.0 (0.9–1.2)
	Probably exposed subgroup	11	2.2 (1.1–4.0)
McDuffie et al., 1990	Saskatchewan farmers applying herbicides	103	0.6
Zober et al., 1990	TCDD reactor accident workers	6	1.6
	High exposure	4	2.0 (0.6–5.2)
	Chloracne	6	1.8 (0.7–4.0)
Wiklund et al., 1989	Pesticide applicators in Sweden	38	0.5 (0.4–0.7)
Bond et al., 1988	Dow 2,4-D production workers (15 years latency)	9	1.2 (0.6–2.3)
	Low cumulative exposure	1	0.7
	Medium cumulative exposure	2	1.0
	High cumulative exposure	5	1.7
Coggon et al., 1986	MCPA production workers	101	1.2 (1.0–1.4)
	Background exposure	39	1.0 (0.7–1.4)
	Low-grade exposure	35	1.1 (0.8–1.6)
	High-grade exposure	43	1.3 (1.0–1.8)

TABLE 7-8 *Continued*

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
Lynge, 1985	Danish production workers		
	Males	38	1.2
	Females	6	2.2
	Manufacture and packing only—males	11	2.1 (1.0–3.7)
Blair et al., 1983	Licensed pesticide applicators in Florida, lawn and ornamental herbicides only	7	0.9 (0.4–1.9)
Axelson et al., 1980	Herbicide sprayers in Sweden	3	1.4 (0.3–4.0)
Bender et al., 1989	Herbicide sprayers in Minnesota	54	0.7 (0.5–0.9)
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	zone A—males	4	0.8 (0.2–2.1)
	zone A—females	0	0.0 (0.0–5.8)
	zone B—males	40	1.2 (0.9–1.7)
	zone B—females	2	0.5 (0.1–1.8)
	zone R—males	208	0.9 (0.8–1.1)
	zone R—females	35	1.1 (0.8–1.5)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	16	0.8 (0.5–1.3)
	West coast	77	0.9 (0.7–1.1)
Studies reviewed in *VAO*			
Bertazzi, 1993	Seveso residents first ten years after accident		
	zone A—males	2	0.8 (0.2–3.4)
	zone A—females	0	—
	zone B—males	18	1.1 (0.7–1.8)
	zone B—females	0	—
	zone R—males	96	0.8 (0.7–1.0)
	zone R—females	16	1.5 (0.8–2.5)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	212	1.3 (1.1–1.5)
Crane et al., 1997b	Australian national service veterans	27	2.2 (1.1–4.3)
Dalager and Kang, 1997	Army Chemical Corps Veterans	11	1.4 (0.4–5.4)
Mahan et al., 1997	Case-control	111	1.4 (1.0–1.9)
Watanabe and Kang, 1996	Vietnam service Army	1,139	1.1
	Non-Vietnam	1,141	1.1
	Vietnam service Marines	215	1.2
	Non-Vietnam	77	0.9
Watanabe and Kang, 1995	Vietnam service Marines v. non-Vietnam	42	1.3 (0.8–2.1)

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and cancers of the lung/bronchus and trachea is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

BONE CANCER

Background

According to the American Cancer Society, approximately 1,300 men and 1,100 women will be diagnosed with bone or joint cancer (ICD·9 170.0–170.9) in the United States in 1998, and 800 men and 600 women will die as a result of this cancer (ACS, 1998). Primary bone cancers are among the least common malignancies. The bones are, however, frequent sites for secondary tumors of other cancers that have metastasized (i.e., have spread from another site). Only the primary cancers are considered here.

Bone cancer is more common in teenagers than adults. The incidence among individuals in the age groups that characterize most Vietnam veterans is quite low, and care should be exercised when interpreting the numbers presented below.

Among the risk factors for adults contracting bone and joint cancer are exposure to ionizing radiation from treatment for other cancers and a history of certain noncancerous bone diseases.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Bone and Joint Cancer

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	1.0	1.1	0.3	0.8	0.8	0.4	1.2	1.2	1.1
females	0.5	0.5	0.5	0.6	0.6	0.4	0.7	0.6	0.4

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Studies of bone cancer and herbicide exposure have included chemical production workers (Coggon et al., 1986; Bond et al., 1988; Zober et al., 1990; Fingerhut et al., 1991; Collins et al., 1993); agricultural workers (Burmeister, 1981; Wiklund, 1983; Ronco et al., 1992; Blair et al., 1993); and Vietnam veterans (Lawrence et al., 1985; Anderson et al., 1986a,b; Breslin et al., 1988). Very few of these studies reported more than a handful of cases, typically resulting in very wide confidence intervals, and there was little evidence of elevated risk from any single study. Point estimates of risk are relatively evenly distributed on both sides of the "no-effect" estimated risk of 1.0.

Update of the Scientific Literature

Occupational Studies

The most important new study is of the IARC combined occupational cohorts (Kogevinas et al., 1997). This study found five cases of bone cancer and an SMR of 1.2 (CI 0.4–2.8) for all workers, with the SMR lower (1.1) in those exposed to TCDD than in those not exposed (1.4). Ramlow and colleague's (1996) study of 770 pentachlorophenol workers reported no deaths from bone cancer. A study of rice growers in northern Italy (Gambini et al., 1997) identified one death. Hertzman's study of 26,000 Canadian sawmill workers presumptively exposed to dioxin-contaminated chlorophenate (Hertzman et al., 1997) reported five bone cancer deaths, with an SMR of 1.3 (CI 0.5–2.7). The SIR, based on four cases, was 1.1 (CI 0.4–2.4). These ratios were based on outcomes determined by record linkage to national data bases.

Environmental Studies

In Bertazzi and colleague's continued follow-up of individuals exposed to TCDD as the result of a 1976 industrial accident near Seveso, Italy (Bertazzi et al., 1997), two deaths occurred in men from the lowest exposure zone, zone R (SMR = 0.5). Among women, one death occurred in contaminated zone B and seven in zone R (SMR = 2.4, CI 1.0–4.9).

Vietnam Veteran Studies

No bone cancer results are reported in any of the DVA Vietnam veteran studies (Dalager et al., 1995a; Watanabe and Kang, 1995, 1996). The most recent study of Ranch Hands (Air Force Health Study, 1996) reports no bone cancer deaths. The Australian veteran studies (Crane et al., 1997a,b) do not specifically report bone cancer outcomes. Only Clapp's update of his study of Massachusetts veterans reports bone cancer outcomes: four cases observed, with an OR of 0.9 (CI 0.1–11.3), comparing Vietnam veterans to Vietnam era veterans who did not serve in Vietnam. No bone cancer results are reported in any of the other Vietnam veteran studies.

Synthesis

There is minimal new information on this very rare disease, for which few data existed before. The new studies do not change the conclusions of *VAO* and *Update 1996* concerning bone cancer.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and bone cancer. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and bone cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

Chondrosarcomas of the Skull

The DVA asked the committee responsible for this report to give special attention to the issue of classification of chondrosarcomas of the skull. "Chondrosarcoma" is the general name for a class of neoplasms derived from cartilage cells or their precursors. Most chondrosarcomas develop in the interior of the bone or on the bone surface. Certain rare chondrosarcomas involve soft tissue.

The studies used by the committee in its consideration of the effects of herbicide or dioxin exposure relied on ICD·9 codes to classify health outcomes. ICD·9 code 170 is the general classification for "malignant neoplasms of bone and articular cartilage." More specifically, chondrosarcomas arising in the skull, mandible, or vertebral column are classified under ICD·9 codes 170.0–170.2, the same codes used for bone cancers at these sites. Soft-tissue sarcomas are classified under ICD·9 codes 171.0–171.9 and 164.1 (heart neoplasms). The definition of code 171, "malignant neoplasm of connective and other soft tissue," explicitly excludes tumors involving articular cartilage but includes cartilage in areas such as the eye and ear.

The committee did not identify any studies that specifically examined herbicide or dioxin exposure and chondrosarcoma incidence. The classifications discussed above mean that any association between herbicide or dioxin exposure and skull chondrosarcomas will be subsumed under the discussion of bone cancer. However, this epidemiologic classification is not intended to be a substitute for the expert judgment of pathologists in individual cases.

SOFT-TISSUE SARCOMAS

Background

The American Cancer Society estimates that 3,700 men and 3,300 women will be diagnosed with soft-tissue sarcoma (STS) (ICD·9 171.0–171.9, 164.1) in

TABLE 7-9 Selected Epidemiologic Studies—Bone Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers	1	
Hertzman et al., 1997	British Columbia sawmill workers		
	Mortality	5	1.3 (0.5–2.7)
	Incidence	4	1.1 (0.4–2.4)
Kogevinas et al., 1997	IARC cohort	5	1.2 (0.4–2.8)
	Workers exposed to TCDD (or higher chlorinated dioxins)		1.1
	Workers not exposed to TCDD (or higher chlorinated dioxins)		1.4
Ramlow et al., 1996	Pentachlorophenol production workers	0	
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states	49	1.3 (1.0–1.8)
Collins et al., 1993	Monsanto 2,4-D production workers	2	5.0 (0.6–18.1)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farm workers	9	0.9
Fingerhut et al., 1991	NIOSH cohort	2	2.3 (0.3–8.2)
Zober et al., 1990	BASF production workers	0	— (0.0–70.0)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0.0–31.1)
Coggon et al., 1986	British MCPA production workers	1	0.9 (0.0–5.0)
Wiklund, 1983	Swedish agricultural workers	44	1.0 (0.6–1.4)[b]
Burmeister, 1981	Farmers in Iowa	56	1.1 (NS)
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso		
	Men in zone R	2	0.5
	Women in zone B	1	— (0.0–14.4)
	Women in zone R	7	2.4 (1.0–4.9)
VIETNAM VETERANS			
New Studies			
Clapp, 1997	Massachusetts Vietnam veterans	4	0.9 (0.1–11.3)
AFHS, 1996	Ranch Hands	0	
Studies reviewed in *VAO*			
Breslin et al., 1988	Army Vietnam veterans	27	0.8 (0.4–1.7)
	Marine Vietnam veterans	11	1.4 (0.1–21.5)
Anderson et al., 1986a	Wisconsin Vietnam veterans	1	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	1	—
Lawrence et al., 1985	New York Vietnam veterans	8	1.0 (0.3–3.0)

NOTE: NS = not significant.
[a] Given when available.
[b] 99% CI.

the United States in 1998 and that 2,000 men and 2,300 women will die from these cancers (ACS, 1998). STSs arise in the soft somatic tissues that occur within and between organs. Three of the most common types of STS—liposarcoma, fibrosarcoma, and rhabdomyosarcoma—occur in similar numbers in men and women. Because of the diverse characteristics of STS, accurate diagnosis and classification can be difficult.

There is no consistent pattern to the incidence of STSs over the age groups that describe most Vietnam veterans.

Among the risk factors for these cancers are exposure to ionizing radiation from treatment for other cancers and certain inherited conditions including Gardner's syndrome, Li-Fraumeni syndrome, and neurofibromatosis. Several chemical exposures have also been identified as possible risk factors (Zahm and Fraumeni, 1997).

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Soft-Tissue Sarcomas (including malignant neoplasms of the heart)

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	3.0	2.9	3.1	3.5	3.8	2.6	4.7	4.0	7.5
females	2.1	1.7	4.4	2.4	2.3	3.6	2.9	2.8	3.0

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

The strongest evidence for an association between STS and exposure to phenoxy herbicides comes from a series of case-control studies conducted in Sweden (Hardell and Sandstrom, 1979; Eriksson et al., 1981, 1990; Hardell and Eriksson, 1988). The studies, involving a total of 506 cases, show an association between STS and exposure to phenoxy herbicides, chlorophenols, or both. The committee concluded that although these studies have been criticized, there is insufficient justification to discount the consistent pattern of elevated risks and the clearly described and sound methods employed. The methodology and findings from these studies are reviewed in detail in *VAO*. These findings are supported by a significantly increased risk in a National Institute for Occupational Safety and Health (NIOSH) study (SMR = 9.2, CI 1.9–27.0) for 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 first exposure (Saracci et al., 1991; Kogevinas et al., 1992), using a fairly crude exposure classification. These are the two largest, as well as the most highly exposed, occupational cohorts. Some studies in other occupational, environmental, and veteran 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).

A nested case-control study of STS within the IARC multicountry worker cohort (Kogevinas et al., 1995) was done using eleven cases of STS and five controls per case. Detailed exposure reconstruction was performed for all cases of STS and a set of controls by a team of industrial hygienists who did not know case or control status (Kauppinen et al., 1994). The team estimated cumulative exposures to TCDD and numerous phenoxy herbicides and related chemicals. There were associations between STS risk and exposure to "any phenoxy herbicide," "any dioxin," and several other definitions of exposure. The authors noted that because many of the workers had multiple exposures and few had single exposures, it is difficult to conclude with confidence that the risk is more strongly associated with any specific exposure to the broad class of phenoxy acids and related compounds. There was evidence of increasing risk with increasing cumulative exposure to several agents, including TCDD and 2,4-D, a herbicide that does not contain TCDD.

Collins et al. (1993) point out that "all but one of the confirmed STS cases among more than 5000 workers in 12 plants mentioned in the Fingerhut et al. [(1991)] study occurred among the 754 persons in the [Monsanto] study" and, based on a detailed analysis of the exposure histories of STS cases, argue that TCDD is unlikely to be responsible, but that 4-aminobiphenyl may be.

Several authors reported additional years of follow-up in occupational cohort studies (Bloemen et al., 1993; Lynge, 1993; Asp et al., 1994). Lynge (1993) found the risk of STS similar to that reported in the earlier study of this cohort of Danish herbicide manufacturers (Lynge, 1985). There were 5 cases of STS observed, versus 2.5 expected (RR = 2.0, CI 0.7–4.8). When the definition of exposure was restricted to those with at least one year of work in exposed areas and a ten-year interval was applied between the start of exposure and the time during follow-up when subjects began to be at risk, there were 3 observed cases, compared to 0.5 cases expected (RR = 6.4, CI 1.3–18.7). These workers were engaged in the manufacture of 2,4-D and a related herbicide, 4-chloro-2-methylphenoxyacetic acid (MCPA), but not 2,4,5-T.

In the United States, a PCMR study was performed of farmers in 23 states, using occupational information from death certificates (Blair et al., 1993). Based on 98 deaths from STS in white male farmers, the PCMR was 0.9 (CI 0.8–1.1). The numbers of deaths due to STS were small and nonsignificant in the other racial and gender groups: nonwhite males and white and nonwhite females.

The Bertazzi et al. (1993) study of cancer incidence in Seveso, Italy, yielded results similar to those reported in earlier publications of this group (Bertazzi et al., 1989a,b; Pesatori et al., 1992). In the small, most heavily exposed group (zone A), no cases of STS were observed when the class is defined as tumors in ICD 171, "malignant neoplasms of connective and other soft tissues." There were two cases of "soft tissue sarcomas of parenchymal origin," which are not included in ICD 171 but, according to some classifications, belong in the group of tumors under consideration (Mack, 1995). It is difficult to evaluate this finding,

because of the problem of estimating a comparable expected incidence for the same tumors, but the authors noted that 1.4 cases would be expected in this cohort when cancers including ICD 171 and cancers of parenchymal origin are combined. In the larger but less exposed group from zone B, no cases of ICD 171 cancers were observed, whereas about 0.5 was expected. Zone R is the largest group, with considerably lower exposures to TCDD on average. Two cases of STS (ICD 171) were observed in females (RR = 1.6, 95% CI 0.3–7.4). In males, six cases were observed, yielding an RR of 2.8 (CI 1.0–7.3). There appeared to be a trend in increasing risk with increasing duration of residence in zone R.

A PMR study that examined the causes of death among veterans on the state of Michigan's Vietnam era bonus list was recently reported (Visintainer et al., 1995). The mortality rates of 3,364 Vietnam veterans were compared to the mortality rates of 5,229 veterans who served elsewhere. Based on eight deaths from STS, the PMR was 1.1 (CI 0.5–2.2). No data were available to identify whether individual Vietnam veterans had been exposed to herbicides.

Update of the Scientific Literature

There have been no studies since *Update 1996* that focused exclusively on the STS–TCDD relationship, although it often receives special attention in studies that look at multiple cancer types. Most of the literature since *Update 1996* consists of longer-term follow-up, or expansion, of previously described cohorts. Because STS is such a rare cancer, continued follow-up typically adds few cases to the total, so most of the previously noted suggestive trends persist in the updated reports. No further studies have been done of the original Swedish cohorts that still form the strongest evidence for this connection.

Occupational Studies

As with the other cancer types, the largest occupational cohort to be studied is that in the IARC international study of 21,863 workers in Europe and the United States (Kogevinas et al., 1997). This cohort includes subjects in the 10-country cohort originally described in 1992 (Kogevinas et al., 1992), as well as several German cohorts that had been reported on separately since *Update 1996* (Becher et al., 1996; Flesch-Janys et al., 1995). The SMR for STS in all workers exposed to any phenoxy herbicide or chlorphenol was 2.0 (CI 0.9–3.8), based on nine deaths. This corresponds to an increased lifetime risk of roughly 2 per 10,000. The SMR was essentially unchanged when the cohort was restricted to workers exposed to TCDD or higher chlorinated dioxins, although this was based on six deaths (CI 0.8–4.4). Workers not exposed to TCDD had an SMR of 1.35, based on two deaths. When the six deaths were analyzed by duration of exposure, there was an apparent trend, with SMRs of 1.2, 4.8, and 6.5 being reported for the durations 1–4 years, 5–9 years, and 10–19 years. However, the authors of this

study also noted that a diagnosis of STS based on death certificates is known to be inaccurate and that two of the deaths reported were not STS when the slides were reviewed. However, they noted that in several cohorts, a few new cases of STS were also discovered when death records were examined intensively. The authors decided to rely on the death certificate-based numbers but warned that their results for STS should be "interpreted with caution."

In a study of 770 pentachlorophenol workers (Ramlow et al., 1996), there were no deaths from STS (0.2 expected). In Ott and Zober's continued follow-up of workers exposed to TCDD in a 1953 industrial accident (Ott and Zober, 1996), no cases of STS have been observed (0.23 expected). In a study of Canadian sawmill workers (Hertzman et al., 1997), 11 deaths from STS were observed, with an SMR of 1.0 (CI 0.6–1.7). Based on the same number of cases, the SIR was 1.0 (CI 0.6–1.7). No dose–response trend was seen between increasing hours of exposure and either mortality or incidence.

Environmental Studies

All other studies of possible herbicide exposure have involved environmental exposures due either to accidents or occupation (e.g., farmers). In Gambini's study of owners of 1,493 rice-growing farms (Gambini et al., 1997), there was one STS death (0.25 expected). In Bertazzi's continued follow-up of the Seveso accident (Bertazzi et al., 1997), four STS deaths were reported (2.1 expected), all among males in the lowest-exposure zone. In this subgroup, the SMR was 2.1 (CI 0.6–5.4). Svensson's study of cancer incidence and mortality among approximately 11,400 Swedish fishermen (Svensson et al., 1995), with presumably a high intake of organochlorine chemicals in the fatty fish they consume found three STS cases (7.5 expected).

Veterans Studies

Several studies of Vietnam veterans have appeared since *Update 1996*. In a study of approximately 2,800 Army Chemical Corps soldiers who went to Vietnam (Dalager and Kang, 1997), STS is not included in the seven types of cancer outcomes reported. With 36 observed cancer deaths, it is highly likely that no STS cases occurred. Watanabe and Kang (1996) did a PMR analysis of more than 60,000 Vietnam era veterans. When Vietnam veterans were compared to non-Vietnam veterans, PMRs near or below 1.0 were observed (based on 59 total deaths). Compared to the U.S. population, a nonstatistically significant PMR of about 1.2 was observed among Vietnam veterans. However, it must be emphasized that this analysis was not of risk of death due to STS, but rather of risk of STS given that death had occurred. A study of 10,716 U.S. Marines who served in Vietnam did not report any STS figures (Watanabe and Kang, 1995). The most recent update of the Ranch Hand study (AFHS, 1996) reported one death from

STS (0.3 expected). Clapp's update of Massachusetts veterans (Clapp, 1997), which previously showed a significant threefold STS excess (Clapp et al., 1991), now reports a nonsignificant OR of 1.6 (CI 0.5–5.4), based on 18 cases.

An Australian study of Vietnam veterans showed no excess in the number of deaths from STS in any service during 1980–1994, with the number of observed deaths ranging from zero to nine in various services and most SMRs <1 (Crane et al., 1997a). A companion study comparing conscripted Australian veterans to military personnel who did not serve in the conflict reported a nonstatistically significant RR of 0.7, based on four deaths among Vietnam veterans and two in the comparison population between 1982 and 1994 (Crane et al., 1997b). Neither of these studies characterized veterans' herbicide exposure.

Synthesis

The bulk of the evidence supporting the STS–dioxin connection still derives from the early Swedish studies. These are somewhat supported by subsequent studies of NIOSH and IARC occupational cohorts, although weaknesses in these studies limit the confidence with which conclusions can be drawn from them. Because soft-tissue sarcomas are rare, it is difficult to discern whether the small increases in the number of cases observed in these studies are due to dioxin

TABLE 7-10 Selected Epidemiologic Studies—All (or unspecified) Skin Cancer Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
Studies reviewed in *VAO*			
Fingerhut et al., 1991	NIOSH cohort	4	0.8 (0.2–2.1)
Saracci et al., 1991	IARC cohort	3	0.3 (0.1–0.9)
Alavanja et al., 1988	USDA agricultural extension agents	5	1.1 (0.5–2.6)
Burmeister, 1981	Farmers in Iowa	105	1.1 (NS)
VIETNAM VETERANS			
New Studies			
Dalager and Kang, 1997	Army Chemical Corps veterans	4	1.5 (0.3–8.6)
Watanabe and Kang, 1996	Army Vietnam veterans	234	1.0
	Marine Vietnam veterans	73	1.3 (1.0–1.6)
Studies reviewed in *VAO*			
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.

exposure or are simply random occurrences. The difficulty of diagnosing this condition also increases the concern that low rates could be appreciably affected by a small number of misclassifications among the vast majority of cancer cases that are not STS. This concern was born out in the IARC cohort, in which, upon review of pathology specimens, two of nine cases were judged not to have STS. There is no evidence that Vietnam veterans from the United States or Australia have experienced an elevated rate of this disease.

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 data available did not permit the committee to determine whether specific forms of STS are or are not associated with TCDD and/or herbicides. Therefore, the committee's findings relate to the class of STS as a whole. Also, because of problems in defining exposure to herbicides in most occupational settings, as well as the difficulty of diagnosing STS, the rarity of the disease, and the restriction of the strongest findings to one country, the committee will continue to closely monitor any substantive new evidence that is relevant to this relationship.

Conclusions

Strength of Evidence in Epidemiologic Studies

No new evidence has been produced to change the committee's earlier judgment that evidence is sufficient to conclude that a positive statistical association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and soft-tissue sarcoma.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and soft-tissue sarcoma is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

SKIN CANCERS

Skin cancers are generally divided into two broad categories, those neoplasms that develop from melanocytes (malignant melanoma) and those that do not. The common nonmelanocytic skin cancers, which include basal cell and squamous cell carcinomas, have a far higher incidence rate than malignant melanoma but are considered less aggressive and therefore more treatable. In *VAO* and *Update 1996*, all skin cancers were assessed together. However, with this review, the committee has decided to address studies assessing the health risk associated with malignant melanoma (Tables 7-12 and 7-13) separately from those assessing

the risk associated with nonmelanocytic cancers (basal and squamous cell carcinoma) (Tables 7-14 and 7-15). Because nonmelanocytic cancers are highly treatable, studies of these cancers have been divided further into those that discuss mortality and those that discuss incidence. Many studies report results by combining all types of skin cancers or do not specify the type of skin cancers assessed. These are also reported here in the interest of completeness (Tables 7-10 and 7-11).

Background

According to American Cancer Society estimates, 24,300 men and 17,300 women will be diagnosed with melanoma (ICD·9 172.0–172.9) in the United States in 1998, and 4,600 men and 2,700 women will die of this cancer (ACS, 1998). Approximately 1,000,000 cases of nonmelanocytic skin cancers (ICD·9 173.0–173.9), primarily basal cell and squamous cell carcinomas, are diagnosed in the United States each year (ACS, 1998). The American Cancer Society estimates that 1,200 men and 700 women will die from these diseases in 1998.

Skin cancers are far more likely to occur in fair-skinned individuals; the risk for whites is roughly 20 times that for dark-skinned African Americans. Incidence also increases with age, although more strikingly for males than females. Other risk factors for melanoma include the presence of certain moles on the skin, a suppressed immune system, and excessive exposure to ultraviolet (UV) radiation, typically from the sun. A family of history of the disease has been identified

TABLE 7-11 Selected Epidemiologic Studies—All (or unspecified) Skin Cancer Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Ott and Zober, 1996	German BASF trichlorophenol production workers	5	1.2 (0.4–2.8)
Studies reviewed in *VAO*			
Hansen et al., 1992	Danish gardeners	32	1.1 (0.8–1.6)
Lynge, 1985	Danish male production workers	14	0.7
Suskind and Hertzberg, 1984	Monsanto production workers	8	1.6
VIETNAM VETERANS			
Studies reviewed in *VAO*			
Wolfe et al., 1990	Air Force Ranch Hand veterans	88	1.5 (1.1–2.0)
CDC, 1988	Army enlisted Vietnam veterans	15	0.8 (0.4–1.7)

NOTE: NS = not significant.
[a] Given when available.

as a risk factor, but it is unclear whether this is due to genetic factors or to similarities in skin type and sun exposure patterns.

Excessive exposure to UV radiation is the single most important risk factor for nonmelanocytic skin cancers. Certain skin diseases and chemical exposures have also been identified as potential risk factors. SEER incidence data are not available for nonmelanocytic skin cancers.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Melanomas of the Skin

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	23	25	1	27	31	2	35	40	2
females	19	22	[b]	18	21	0.4	20	23	3

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.
[b] Insufficient data to provide a meaningful incidence rate.

MELANOMA

Summary of *VAO* and *Update 1996*

A number of mortality studies of agricultural workers showed either a deficit or no excess in skin cancer mortality (Wiklund, 1983; Blair et al., 1993; Asp et al., 1994). A study of Vietnam veterans with 181 cases of melanoma also showed either a deficit or no excess in skin cancer mortality among Army and Marine veterans (Breslin et al., 1988). One mortality study of environmental exposures with very few cases (N = 3) showed nonsignificant excesses of skin cancer (Bertazzi et al., 1989a).

If most of the deaths reported in Table 7-10, where all skin cancers are combined or where the type of skin cancer is unspecified, are assumed to be due to melanoma, then there are a few additional studies to examine. Two occupational cohorts with TCDD exposures showed deficits in melanoma deaths (Fingerhut et al., 1991; Saracci et al., 1991). Two studies of farm-related occupations showed small (RR = 1.1), nonsignificant increases in risk (Burmeister, 1981; Alavanja et al., 1988). The two Wisconsin Vietnam veteran studies reported, on one hand, a statistically nonsignificant elevation (RR = 1.3) and, on the other hand, a deficit (RR = 0.9) in melanoma based on five to six cases (Anderson et al., 1986a,b).

In terms of cancer incidence, a deficit of skin cancer cases was found among Danish farmers (Ronco et al., 1992). A study of Danish phenoxy herbicide and chlorophenol production workers showed a statistically significant excess of melanoma. This result, based on four cases, was observed in the subgroup of men who had been employed for at least one year, using a ten-year latency period (SIR = 4.3, CI 1.2–10.9) (Lynge, 1985). In addition, Air Force Ranch Hands had

four cases of melanoma, resulting in increased, but nonsignificant risk (SIR = 1.3, CI 0.3–5.2).

Update of the Scientific Literature

Mortality Studies

Since *Update 1996*, several studies of mortality in relevant cohorts have been published. Some of these are updates of previously reported cohorts. For example, the Seveso, Italy, cohort has been updated (Bertazzi et al., 1997). For zones A, B and R the only increased risk from melanoma was for zone A females based on only case (RR = 9.4, CI 0.1–52.3).

The IARC multinational cohort (Kogevinas et al., 1997) of production workers exposed to phenoxy herbicides, chlorophenols, and dioxins reported no increased risk of malignant melanoma among workers exposed to phenoxy herbicides contaminated with TCDD or higher chlorinated dioxins (SMR = 0.5, CI 0.2–3.2), or among workers with exposure to phenoxy herbicides and chlorophenols but minimal or no exposure to TCDD or higher chlorinated dioxins (SMR = 1.0, CI 0.3–2.4). These subcohorts had four or five cases of melanoma each.

New studies reviewed by the committee include a study of sawmill workers exposed to higher chlorinated PCDD's in chlorophenate wood preservatives. The reported SMR for melanoma in this cohort was 1.4 (CI 0.9–2.0) (Hertzman et al., 1997). A study of Swedish fishermen found a decreased risk of death from melanoma compared to the general Swedish population (Svensson et al., 1995). The fishermen included those from the west coast who reportedly ate lean fish with potentially higher arsenic contamination, and those from the east coat (Baltic) who consumed fatty fish with potentially higher levels of TCDD and other persistent organochlorine compounds. No actual measurements of TCDD or arsenic in the fish or fishermen were made.

The Australian veterans study (Crane et al., 1997a), which compared all Vietnam veterans to the Australian population, reported an elevated melanoma mortality for 1980–1994 (SMR = 1.3, CI 1.0–1.8). When the reference group was non-Vietnam veterans (Crane et al., 1997b), melanoma mortality for 1982–1994 was not elevated (RR = 0.5, CI 0.2–1.3).

Unfortunately, two of the new studies of Vietnam veterans either combined all skin cancer cases or did not specify the type of skin cancer studied. If it is assumed that most of these cancer deaths were due to melanoma, then these studies should be included in this review. A study of the Army Chemical Corps, the group responsible for spraying herbicides by helicopter and around base camp perimeters, found an elevated skin cancer risk of 1.5 (CI 0.3–8.6). This result was based on four cases and used non-Vietnam-based Chemical Corps veterans as the reference group (Dalager and Kang, 1997). When the U.S. male population was used as a reference group, the risk was 2.6 (CI 0.7–6.7).

Watanabe and Kang (1996) conducted a proportional mortality study of Vietnam veterans and found that Marine Vietnam veterans had a significantly higher mortality from all skin cancers compared to Marine non-Vietnam veterans (PMR = 1.3, CI 1.0–1.6), all non-Vietnam veterans (PMR = 1.3, CI 1.0–1.7) and the U.S. male population (PMR = 1.3, CI >1.0). Although control groups were presumably less exposed to Agent Orange and herbicides, no significant differences were observed between Army Vietnam veterans and any control group. Both Army and Marine veterans showed a trend toward higher PMRs with increased latency (i.e., time from last year in Vietnam to year of death).

Incidence

New studies include a study of sawmill workers exposed to higher chlorinated PCDDs that contaminate chlorophenate wood preservatives. The reported SIR for melanoma in this cohort was 1.0 (CI 0.7–1.2) (Hertzman et al., 1997). Also, the study of Swedish fishermen found no elevated incidence of melanoma compared to the general Swedish population (Svensson et al., 1995).

Among Vietnam veterans, there was a follow-up study of cancer incidence among Massachusetts Vietnam veterans. When Massachusetts Vietnam veterans were compared to other Vietnam era veterans, the authors reported an OR of 1.4 (CI 0.7–2.9) for melanoma, based on 21 cases (Clapp, 1997).

Synthesis

Known etiologic agents associated with melanoma include UV radiation or sunlight and polyaromatic hydrocarbons (PAHs). Cytochrome P4501A1 (CYP1A1), a P450 isozyme, under the control of the aryl hydrocarbon receptor (AhR), is expressed in the skin. It has been linked to the development of skin cancer and skin sensitization by PAHs (Gonzalez et al., 1996). Since TCDD toxicity is also mediated by the AhR, it is plausible that exposure to TCDD could be associated with increased risk of melanoma.

The epidemiologic data are not strong in this area. To date, only three studies show statistically significant increases in melanoma mortality. These include a study of phenoxy herbicide production workers, with four cases of melanoma (Lynge, 1985), and two Vietnam veteran studies. The Australian Vietnam veteran study had an SMR of 1.3 (CI 1.0–1.8) (Crane et al., 1997a). This increased risk was not found when the reference group was non-Vietnam veterans (Crane et al., 1997b). In the United States, a study of Marine and Army veterans found that only Marine veterans had an increased risk of melanoma (PMR = 1.3, CI 1.0–1.6) (Watanabe and Kang, 1996). None of these studies controlled for the greatest known risk factor for melanoma, sunlight exposure. Therefore the committee encourages future studies of the melanoma risk of occupational and Vietnam veteran populations to make an effort to control confounding from UV (sunlight) exposures.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and melanoma. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

TABLE 7-12 Selected Epidemiologic Studies—Melanoma Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Hertzman et al., 1997	Sawmill workers	17	1.4 (0.9–2.0)
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	5	0.5 (0.2–3.2)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	4	1.0 (0.3–2.4)
Svensson et al., 1995	Swedish fisherman		
	East coast	0	0.0 (0.0–1.73)
	West coast	6	0.7 (0.2–1.5)
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states (white males)	244	1.0 (0.8–1.1)
Studies reviewed in *VAO*			
Wigle et al., 1990	Saskatchewan farmers	24	1.1 (0.7–1.6)
Wiklund, 1983	Swedish agricultural workers	268	0.8 (0.7–1.0)[b]
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	Males zone R	3	1.1 (0.2–3.2)
	Females zone R	3	0.6 (0.1–1.8)
Studies reviewed in *VAO*			
Bertazzi et al., 1989a	Seveso male residents—zones A, B, R	3	3.3 (0.8–13.9)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	51	1.3 (1.0–1.8)
Crane et al., 1997b	Australian national service veterans	16	0.5 (0.2–1.3)
Studies reviewed in *VAO*			
Breslin et al., 1988	Army Vietnam veterans	145	1.0 (0.9–1.1)
	Marine Vietnam veterans	36	0.9 (0.6–1.5)

NOTE: NS = not significant.
[a] Given when available.
[b] 99% CI.

TABLE 7-13 Selected Epidemiologic Studies—Melanoma Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Hertzman et al., 1997	Sawmill workers	38	1.0 (0.7–1.2)
Svensson et al., 1995	Swedish fisherman		
	East coast	0	0 (0.0–0.7)
	West coast	20	0.8 (0.5–1.2)
Studies reviewed in *Update 1996*			
Lynge, 1993	Danish male production workers	4	4.3 (1.2–10.9)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish self-employed farmers	72	0.7 ($p < .05$)
VIETNAM VETERANS			
New Studies			
Clapp, 1997	Massachusetts Vietnam veterans	21	1.4 (0.7–2.9)
Studies reviewed in *VAO*			
Wolfe et al., 1990	Air Force Ranch Hand veterans	4	1.3 (0.3–5.2)

NOTE: NS = not significant.
[a] Given when available.

Biologic Plausibility

Known etiologic agents associated with melanoma include UV radiation or sunlight and PAHs. CYP1A1, a P450 isozyme under the control of the AhR, is expressed in the skin and has been linked to the development of skin cancer and skin sensitization by PAHs (Gonzalez et al., 1996). Since TCDD toxicity is also mediated by the AhR, it is plausible that TCDD exposures could be associated with increased risk of melanoma. A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and melanoma is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

BASAL AND SQUAMOUS CELL (NONMELANOMA) SKIN CANCER

Summary of *VAO* and *Update 1996*

Although the mortality from nonmelanoma skin cancer is said to be quite low, because of the highly successful treatment regimes available, several studies have reported cancer risk for the ICD·7 191 or ICD·8/ICD·9 173, the codes for other (nonmelanoma) skin cancers. Two of the mortality studies previously reviewed were of agricultural workers presumed to be exposed to herbicides. A large study of Swedish farmers, with 708 cases, found an SMR of 1.1 (99% CI

1.0–1.2) (Wiklund, 1983). In addition, a study of farmers in 23 U.S. states found a significantly elevated risk of nonmelanoma skin cancers in white male farmers with a PCMR of 1.1 (CI 1.0–1.2) based on 425 deaths (Blair et al., 1993). Although these were large studies, the strength of the association was weak and no verification of exposure status with regard to herbicides was done. Likewise, there was no controlling for confounding from UV (sunlight) exposure.

Since nonmelanocytic skin cancers are highly curable, studies of cancer incidence may be more helpful than mortality studies in evaluating the risk of basal and squamous cell skin cancers. A study of Danish farmers showed significantly reduced risk of these "other" skin cancers (Ronco et al., 1992). In the Seveso cohort, a small number of cases have been identified, resulting in an increased risk in some analyses but no statistically significant finding (Pesatori et al., 1992; Bertazzi et al., 1993).

The strongest information in *VAO* and *Update 1996* comes from the Air Force Ranch Hand study, which examined Vietnam veterans responsible for aerial herbicide spraying (Wolfe et al., 1990). Ranch Hands were compared to non-Ranch Hand Air Force air cargo personnel serving in Southeast Asia. Wolfe and colleagues found an OR of 1.5 (CI 1.1–2.0) for skin cancer of all types, which they called "sun-exposure neoplasms" (basal and squamous cell carcinomas, melanoma, and malignant epithelial neoplasms not otherwise specified). The Ranch Hand study also reported an OR of 1.5 (CI 1.0–2.1) for basal cell carcinoma alone. Squamous cell carcinoma had an elevated but nonsignificant OR of 1.6 (CI 0.5–5.1) due to the small number of cases ($N = 6$). To control for confounding by sun exposure, in addition to using a Vietnam-based reference group, these analysis were adjusted for two sun reaction indices and average residential latitude.

A number of studies reported results with all skin cancers combined or did not specify the type of skin cancer (Tables 7-10 and 7-11). If most of these incident cases are assumed to be the more common basal or squamous cell carcinomas, then such studies should be considered in this review. For example, the CDC (1988) studied Army personnel who served in Vietnam compared to non-Vietnam servicemen and found an OR of 0.8 (CI 0.5–1.2) for skin cancer prevalence on exam.

Two of the three occupational studies examining all types of skin cancer combined or unspecified skin cancer reported a nonstatistically significant increase in the incidence of skin cancer. The risk of skin cancer was elevated, but no confidence interval was given for the Nitro, West Virginia, work force involved in manufacturing 2,4,5-T (Suskind and Hertzberg, 1984). Hansen et al. (1992) found increased incidence of skin cancer among Danish gardeners (standardized morbidity ratio [SMbR] = 1.3 for males, CI 0.9–1.8). Studies of two phenoxy herbicide plants in Denmark found no statistically significant increase in skin cancer among exposed production workers (Lynge, 1985).

Update of the Scientific Literature

Mortality Studies

Since *Update 1996*, several mortality studies of relevant cohorts have been published. The IARC multinational cohort (Kogevinas et al., 1997) of workers exposed to phenoxy herbicides, chlorophenols, and dioxins reported an SMR of 1.2 (CI 0.3–3.2) for nonmelanocytic skin cancer among workers exposed to phenoxy herbicides contaminated with TCDD or higher chlorinated dioxins, based on four cases. There were no cases of skin cancer among those with minimal or no exposure to TCDD or higher chlorinated dioxins.

Svensson and colleagues (1995) reported a study of Swedish fishermen that compared those from the west coast, who were reported to eat lean fish containing potentially higher arsenic contamination, and those from the east coat (Baltic), who consumed fatty fish containing potentially higher levels of TCDD and other organochlorine compounds (Svensson et al., 1995). They report a nonsignificantly elevated risk of death from nonmelanoma skin cancers for west coast fishermen, compared to the general Swedish population (SMR = 3.1, CI 1.0–7.1). No cases were reported for east coast fishermen. No actual measurements of TCDD or arsenic in the fish or fishermen were made.

Cancer Incidence

The study of Swedish fishermen also reported a statistically significant increase in the incidence of nonmelanocytic skin cancer (RR = 2.3, CI 1.4–3.5) among east coast fisherman who ate more fatty fish containing potentially higher levels of TCDD and other organochlorine compounds. West coast fisherman had a nonsignificant elevation in skin cancer incidence (RR = 1.1, CI 0.9–1.4).

Zhong and Rafnsson (1996) reported on the cancer incidence among Icelandic pesticide users. This cohort included individuals who were allowed to buy and handle toxic chemicals including pesticides. Both 2,4,5-T and 2,4-D were used in Icelandic agriculture during the study period. Based on five cases of nonmelanocytic skin cancer, an nonstatistically significant elevation in skin cancer incidence was reported (SIR = 2.8, CI 0.9–6.6).

The strongest recent study in this area was a community case-control study of squamous cell and basal cell (nonmelanocytic) carcinoma, undertaken in Alberta, Canada (Gallagher et al., 1996). The study used a questionnaire to evaluate more than 50 different potential exposures. After adjustment for age, skin and hair color, and mother's ethnic origin, elevated risks for squamous cell carcinoma were seen in subjects ever exposed at work, at home, or by hobby to herbicides (OR = 1.5, CI 1.0–2.3); fungicides or seed treatments (OR = 1.4, CI 0.9–2.10); and insecticides (OR = 1.7, CI 1.1–2.7). Other exposures associated with a statistically significant increase in the risk of squamous cell carcinoma include coal

dust (OR = 1.6 CI 1.0–2.4) and diesel fumes (OR = 1.7 CI 1.1–2.5). No statistically significant associations of herbicide, fungicide, or insecticide exposure with risk of basal cell cancer were found. Exposures associated with a statistically significant increased risk of basal cell carcinoma include dry cleaning agents (OR = 4.6, CI 1.1–19.7), fiberglass dust (OR = 2.0, CI 1.1–3.9), luminous paint (OR = 6.7, CI 1.2–38.0), and asbestos dust (OR = 1.9, CI 1.0–3.5). No multivariate exposure analysis was done in this study.

For studies with a sufficient number of exposed subjects, the total duration of exposure until the time of diagnosis was weighted by the source (intensity) of exposure (direct job, workplace environment, hobby or home) and the duration of exposure (<1, 1–4, 5–19, or ≥20 hours per week). This was converted to lifetime hours of exposure to the agent, and exposed subjects were dichotomized into low and high levels of exposure based on this lifetime exposure level. After adjustment for age, skin and hair color, mother's ethnic origin and sunlight exposure in the 10 years prior to diagnosis, statistically significant trends in risk for squamous cell carcinoma were seen with increasing exposure to herbicides, fungicides, seed treatments, and insecticides. For herbicides, the low-exposure group (33 cases) had an OR of 1.9 (CI 1.0–3.6), whereas the high-exposure group (46 cases) had an OR of 3.9 (CI 2.2–6.9) with a trend test p value of <.001. For fungicides and seed treatments, the low-exposure group (40 cases) had an OR of 0.8 (CI 0.4–1.4), and the high-exposure group (56 cases) had an OR of 2.4 (CI 1.4–4.0), with a trend test p value of .003. For insecticides, the low-exposure group (21 cases) had an OR of 0.7 (CI 0.3–1.4), and the high-exposure group (36 cases) had an OR of 2.8 (CI 1.4–5.6), with a trend test p value of .02. In examining the analysis for herbicides, it was noted that the dichotomous evaluation (ever or never) produced an OR lower than that of the low-exposure category in the exposure–response analysis, which also included an adjustment for occupational sunlight exposure. The change in OR between the two analyses suggests an unexpected negative confounding between sunlight and herbicide exposure. This casts some doubt on the strength of the finding and the reliability of the herbicide exposure assessment.

Synthesis

Known etiologic agents associated with basal and squamous cell carcinoma include arsenic and UV radiation or sunlight. Cacodylic acid was an organic arsenic herbicide widely used in Vietnam. CYP1A1, a P450 isozyme, under the control of the AhR is expressed in the skin. It has been linked to the development of skin cancer and skin sensitization by polyaromatic hydrocarbons (Gonzalez et al., 1996). Since TCDD toxicity is also mediated by the AhR, it is plausible that TCDD exposures could be associated with increased risk of skin cancer.

In *VAO* and *Update 1996*, an increased risk of mortality from nonmelanoma skin cancers was found among agricultural workers. However, the strength of the association was weak and herbicide exposure was not verified. In addition, these

studies did not control for the most likely source of the slightly elevated risk, exposure to sunlight. The Air Force Ranch Hand study (Wolfe et al., 1990) was also reported in *VAO* and *Update 1996*. This study showed a small, but significant, increased incidence of basal cell carcinoma (OR = 1.5, CI 1.0–2.1). The analysis controlled for confounding by sun exposure by using a Vietnam-based reference group (non-Ranch Hand Air Force air cargo crews in Southeast Asia), as well as two personal sun reaction indices and average residential latitude.

The strongest evidence linking herbicide exposure and these other skin cancers comes from a recent community-based case-control study (Gallagher et al., 1996). This study controlled for sun exposure, skin and hair color, and mother's ethnic origin, and found increasing risk of squamous cell carcinoma with increasing lifetime exposure to herbicides. At issue in this study are the large number of different potential exposures examined (>50) and the lack of any multivariate exposure analysis. A number of these exposures might be expected to be statistically significant based on chance alone. However, the exposure–response gradients observed lend credibility to the finding. Other concerns are misclassification and bias introduced through the use of exposure interviews and the attendant exposure algorithm to estimate exposure. Since the associations with herbicides in this study were seen with squamous but not basal cell carcinomas, this argues

TABLE 7-14 Selected Epidemiologic Studies—Other Nonmelanoma (basal and squamous cell) Skin Cancer Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Hertzman et al., 1997	Sawmill workers	38	1.0 (0.7–1.2)
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	4	1.2 (0.3–3.2)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	0	—
Svensson et al., 1995	Swedish fisherman		
	East coast	0	0.0 (0–15.4)
	West coast	5	3.0 (1.0–7.1)
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states (white males)	425	1.1 (1.0–1.2)
Studies reviewed in *VAO*			
Coggon et al., 1986	British MCPA chemical workers	3	3.1 (0.6–9.0)
Wiklund, 1983	Swedish agricultural workers	708	1.1 (1.0–1.2)[b]

NOTE: NS = not significant.
[a] Given when available.
[b] 99% CI.

against exposure misclassification or bias. On the other hand, the lack of an association between herbicide exposure and basal or squamous cell cancers casts some doubt on the biologic plausibility of the association. Of note here is the conflicting finding of a significantly elevated risk of basal cell, but not squamous cell, carcinoma in the Ranch Hand study. Thus, although the committee agrees that the Gallagher et al. (1996) study is the best to date, concerns still remained regarding the control of confounding and the adequacy of exposure assessment. Therefore, the committee encourages further study of basal and squamous cell skin cancer incidence among working and Vietnam veteran populations. In any future studies, careful attention should be paid to exposure assessment, as well as to controlling for confounding from UV exposure. In addition, efforts to examine the carcinogenicity of organic arsenicals should be encouraged.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate/insufficient evidence of an association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and skin cancer. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

Known etiologic agents associated with basal and squamous cell carcinoma include arsenic and UV radiation or sunlight. Cacodylic acid was an organic arsenic herbicide used widely in Vietnam. CYP1A1, a P450 isozyme under the control of the AhR, is expressed in the skin. It has been linked to the development of skin cancer and skin sensitization by PAHs (Gonzalez et al., 1996). Since TCDD toxicity is also mediated by the AhR, it is plausible that TCDD exposures could be associated with increased risk of skin cancer. A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and skin cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

BREAST CANCER

Background

Breast cancer (ICD·9 174.0–174.9 for females) is the single most common cause of cancer among women in the United States, excluding nonmelanocytic skin cancers. The American Cancer Society estimates that 178,700 women will

TABLE 7-15 Selected Epidemiologic Studies—Other Nonmelanoma (basal and squamous cell) Skin Cancer Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Zhong and Rafnsson, 1996	Icelandic pesticide users	5	2.8 (0.9–6.6)
Svensson et al., 1995	Swedish fisherman		
	East coast	22	2.3 (1.4–3.5)
	West coast	69	1.1 (0.9–1.4)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish self-employed farmers	493	0.7 ($p < .05$)
ENVIRONMENTAL			
New Studies			
Gallagher et al., 1996	Alberta, Canada, residents—Squamous cell Carcinoma		
	All herbicide exposure	79	1.5 (1.0–2.3)
	Low herbicide exposure	33	1.9 (1.0–3.6)
	High herbicide exposure	46	3.9 (2.2–6.9)
	All fungicide exposure	96	1.4 (0.9–2.1)
	Low fungicide exposure	40	0.8 (0.4–1.4)
	High fungicide exposure	56	2.4 (1.4–4.0)
	Alberta, Canada, residents—Basal cell Carcinoma		
	All herbicide exposure	70	1.1 (0.8–1.7)
	All fungicide exposure	76	0.9 (0.6–1.3)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male residents		
	zone A	1	2.4 (0.3–17.2)
	zone B	2	0.7 (0.2–2.9)
	zone R	20	1.0 (0.6–1.6)
Studies reviewed in *VAO*			
Pesatori et al., 1992	Seveso male residents—zones A and B	3	1.0 (0.3–3.0)
	Female residents—zones A and B	3	1.5 (0.5–4.9)
VIETNAM VETERANS			
Studies reviewed in *VAO*			
Wolfe et al., 1990	Air Force Ranch Hand veterans		
	Basal cell carcinoma	78	1.5 (1.0–2.1)
	Squamous cell carcinoma	6	1.6 (0.5–5.1)

NOTE: NS = not significant.
[a] Given when available.

be diagnosed with breast cancer in the United States in 1998 and that 43,500 will die from the disease (ACS, 1998). Overall, these numbers represent approximately 30 percent of the incidence of new cancer and 16 percent of cancer deaths. Among women aged 40–55, it is the leading cause of cancer death.

Breast cancer incidence generally increases with age. In the age groups that characterize most Vietnam veterans, the incidence for whites is slightly higher than for African Americans. Risk factors other than aging include a personal or family history of breast cancer and reproductive history (specifically, early onset of menarche, late onset of menopause, and either no pregnancies or first full-term pregnancy after 30 years of age). A pooled analysis of six large-scale prospective studies of invasive breast cancer found that alcohol consumption was associated with a linear increase in incidence in women, over the range of consumption reported by most women (Smith-Warner et al., 1998). The potential role of other personal behaviors and environmental factors in breast cancer incidence is being studied extensively.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Breast Cancer in Females

45–49 years of age			50–54 years of age			55–59 years of age		
all races	white	black	all races	white	black	all races	white	black
199	203	203	245	253	217	284	291	267

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Data relating herbicide exposure to cancer among women are extremely limited. The committee has attempted to examine cancer among women separately from cancer among men. However, although the data available for men are sparse, data for women are almost nonexistent. Many studies have excluded women from analysis because of their small numbers in the groups under study. For example, in their follow-up of workers from 12 companies, Fingerhut et al. (1991) identified 67 women who were then excluded from the report. Likewise, Moses et al. (1984) excluded three women from their follow-up analysis of workers, and Zack and Suskind (1980) excluded the one woman who was living at the end of this study. Among the studies based on follow-up of workers, women contributed a minor portion of the data.

Occupational Studies

Manz et al. (1991) described a retrospective cohort study of chemical workers employed in an herbicide plant in Hamburg, Germany. The SMR, based on nine breast cancer deaths, was elevated, at 2.2 (CI 1.0–4.1). The small percentage

(7 percent) of women in this study who worked in high-exposure departments precluded separate examination of those with high exposure.

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 (contributing 17,624 person-years of follow-up) with the National Cancer Register. Thirteen cases of breast cancer were diagnosed, yielding an SMR of 0.9.

Saracci et al. (1991) reported one breast cancer death among 1,527 women employed in 11 plants with potential exposure to phenoxy herbicides or TCDD in the multicenter IARC occupational study; the mortality rate was lower than among unexposed women in this study. Additional data on morbidity in a subset (N = 701) of these women were described by Kogevinas et al. (1993), who found no excess incidence of breast cancer: seven cases resulted in an SIR of 0.9 (CI 0.4–1.9). Among those female workers exposed to chlorophenoxy herbicides contaminated with TCDD (N = 169), there was an excess overall cancer incidence, based on nine cases (SIR = 2.2, CI 1.0–4.2), with one case of breast cancer (SIR = 0.9, CI 0.0–4.8).

Among women farm workers in Denmark (Ronco et al., 1992), 429 cases of breast cancer were diagnosed, and the SIR of 0.8 was significantly less than unity. The actual level of exposure of these women to herbicides is not defined, however, and it is possible that the reduced incidence of breast cancer 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 for economically active women in Sweden (Wiklund, 1983), the SIR for breast cancer among farm workers was 0.8. This result is not adjusted for reproductive risk factors for these cancers, and the actual exposures of interest are not defined.

In the United States, a PCMR study was performed using death certificate data for male and female farmers from 23 states (Blair et al., 1993). Occupational and industry data were coded based on the information listed on death certificates. Among white female farmers, 71 deaths from breast cancer yielded a PCMR of 1.0 (CI 0.8–1.3); among nonwhite female farmers, the PCMR, based on 30 breast cancer deaths, was significantly decreased at 0.7 (0.5–1.0).

The 10-year Seveso follow-up (Bertazzi et al., 1989b) 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. Because of the small number of cancer deaths (three) in females in zone A, no conclusions 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, the least contaminated area, 28 women died from breast cancer, giving a significantly reduced estimated RR of 0.6 (CI 0.4–0.9).

In another report from Seveso (Bertazzi et al., 1993), cancer incidence during the first 10 years following exposure to TCDD was investigated. In zones A, B and R, the number of breast cancers diagnosed and the corresponding RRs, were respectively, one case in zone A (RR = 0.5, CI 0.1–3.3); 10 in zone B (RR = 0.7, CI 0.4–1.4); and 106 in zone R (RR = 1.1, CI 0.9–1.3), the least contaminated area.

Vietnam Veteran Studies

Thomas et al. (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 those for Vietnam era veterans who served elsewhere were derived from proportional hazards multivariate models adjusted for rank (officer or enlisted), occupation (nurse or nonnurse), duration of service (at least 10 years), age at entry to follow-up, and race. Of these women, 80 percent were classified as officers or nurses, and the majority served between 3 and 19 years. Slightly more than one-fourth of the cancer deaths among Vietnam veterans were due to breast cancer. The RR was not significantly elevated (RR = 1.2, CI 0.6–2.5) compared to that for other Vietnam era veterans.

Cancer mortality rates for 4,586 female Vietnam veterans were recently compared with rates for 5,325 female veterans who served elsewhere (Dalager et al., 1995a). This study extended the follow-up of Thomas et al. (1991) for four years, through 1991. There were 196 deaths observed among the Vietnam veterans. Based on 26 deaths from breast cancer among Vietnam veterans, the RR was 1.0 (CI 0.6–1.8).

Update of the Scientific Literature

Few new data have been published since *Update 1996*. The 15-year follow-up of the Seveso, Italy, population indicates no excess of breast cancer: the RRs are 0.6 for the most highly exposed (zone A), and 0.8 for the middle- and low-exposure groups (zones B and R), compared to an unexposed population. Although there was 1 case in zone A, zones B and R had 9 and 67 cases, respectively, leading to fairly stable measures of association. In particular, the 95% CI for the female breast cancer RR of 0.8 in zone R was 0.6–1.0. Thus, evidence from this study suggests a possible protective effect of TCDD exposure.

In contrast, the multinational study conducted by IARC observed an SMR of 2.2 for female breast cancers (95% CI 1.0–4.1) and 2.6 for male breast cancers (CI 0.3–9.3), for workers exposed to TCDD or higher chlorinated dioxins (Kogevinas et al., 1997). The finding for women workers was confined to women from one cohort in Germany, where the SMR was 2.8 (CI 1.3–5.4). This was the only cohort in the IARC study that had a substantial number of female production workers with exposure to TCDD or higher chlorinated dioxins.

Data from Australian Vietnam veterans also indicate an elevation of male breast cancer (Crane et al., 1997a): the SMR was 5.5 (95% CI 1.1–16.1), based on three deaths during 1980–1994. The SRMR comparing male breast cancer deaths to all other deaths was 5.2 (CI 1.04–>10.0); compared to other cancer deaths, it was 4.6.

Synthesis

Through *Update 1996*, there had been a few occupational studies, two environmental studies, and two veteran studies of breast cancer among women exposed to herbicides and/or TCDD (Table 7-16). Most of these studies reported a RR of approximately 1.0 or less, but it is uncertain whether or not female members of the cohorts had substantial chemical exposure. Further follow-up of the Seveso cohort indicates, if anything, a protective effect. However, an expanded multinational occupational study found an increased risk of breast cancer in both males (nonsignificant) and females (significant). This cohort may have been the only occupational one, among those in which breast cancer was analyzed, that had substantial exposure to TCDD. Also notable is the finding, both in this occupational cohort and among Australian Vietnam veterans, of an elevated risk of male breast cancer, despite its rarity. In contrast, TCDD appears to exert a protective effect on the incidence of mammary tumors in experimental animals (see Chapter 3), which is consistent with the tendency for RRs to be less than 1.0. In summary, data continue to be inconclusive regarding whether an association exists between exposure to the herbicides used in Vietnam and the occurrence of breast cancer.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and breast cancer. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

In laboratory animals, TCDD has been shown to have a wide range of effects on growth, hormone systems, and other factors associated with the regulation of activities in normal cells. Because animal data suggest that TCDD may act as an antiestrogen, and because it has been shown to inhibit the growth of breast cancer cell lines in tissue culture, the extrapolation to prevention of breast cancers in

TABLE 7-16 Selected Epidemiologic Studies—Breast Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort, female; identical to Manz et al., 1991	9	2.2 (1.0–4.1)
	IARC cohort, male	2	2.6 (0.3–9.3)
Studies reviewed in *Update 1996*			
Blair et al., 1993	Female U.S. farmers in 23 states		
	Whites	71	1.0 (0.8–1.3)
	Nonwhites	30	0.7 (0.5–1.0)
Kogevinas et al., 1993	Female herbicide spraying and production workers	7	0.9 (0.4–1.9)
	Probable exposed to TCDD	1	0.9 (0.0–4.8)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish family farm workers	429	0.8 ($p < .05$)
Manz et al., 1991	German production workers	9	2.2 (1.0–4.1)
Saracci et al., 1991	IARC cohort	1	0.3 (0.0–1.7)
Lynge, 1985	Danish production workers	13	0.9
Wiklund, 1983	Swedish agricultural workers	444	0.8 (0.7–0.9)[b]
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso female residents, mortality		
	zone A	1	0.6 (0.0–3.1)
	zone B	9	0.9 (0.4–1.5)
	zone R	67	0.8 (0.6–1.0)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso female residents; follow-up of Bertazzi et al., 1989b		
	zone A	1	0.5 (0.1–3.3)
	zone B	10	0.7 (0.4–1.4)
	zone R	106	1.1 (0.9–1.3)
Studies reviewed in *VAO*			
Bertazzi et al., 1989b	Seveso female residents		
	zone B	5	0.9 (0.4–2.1)
	zone R	28	0.6 (0.4–0.9)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	3	5.5 (1.1–16.1)
Studies reviewed in *Update 1996*			
Dalager et al., 1995a	Women Vietnam veterans	26	1.0 (0.6–1.8)
Studies reviewed in *VAO*			
Thomas et al., 1991	Women Vietnam veterans	17	1.2 (0.6–2.5)

[a] Given when available.
[b] 99% CI.

humans is plausible; however, such an effect has not been clearly demonstrated. Furthermore, a recent occupational study that found a twofold increase in breast cancer in women and two reports of elevated breast cancer mortality in males provide evidence that such extrapolation is inappropriate at this time. A more thorough discussion of TCDD's antiestrogenic properties and of the effect of TCDD or herbicides on breast cells is contained in Chapter 3; a summary discussion of biologic plausibility is presented in the conclusion to this chapter.

Increased Risk of Disease Among Vietnam Veterans

Studies in female Vietnam veterans have not shown an elevated risk of breast cancer, but the length of follow-up may not have been sufficient for an effect, if it exists, to become evident. A report on Australian male Vietnam veterans found three breast cancer deaths, where less than one was expected. Further follow-up among both male and female veterans from the Vietnam era is needed before conclusions can be drawn. A more thorough discussion of the issue of increased risk of disease among Vietnam veterans is included in Chapter 1.

CANCERS OF THE FEMALE REPRODUCTIVE SYSTEM

Background

This section addresses cancers of the cervix (ICD·9 180.0–180.9), endometrium (also referred to as the corpus uteri, ICD·9 182.0–182.1, 182.8), and ovaries (ICD·9 183.0). Statistics for other cancers of the female reproductive system are presented as well. The American Cancer Society estimates the following numbers of new female reproductive system cancer in the United States for 1998 (ACS, 1998):

Site	New cases	Deaths
Cervix	13,700	4,900
Endometrium	36,100	6,300
Ovary	25,400	14,500
Other female genital	5,200	1,400

Taken together, these numbers represent roughly 13 percent of new cancer diagnoses and 10 percent of cancer deaths in women.

Incidence patterns and risk factors vary for these diseases. Cervical cancers occur more often in African-American women than in whites, whereas whites are more likely to develop endometrial and ovarian cancers. The incidence of endometrial and ovarian cancer is also dependent on age, with older women at greater risk. Other risk factors for these cancers vary. Human papillomavirus infection is the most important risk factor for cervical cancer. Diet, a family

history of the disease, and breast cancer are among the risk factors for endometrial and ovarian cancers.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Female Genital System Cancers

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
cervix	17	16	23	17	15	23	16	13	27
endometrium	24	25	12	41	44	19	62	65	40
ovary	22	24	13	29	29	26	38	40	21
other genital	3	3	5	4	5	4	6	6	7
overall[b]	66	67	53	91	92	71	121	125	95

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.
[b] Totals may differ from the sum of the column because of independent rounding.

Summary of *VAO* and *Update 1996*

Occupational studies that examined the relationship between exposure to herbicides and uterine or ovarian cancers were extremely limited, largely because most occupational cohorts have included few exposed female workers. In a case-control study specifically designed to address the relation between herbicide exposure and risk of ovarian cancer, Donna et al. (1984) compared exposure histories of 60 women with ovarian cancer to controls (i.e., women with cancers at other sites, including breast, endometrium, cervix, and other organs). Overall, 18 women with ovarian cancer were classified as definitely or probably exposed, compared to 14 controls, giving an OR of 4.4 (CI 1.9–16.1). These findings of elevated risk for ovarian cancer, although striking, have not been replicated in studies conducted since that time. Other studies reporting no association between cancers of female reproductive organs and exposure to herbicides are Wiklund (1983), Saracci et al. (1991), and Ronco et al. (1992). In a cohort of farm workers in Denmark, Ronco et al. (1992) observed SIRs for cervical cancer, uterine cancer, and ovarian cancer, each based on 100 or more cases, that were all significantly less than 1.0.

In the first 10 years following the 1976 Seveso, Italy, accident, Bertazzi et al. (1989b, 1993) observed no increase in cancers of the uterus or cervix among female residents of zones A, B, or R, based on 2, 2, and 21 cases, respectively; the RRs were 2.6, 0.4, and 0.6, respectively. No cases of ovarian cancer were diagnosed among women in zones A or B. Based on 20 cases, the RR for ovarian cancer in zone R, the least contaminated area, was 1.1 (CI 0.7–1.7).

A cohort study of cancer incidence was conducted among employees of two phenoxy herbicide manufacturing facilities in Denmark (Lynge, 1993). This cohort included 1,071 women who were followed for 1947–1987. A statistically significant increase in risk of cervical cancer was found, based on seven cases

(SIR = 3.2, CI 1.3–6.6). An overlapping cohort study of cancer incidence and mortality was conducted among 701 women in the IARC cohort (66 percent were included in the study by Lynge, 1993), who were occupationally exposed to chlorophenoxy herbicides, chlorophenols, and dioxins (Kogevinas et al., 1993). One death was observed from each of the following types of cancer: cervical (SMR = 80), uterus nonspecified (SMR = 192), and ovary (SMR = 74).

In a study of death certificates, Blair et al. (1993) compared occupational groups and found that farmers in 23 states in the United States had an elevated risk for cervical cancer mortality, based on 21 deaths, among nonwhites only (PCMR = 2.0, CI 1.3–3.1). In whites, the PCMR, based on six deaths, was 0.9 (CI 0.3–2.0). The numbers of deaths from cancer of the uterine corpus were small and nonsignificant for both white and nonwhite female farmers.

Cancer mortality rates among 4,586 female Vietnam veterans were recently evaluated, as well as rates among 5,325 female veterans who had served elsewhere (Dalager et al., 1995a). Based on four cases of cancer of the uterine corpus, the RR for Vietnam veterans compared to that of the general U.S. population was 2.1 (CI 0.6–5.4).

Update of the Scientific Literature

Bertazzi et al. (1997) have updated their follow-up of the Seveso population. With 15 years of follow-up of the more than 20,000 exposed women, this cohort provides no evidence that TCDD is associated with deaths from either uterine or ovarian cancer. In zone A, which had the highest exposures, and zone B, with probably the second-highest exposures, the numbers of uterine cancer deaths were lower than expected. In zone R, where exposures were lower, but still greater than those not exposed to the accident, 27 uterine cancers were observed, with 23.7 expected, for a RR of 1.1 (95% CI 0.8–1.7). The numbers of deaths from ovarian cancer were 1 and 0 in zones A and B, respectively, where 0.4 and 2.7 were expected, yielding an elevated but unstable RR for zone A of 2.3 (CI 0.0–12.8). In zone R, the RR was 1.0 (CI 0.6–1.6). If the TCDD did initiate cancers of female reproductive organs, the elapsed time may still be insufficient for these tumors to have come to clinical attention. In particular, women exposed to TCDD during adolescence may be at increased risk for cancers that will not be detected until 20 or more years after the exposure.

The only other new data on female reproductive cancers come from an expanded multinational occupational study conducted by IARC of cohorts from 12 countries (Kogevinas et al., 1997). A total of 36 cohorts were included; 15 of these included females, and 11 of these cohorts had exposures to TCDD or higher chlorinated dioxins. No deaths from cancer of the uterine cervix or the ovary were observed among women workers exposed to TCDD or higher chlorinated dioxins. An SMR of 3.41, based on three cases with exposure to TCDD or higher chlorinated dioxins, was observed for cancer of the endometrium and uterus

(ICD·9 179, 181–182). Two of these three cases occurred in one cohort in Germany, which included most of the TCDD-exposed female production workers.

Synthesis

There has been considerable recent interest in the potential association of exposure to chlorinated hydrocarbons, including TCDD, and female reproductive cancers and other health outcomes in women. For example, teratogenic effects due to maternal exposures to TCDD have been well documented in experimental animals (see Chapter 3). Endometriosis has recently been demonstrated in monkeys exposed to TCDD (Rier et al., 1993), and research on this disease has been proposed for women in the Seveso cohort (Bois and Eskenazi, 1994). Epidemiologic studies regarding female reproductive tract cancers are summarized in Tables 7-17, 7-18, and 7-19. The evidence from these studies remains inconclusive, despite some strong associations with ovarian and uterine cancers, largely because most of the published studies include a small number of cases and/or have poor exposure characterization or too short a follow-up period. The committee concludes that more research is needed on populations of women with documented exposure to herbicides and TCDD.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides used in Vietnam (2,4-D, 2,4,5-T and its

TABLE 7-17 Selected Epidemiologic Studies—Cancers of the Cervix

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort	0	0
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	Whites	6	0.9 (0.3–2.0)
	Nonwhites	21	2.0 (0.3–3.1)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish farmers		
	Self-employed farmers	7	0.5
	Family workers	100	0.5
	Employees	12	0.8
Wiklund, 1983	Swedish agricultural workers	82	0.6

TABLE 7-18 Selected Epidemiologic Studies—Cancers of the Uterus

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort (includes cancers of the endometrium)	3	3.4 (0.7–10.0)
Studies reviewed in *VAO*			
Blair et al., 1993	U.S. farmers in 23 states		
	Whites	15	1.2
	Nonwhites	17	1.4
Ronco et al., 1992	Danish farmers		
	Self-employed farmers	8	0.6
	Family workers	103	0.8
	Employees	9	0.9
Wiklund, 1983	Swedish agricultural workers	135	0.9
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents	0	0.0
VIETNAM VETERANS			
Studies reviewed in *Update 1996*			
Dalager et al., 1995a	Women Vietnam veterans	4	2.1 (0.6–5.4)

TABLE 7-19 Selected Epidemiologic Studies—Ovarian Cancer

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort	0	0.0
Studies reviewed in *Update 1996*			
Kogevinas et al., 1993	IARC cohort	1	0.7
Lynge, 1993	Danish male production workers	7	3.2
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish farmers		
	Self-employed farmers	12	0.9
	Family workers	104	0.8
	Employees	5	0.5
Donna et al., 1984	Female residents near Alessandria, Italy	18	4.4 (1.9–16.1)
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	zone A	1	2.3 (0.0–12.8)
	zone B	0	0.0
	zone R	21	1.0 (0.6–1.6)

contaminant TCDD, cacodylic acid, and picloram) and uterine and ovarian cancers. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and uterine and ovarian cancers is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are inadequate data at this time to evaluate the possible increased risk of female reproductive cancers in Vietnam veterans, an ongoing study in female veterans of the Vietnam era may shed light on this issue. A more thorough discussion of the issue of increased risk of disease among Vietnam veterans is included in Chapter 1.

PROSTATE CANCER

Background

According to the American Cancer Society estimates, 184,500 new cases of prostate cancer (ICD·9 185) will be diagnosed in the United States in 1998, and 39,200 men will die from the disease (ACS, 1998). This makes prostate cancer the most common cancer among men, excluding nonmelanocytic skin cancers. It is expected to account for approximately 29 percent of new diagnoses and 13 percent of cancer deaths in 1998.

Prostate cancer incidence varies dramatically as a function of age and race. The risk increases fivefold between 45–49 and 50–54 years of age, and nearly triples between 50–54 and 55–59 years of age. As a group, African-American men have the highest recorded incidence of prostate cancer in the world (Miller et al., 1996). Their risk is roughly twice that of whites in the United States, 4 times higher than Alaskan natives, and nearly 7.5 times higher than Korean Americans.

Little is known about the causes of prostate cancer. Other than race and age, risk factors include a family history of the disease and a diet high in fats.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Prostate Cancer

45–49 years of age			50–54 years of age			55–59 years of age		
all races	white	black	all races	white	black	all races	white	black
16	16	26	82	78	143	225	216	404

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Occupational Studies

For prostate cancer, several studies have shown elevated risk in agricultural or forestry workers. Mortality was increased in studies of United States Department of Agriculture (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 related to being an extension agent (OR = 1.0, CI 0.7–1.5) or a soil conservationist (OR = 1.0, CI 0.6–1.8), although the risk was elevated for forest conservationists (OR = 1.6, CI 0.9–3.0). The risk of prostate cancer was more highly elevated for those whose employment ended prior to 1960 and who had worked for at least 15 years as conservationists (OR = 2.1 for forest workers and 2.9 for soil workers). 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 that was not connected to a specific agricultural exposure. Higher RRs were observed after restricting analysis to those born before 1890 (OR = 1.5) or those age 65 and older (OR = 1.3).

A PCMR study was performed with farmers in 23 states, using occupational information from death certificates (Blair et al., 1993). Based on 3,765 deaths from prostate cancer in white male farmers, out of 119,648 studied, the PCMR was significantly increased, at 1.2 (CI 1.1–1.2). Based on 564 deaths from prostate cancer in nonwhite male farmers, out of 11,446 studied, the PCMR was also significantly increased to 1.1 (CI 1.1–1.2). This increased risk for prostate cancer was observed in 22 of the 23 states studied.

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 the risk was found to rise with increasing number of acres sprayed. For the entire cohort, the RR for prostate cancer and spraying at least 250 acres was 1.2 (CI 1.0–1.5). Adjustment for potential confounders showed no evidence of confounding for the association. Additional analyses were restricted to a one-third sample of farmers most likely to be exposed to phenoxy or other herbicides (RR = 1.3, CI 1.0–1.8 for ≥250 acres sprayed). To focus on those farmers most likely exposed to herbicides, additional analyses were restricted to those with no employees (RR = 1.4, CI 1.0–1.9 for ≥250 acres sprayed); no customary expenses for assisting in work, which might include spraying (RR = 1.6, CI 1.1–2.2 for ≥250 acres sprayed); age between 45 and 69 years (RR = 1.7, CI 1.1–2.8 for ≥250 acres sprayed); and a combination of the three restrictions (RR = 2.2, CI 1.3–3.8 for ≥250 acres sprayed). In each of these comparisons, a statistical test for trend (increasing risk with increasing number of acres sprayed) was significant.

Although a number of occupational and other studies have examined prostate cancer in relation to potential herbicide exposures, many had very few cases.

These include numerous studies of chemical production workers (Bond et al., 1983; Lynge, 1985; Zober et al., 1990; Manz et al., 1991; Buena de Mesquita et al., 1993; Collins et al., 1993; Becher et al., 1996); pesticide appliers (Blair et al., 1983; Swaen et al., 1992; Asp et al., 1994); and paper and pulp workers (Henneberger et al., 1989; Solet et al., 1989). These small numbers may reflect an insufficient latency period or a cohort that is too young to observe excess prostate cancer. They also may reflect the fact that since prostate cancer tends not to be fatal, studies of mortality tend to have low statistical power to detect an effect if it exists, compared to other cancer sites. Other problems in certain studies are low specificity of exposure definition and, in at least one report, a very short duration of exposure (median duration for 2,4-D and 2,4,5-T was six weeks in Asp et al., 1994).

Environmental Studies

Cancer incidence and mortality were described for the Seveso population in three previous studies (Bertazzi et al., 1989a,b; Pesatori et al., 1992). Cancer incidence for the first 10 years after exposure to TCDD was thoroughly updated for the Seveso, Italy, cohort (Bertazzi et al., 1993). In zones A and B (the more highly exposed areas), four cases of prostate cancer were diagnosed, and the RR was 1.4 (CI 0.5–3.9) (Pesatori et al., 1992). In zone R (the less exposed area), based on 16 cases of prostate cancer (Bertazzi et al., 1993), the RR was 0.9 (CI 0.5–1.5).

Vietnam Veteran Studies

Studies of prostate cancer mortality among Vietnam veterans have not consistently shown an association (Anderson et al., 1986a,b; Breslin et al., 1988). A proportionate mortality study examining causes of death among veterans on the state of Michigan's Vietnam era bonus list (Visintainer et al., 1995) compared cause-specific mortality rates for 3,364 Vietnam veterans with rates for 5,229 age-matched veterans who had served elsewhere. There were 19 deaths from male genital cancers among Vietnam veterans (PMR = 1.1, CI 0.6–1.7). Rates for prostate cancer were not reported separately from testicular cancer.

Update of the Scientific Literature

Several new reports have been published, examining prostate cancer mortality, incidence, or both, in populations with possible or documented exposures to TCDD. Six of these involved occupational exposures (Becher et al., 1996; Ott and Zober 1996; Zhong and Rafusson, 1996; Gambini et al., 1997; Hertzman et al., 1997; Kogevinas et al., 1997): one was an occupational cohort with dietary exposures (Svensson et al., 1995), one was a follow-up of the Seveso cohort, and four were examined deaths among Vietnam veterans (AFHS, 1996; Watanabe

and Kang, 1996; Crane et al., 1997a,b). One occupational study grouped all male genital cancers for examining mortality (Hertzman et al., 1997). None of these provides strong evidence of increased risk for prostate cancer.

The smallest study involved 243 workers exposed during and after a 1953 reactor accident in a West German plant that produced trichlorophenol (Ott and Zober, 1996). Exposures were originally documented on the basis of chloracne, which was observed in nearly half the cohort, although it was several years after the accident that TCDD was identified as the cause of the chloracne. Exposures occurred during three periods: (1) clean-up operations and repair activities that lasted several months; (2) incidental maintenance activities after the completion of the restoration; and (3) demolition of the reactor in 1968–1969. Biomonitoring data were collected later and used to estimate internal doses of dioxin at the time of each worker's peak exposures. In this small cohort, there have been no deaths from prostate cancer, with follow-up through 1992. Four cases were identified during 1960–1992, resulting in a SIR of 1.1 (CI 0.3–2.8). When broken down by dose, there is no clear dose–response relationship: the highest SIR (2.5) occurs in the lowest-dose group (<0.1 μg/kg), and there are no cases in the highest-dose group (>1 μg/kg). On the other hand, the small size of the cohort coupled with the low number of expected cases would indicate that there is not sufficient power to examine the dose–response relation in this follow-up.

In a reanalysis of the study published by Manz et al. (1991), Becher et al. (1996) report on 2,479 workers exposed to phenoxy herbicides and associated contaminants in four German chemical production plants. The SMRs in the four cohorts ranged from zero to 1.5, based on nine prostate cancer deaths. Combining the data from these plants yields an SMR of 1.3. Again, the small number of expected deaths from prostate cancer suggests a fairly young cohort. These four cohorts were also included in the large IARC study of 36 cohorts in 12 countries (Kogevinas et al., 1997). With 21,863 workers, of whom 13,831 were exposed to TCDD or higher chlorinated dioxins, and a total of 488,482 person-years of follow-up, 43 prostate cancer deaths were observed, yielding an SMR of 1.1 (CI 0.8–1.5). This SMR was similar to the one for workers not exposed to TCDD or higher chlorinated dioxins: 1.1 (CI 0.7–1.6). Biomonitoring data were collected on more than 500 workers, but were not used to categorize individual workers. Production workers did not appear to have considerably higher exposures than sprayers. An overall "healthy worker effect" was not observed in this study.

The fourth study involving occupational exposures was conducted on rice growers in northern Italy (Gambini et al., 1997). The cohort consisted of 1,487 male workers, with 31,648 person-years of observation, over half of which were at ages 60 or older. Individual exposures were not estimated, but the use of herbicides was documented to begin in the early 1950s and to include 2,4-D (used in the 1960s), 2,4,5-T (1960s and 1970s), MCPA (1960s–1990s), and MCPP (mecoprop, 1960s–1980s). The 19 observed prostate cancer deaths resulted in an SMR of 1.0 (CI 0.6–1.5). No analysis was conducted based on years of work.

This cohort showed a prominent healthy worker effect, suggesting a possible underestimate of the relative mortality compared to the general population.

A study of 2,449 Icelandic pesticide users, of whom 1,860 were men, took advantage of the country's Cancer Registry to analyze morbidity (Zhong and Rafnsson, 1996). A deficit of prostate cancer cases was observed, with an SIR of 0.7 (CI 0.2–1.2) based on 10 cases. Data on actual work tasks were not available for most of the cohort.

A cohort of sawmill workers from British Columbia, Canada, with chlorophenate exposures was evaluated with respect to both morbidity and mortality (Hertzman et al., 1997). An SMR for prostate cancer was not given, but for all male genital cancers combined it was 1.2 (CI 1.0–1.4). (Prostate cancers generally represent about 90 percent of male genital cancers.) The SIR for prostate cancer was 1.0 (CI 0.9–1.1). Although data were collected on sawmill workers not exposed to chlorophenates, the size of this unexposed group was much smaller than for exposed workers, and data were not presented on its prostate cancer incidence or mortality that would have enabled internal comparisons to be made for prostate cancer.

Svensson et al. (1995) analyzed mortality and morbidity from two cohorts of fisherman: those from the east coast of Sweden, who consume a diet rich in fatty fish, and those from the west coast of Sweden, whose diets involve leaner fish, but who are otherwise socioeconomically quite similar. Plasma toxic equivalents of PCBs and PCDD/F have been documented to be twice as high in east coast as in west coast fishermen or referents. The SMR for prostate cancer among east coast fishermen was 1.0 (CI 0.5–1.8), compared to 1.1 (CI 0.9–1.3) for west coast fishermen. The SIRs for these two groups, respectively, were 1.1 (CI 0.8–1.5) and 1.0 (CI 0.9–1.1).

In the Seveso cohort, the numbers of deaths from prostate cancer among men in zones A, B, and R were 0, 6, and 39, for SMRs of 0, 1.2 (CI 0.5–2.7), and 1.2 (CI 0.8–1.6), respectively.

Finally, two new studies on Vietnam veterans reported data on prostate cancer. Watanabe and Kang (1996) published a study of deaths among Vietnam veterans (N = 33,833) and a comparison group of Vietnam era veterans who served outside Southeast Asia (N = 36,797). This was not a cohort study, since there is no register of those who served in the military during the Vietnam period. Most of these veterans had served in the Army, but about 20 percent had served in the Marine Corps. Proportionate mortality comparisons were made between the two groups of deaths. Results for prostate cancer were: PMR = 1.1 for those who served in the Army in Vietnam and PMR = 1.2 for those who served there as Marines; among those who served outside Southeast Asia, PMRs were 1.2 for Army veterans, and 1.3 for Marine veterans. Those who served in Vietnam were generally similar to those who did not, although slightly more African Americans served in Vietnam and the length of service was substantially longer for those who served in Vietnam compared with those who served elsewhere (e.g., 1.9

percent versus 13.8 percent served one year or less in the Army, and 3.8 percent versus 27.1 percent served 1 year or less in the Marines). In an analysis by years of latency, the highest PMR among those who served in the Army was for the longest latency (at least 16 years from the last year in Vietnam): 1.1.

A recent update on the Ranch Hand cohort found an SMR of 4.0 (two observed deaths) for prostate cancer, when considering only the period after 20 years' latency (AFHS, 1996). A brief report of a case-control study of Massachusetts veterans (Clapp, 1997), which used gastrointestinal cancers as controls, found an OR of 0.8, comparing those who had served in Vietnam (15 cases) with those who had served elsewhere in the same era (118 cases). The latter analysis could be problematic since some studies have shown rectal cancer to be increased in association with herbicide exposures.

Crane et al. (1997a) conducted a detailed analysis of Australian male Vietnam veteran mortality. In the early follow-up period from 1964 to 1979, one death from prostate cancer was observed. However, from 1980 to 1994, there were 36 such deaths, with an SMR of 1.5 (CI 1.1–2.1). By service, the figures for Army, Navy, and Air Force personnel were, respectively, 26 deaths, SMR = 1.6 (CI 1.1–2.4); 8 deaths, SMR = 2.2 (CI 0.9–4.3); and 2 deaths, SMR = 0.5 (CI 0.1–1.9). A companion study comparing conscripted Australian veterans of Vietnam with military personnel who did not serve there reported no deaths in the Vietnam veteran population and one in the comparison population between 1982 and 1994 (Crane et al., 1997b). Although smoking data were not available, prostate cancer is not associated with smoking; therefore, there is no concern about smoking being a confounder of these results.

Synthesis

Results of studies published to date are summarized in Table 7-20. At the time of *Update 1996*, several of the agricultural studies indicated some elevation in risk of prostate cancer, with some subgroups that had the greatest probability and longest duration of herbicide exposure showing the highest risks (Morrison et al., 1993). Additional agricultural studies published since *Update 1996*, one on rice growers in northern Italy and the other on Icelandic pesticide users, show no evidence of increased risk of prostate cancer, although it should be noted that exposure information was poor and both cohorts demonstrated a rather strong healthy worker effect.

The earlier major studies of production workers (Fingerhut et al., 1991; Manz et al., 1991; Saracci et al., 1991) showed small, but not statistically significant, elevations in risk. Since *Update 1996*, several additional studies, some of which overlap earlier ones (Becher et al., 1996; Kogevinas et al., 1997; and Ott and Zober, 1996), continue to show weak evidence of effects on prostate cancer mortality, with RRs generally <1.5 and mostly in the range of 1.1–1.2.

Two cohort studies reported both morbidity and mortality (Svensson et al.,

1995; Hertzman et al., 1997). Among Swedish fisherman, no association was seen between exposure and prostate cancer mortality (SMR = 1.0) or morbidity (SIR = 1.1); among sawmill workers, no association was seen with morbidity (SIR = 1.0), whereas the association with mortality from male genital tract cancers was significant (SMR = 1.2, CI 1.0–1.4).

In general, when observed associations are this weak, biases could have induced artifactual associations. In this case, it is unclear what those biases may be in such varied populations involving quite different exposure scenarios. In studies using mortality as an outcome, detection bias is unlikely to explain any elevated risks. On the other hand, factors that increase incidence might not be the same as those related to subsequent mortality among those who have the disease. The recent introduction and widespread adoption of PSA (prostate specific antigen) for screening purposes has brought about an apparent increase in incidence in the United States; the pattern of long-term impact on incidence or mortality is difficult to predict for any country or population and will depend on the rapidity with which this screening tool is adopted, its differential across different ages of men, and the aggressiveness of tumors detected early by this test (Gann, 1997). Differences among countries in the rate of use of PSA could produce more variable, less consistent results in studies of exogenous exposures and prostate cancer.

Several new studies have been published on Vietnam veterans. Too few cases were seen among Ranch Hands to provide useful information about this outcome (AFHS, 1996). In a larger study of deaths among U.S. Vietnam veterans, those who had last served in Vietnam more than 16 years earlier had a weakly elevated PMR of 1.1. PMR studies should be viewed cautiously, however, since they include only deaths; in this type of study, findings for any one outcome are strongly influenced by associations between the exposure of interest and other causes of death, particularly the most common ones. In the cohort study of Australian male veterans, which had a much stronger design, an elevated rate of prostate cancer mortality was observed among those who served in Vietnam (SMR = 1.5, CI 1.1–2.1, based on 36 prostate cancer deaths). Unlike results for respiratory cancer, the lack of smoking data for this cohort does not pose a threat to the validity of these findings, since prostate cancer is not associated with smoking. It should also be kept in mind that most Vietnam veterans have not yet reached the age when this cancer tends to appear, and that morbidity may represent a more sensitive outcome than mortality for this cancer site.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and

TABLE 7-20 Selected Epidemiologic Studies—Prostate Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers	19	1.0 (0.6–1.5)
Hertzman et al., 1997	Canadian sawmill workers		
	Mortality	282	1.0 (0.9–1.1)
	Morbidity for male genital tract cancers	116	1.2 (1.0–1.4)
Kogevinas et al., 1997	IARC cohort	43	1.1 (0.8–1.5)
Becher et al., 1996	German chemical production workers	9	1.3
Ott and Zober, 1996	BASF cleanup workers	4	1.1 (0.3–2.8)
Zhong and Rafusson, 1996	Icelandic pesticide users	10	0.7 (0.3–1.2)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	5	0.8 (0.3–1.8)
Blair et al., 1993	U.S. farmers in 23 states		
	Whites	3,765	1.2 (1.1–1.2)
	Nonwhites	564	1.1 (1.1–1.2)
Bueno de Mesquita et al., 1993	Dutch production workers	3	2.6 (0.5–7.7)
Collins et al., 1993	Monsanto 2,4-D production workers	9	1.6 (0.7–3.0)
Studies reviewed in *VAO*			
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 ($p < .05$)
Swaen et al., 1992	Dutch herbicide applicators	1	1.3 (0.0–7.3)
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)
Manz et al., 1991	German production workers	7	1.4 (0.6–2.9)
Saracci et al., 1991	IARC cohort	30	1.1 (0.8–1.6)
Zober et al., 1990	BASF production workers	0	— (0.0–7.5)
Alavanja et al., 1989	USDA forest conservationists		1.6 (0.9–3.0)
	Soil conservationists		1.0 (0.6–1.8)
Henneberger et al., 1989	Paper and pulp workers	9	1.0 (0.7–2.0)
Solet et al., 1989	Paper and pulp workers	4	1.1 (0.3–2.9)
Alavanja et al., 1988	USDA agricultural extension agents		1.0 (0.7–1.5)
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)
Robinson et al., 1986	Paper and pulp workers	17	1.2 (0.7–2.0)
Lynge, 1985	Danish production workers	9	0.8
Blair et al., 1983	Florida pesticide applicators	2	0.5
Burmeister et al., 1983	Iowa residents		1.2 ($p < .05$)
Wiklund, 1983	Swedish agricultural workers	3,890	1.0 (0.9–1.0)[b]
Burmeister, 1981	Iowa farmers	1,138	1.1 ($p < .01$)

continued

TABLE 7-20 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	zone A	0	0 (0.0–5.2)
	zone B	6	1.2 (0.5–2.7)
	zone R	39	1.2 (0.8–1.6)
Svensson et al., 1995	Swedish fishermen mortality	12	1.0 (0.5–1.8)
	Swedish fishermen incidence	38	1.1 (0.8–1.5)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male residents—zone R	16	0.9 (0.5–1.5)
Studies reviewed in *VAO*			
Pesatori et al., 1992	Seveso male residents—zones A and B	4	1.4 (0.5–3.9)
Bertazzi et al., 1989a	Seveso male residents—zones A, B, and R	19	1.6 (1.0–2.7)
Bertazzi et al., 1989b	Seveso male residents—zone B	3	2.2 (0.7–6.9)
VIETNAM VETERANS			
New Studies			
Clapp, 1997	Massachusetts Vietnam veterans		
	Exposed cancers	15	0.8 (0.4–1.6)
	Total cancer	133	
Crane et al., 1997a	Australian military veterans	36	1.5 (1.1–2.1)
	Army	26	1.6 (1.1–2.4)
	Navy	8	2.2 (0.9–4.3)
	Air Force	2	0.5 (0.1–1.9)
AFHS, 1996	Ranch Hands	2	4.0
Watanabe and Kang, 1996	Army Vietnam veterans	58	0.9
	16+ years after discharge		1.1
Studies reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans	19	1.1 (0.6–1.7)
Studies reviewed in *VAO*			
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 Percent CI.

picloram) and prostate cancer. Although the associations are not large, a number of studies provide evidence that is suggestive of a slight increase in either morbidity or mortality from prostate cancer. The evidence regarding association is drawn from occupational studies in which subjects were exposed to a variety of herbicides and herbicide components and is also based on data from studies of Vietnam veterans. An important consideration is the fact that prostate cancer tends not to be fatal; thus, mortality studies have lower statistical power to detect a comparable effect than a similar-sized morbidity study would have.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and prostate cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

Increased Risk of Disease Among Vietnam Veterans

Studies that have been conducted on Vietnam veterans have had a low likelihood of detecting an increased risk of prostate cancer, if service in Vietnam is associated with this cancer, because of the weak study designs and the relatively young age of Vietnam veterans. The statistically significantly elevated prostate cancer SMR for Australian male Vietnam veterans suggests that U.S. Vietnam veterans may be at increased risk. Further follow-up that includes, in particular, studies of morbidity among living veterans, would help to define the risk. A more thorough discussion of the issue of increased risk of disease in Vietnam veterans is included in Chapter 1.

TESTICULAR CANCER

Background

The American Cancer Society estimates that 7,600 men will be diagnosed with testicular cancer (ICD·9 186.0–186.9) in the United States in 1998 and that 400 will die from the disease (ACS, 1998).

Testicular cancer is far more likely in men younger than 40 than in those who are older. On a lifetime basis, the risk for white men is about four times greater than for African Americans. Cryptorchidism, or undescended testicles, is a major risk factor for testicular cancer. Family history of the disease also appears to play a role. Several other hereditary and environmental factors have been suggested, but research regarding them is inconsistent (Bosl and Motzer, 1997).

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Testicular Cancer

45–49 years of age			50–54 years of age			55–59 years of age		
all races	white	black	all races	white	black	all races	white	black
5.7	6.4	0.9	3.3	3.8	0.4	2.2	2.6	0.5

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

A case-control study of 137 testicular cancer cases and 130 hospital controls (Tarone et al., 1991) found an OR of 2.3 (CI 1.0–5.5) for service in Vietnam. Risk for testicular cancer was not significantly elevated by service branch. Another

case-control study among veterans investigated the association between potential Agent Orange exposure and the risk of testicular cancer (Bullman et al., 1994), using subjects chosen from the DVAs Agent Orange Registry. This included 97 veterans with testicular cancer and 311 veterans with no clinical diagnosis recorded on the registry. Risk of testicular cancer was not significantly increased for ground troops, combat duty, service in the III Corps area (a heavily sprayed area), or proximity to other areas where Agent Orange was sprayed. Only Navy veterans had a statistically significant increased risk of testicular cancer (OR = 2.6, CI 1.1–6.2), based on 12 cases among 27 veterans. One of these 27 served in the "brown-water" Navy and may have had Agent Orange exposure due to spraying of riverbanks. In general, other veteran studies and most of the occupational and environmental studies showed no association between exposure and outcome, but the sample sizes of some of these studies were generally small. Results of other studies of testicular cancer have been equivocal. These include studies of chemical production workers in the United States and other countries (Bond et al., 1988; Saracci et al., 1991); agricultural workers (Wiklund, 1983; Ronco et al., 1992; Blair et al., 1993); residents of Seveso (Pesatori et al., 1992; Bertazzi et al., 1993); and Vietnam veterans (Anderson et al., 1986a,b; Boyle et al., 1987; Breslin et al., 1988; Watanabe et al., 1991).

Update of the Scientific Literature

Occupational Studies

The IARC study (Kogevinas et al., 1997) found seven deaths due to testicular cancer, SMR = 1.3 (CI 0.5–2.7). Ramlow et al. (1996) found no cases of death due to testicular cancer, with about 0.2 expected. Hertzman and colleagues' (1997) study of Canadian sawmill workers reported an SIR of 1.0 (CI 0.6–1.4) based on 18 cases. "Male genital cancers" (possibly including prostate) had an SMR of 1.0 (CI 0.8–1.1) with 116 cases.

Environmental Studies

The Seveso follow-up (Bertazzi et al., 1997) did not report testicular cancer separately, but the SMR for genitourinary cancers was less than 1.0. A study of Icelandic pesticide appliers (Zhong and Rafnsson, 1996) reported two cases, with an SIR of 1.2 (CI 0.1–4.3).

Vietnam Veterans' Studies

In studies of Vietnam veterans, Dalager and Kang (1997) found two deaths due to testicular cancer among Army Chemical Corps veterans (SMR = 4.0, CI 0.5–14.5). Watanabe and Kang (1996) reported PMRs of 1.1 and 1.0 for Army

TABLE 7-21 Selected Epidemiologic Studies—Testicular Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Hertzman et al., 1997	British Columbia sawmill workers		
	Mortality	116	1.0 (0.8–1.1)
	Incidence	18	1.0 (0.6–1.4)
Kogevinas et al., 1997	IARC cohort	7	1.3 (0.5–2.7)
Ramlow et al., 1995	Pentachlorophenol production workers	0	—
Studies reviewed in *Update 1996*			
Blair et al., 1993	U.S. farmers in 23 states		
	White males	32	0.8 (0.6–1.2)
	Nonwhite males	6	1.3 (0.5–2.9)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish self-employed farm workers	74	0.9
Saracci et al., 1991	IARC cohort	7	2.3 (0.9–4.6)
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)
Wiklund, 1983	Swedish agricultural workers	101	1.0 (0.7–1.2)[b]
ENVIRONMENTAL			
New Studies			
Zhong and Rafnsson, 1996	Icelandic pesticide users	2	1.2 (0.1–4.3)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents		
	zone A	0	—
	zone B	1	—
	zone R	9	1.4 (0.7–3.0)
Studies reviewed in *VAO*			
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			
New Studies			
Clapp, 1997	Massachusetts Vietnam veterans—incidence	30	1.2 (0.4–3.3)
Crane et al., 1997a	Australian military veterans	4	NS
Crane et al., 1997b	Australian national service veterans	4	1.3
Dalager and Kang, 1997	Army Chemical Corps veterans	2	4.0 (0.5–14.5)
Watanabe and Kang, 1996	Vietnam service Army		1.1
	Vietnam service Marines		1.0
Studies reviewed in *Update 1996*			
Bullman et al., 1994	Navy veterans	12	2.6 (1.1–6.2)
Studies reviewed in *VAO*			
Tarone et al., 1991	Patients at three Washington, D.C., area hospitals		2.3 (1.0–5.5)
Watanabe et al., 1991	Army Vietnam veterans	109	1.2
	Marine Vietnam veterans	28	0.8

continued

TABLE 7-21 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Breslin et al., 1988	Army Vietnam veterans	90	1.1 (0.8–1.5)
	Marine Vietnam veterans	26	1.3 (0.5–3.6)
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)

[a] Given when available.
[b] 99% CI.
[c] "Male genital cancers"

and Marine Vietnam veterans, respectively, based on 142 total deaths. Crane and colleagues report 4 deaths due to testicular cancer among Australian military veterans, with an SMR near unity (1997a). A study comparing conscripted Australian veterans of Vietnam with military personnel who did not serve there reports a RR of 1.3 based on a single death in each of the populations (Crane et al., 1997b). Clapp (1997) reported 30 incident cases in Massachusetts Vietnam veterans, OR = 1.2 (CI 0.4–3.3), compared to veterans who had not served in Vietnam.

Several studies of military working dogs have been reported, which showed abnormal testicular pathology and a moderate excess of seminomas in dogs that died that had worked in Vietnam (Mahaney 1990; Hayes et al., 1990, 1994, 1995a,b). Most ORs were around 2, and the design was that of a proportionate mortality study (i.e., all risks were based on the proportion of dogs that had died from any cause with a given testicular finding at necropsy). There were no measures of exposure to environmental agents.

Synthesis

There is minimal new information on this rare cancer, and what there is provides little evidence supporting a herbicide–testicular cancer connection. The findings in dogs are not felt to carry great weight in the absence of exposure data and without observed excesses in human populations.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and testicular cancer.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and testicular cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

URINARY BLADDER CANCER

Background

Urinary bladder cancer (ICD·9 188.0–188.9) is the most common of the genitourinary tract cancers. According to American Cancer Society estimates, 39,500 men and 14,900 women will be diagnosed with this cancer in the United States in 1998 and 8,400 men, and 4,100 women will die from the disease (ACS, 1998). In males, where this cancer is about three times more likely to occur than in females, these numbers represent approximately 6 percent of new cancer diagnoses and 3 percent of deaths. Overall, bladder cancer is the fifth most common cancer and the fifth leading cause of cancer death in the United States

Among males in the age groups that characterize most Vietnam veterans, bladder cancer incidence is about twice as high in whites as in African Americans. Rates are slightly higher in white than African-American women. Bladder cancer incidence increases greatly with age for individuals older than 40. For the age groups shown below, the incidence rate in each five-year grouping is roughly double that of the age group before it.

The most important known risk factor for bladder cancer is smoking. About one-half of bladder cancers in men and one-third in women are thought to be due to smoking (Miller et al., 1996). Occupational exposure to aromatic amines (also called arylamines) is also associated with higher incidence. High-fat diets have been implicated as risk factors, along with exposure to the parasite *Schistosoma haematobium*.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Urinary Bladder Cancer

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	14	16	7	29	31	16	52	56	35
females	4	5	3	9	9	7	17	18	10

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

For bladder cancer, Fingerhut et al. (1991) found a small excess mortality in their study of chemical production workers exposed to TCDD. In the total cohort of 5,172 workers, the SMR was 1.6 (CI 0.7–3.0), based on nine cases. In workers

with at least one year of employment and 20 years' latency, there were four cases (SMR = 1.9, CI 0.5–4.8). Other studies of bladder cancer have produced inconclusive results. Occupational studies include chemical production workers in the United States and other countries (Moses et al., 1984; Suskind and Hertzberg, 1984; 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; Blair et al., 1993); pesticide appliers (Blair, 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) and Bertazzi et al. (1989b, 1993) studies 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 a study of veterans in Wisconsin (Anderson et al., 1986a,b). The results of these studies are summarized in Table 7-22.

As a subgroup of the IARC cohort, a cohort of workers was identified who manufactured chlorophenoxy herbicides in two factories in the Netherlands (Bueno de Mesquita et al., 1993). Among 963 exposed male workers, there was one case of bladder cancer (SMR = 1.2, CI 0.0–6.7).

The mortality experience of 754 male production workers at a Monsanto plant was evaluated (Collins et al., 1993). One hundred and twenty-two of these workers developed chloracne as a result of an accidental release of TCDD in 1949. Based on 16 deaths due to bladder cancer, the SMR was 6.8 (CI 3.9–11.1). Eleven of these cases had documented exposure to 4-aminobiphenyl, a known bladder carcinogen; therefore, TCDD exposure was not the primary suspected risk factor. The SMR was not significantly elevated for the other five cases not exposed to 4-aminobiphenyl.

An 18-year follow-up study of cancer incidence and mortality in 1,909 Finnish herbicide appliers was reported (Asp et al., 1994). Employees had previously been identified as being exposed to 2,4-D and 2,4,5-T (Riihimaki et al., 1982). The median total exposure to herbicides was six weeks. Based on 12 cases of bladder cancer, the SIR was 1.6 (CI 0.8–2.8).

Update of Scientific Literature

Occupational Studies

The IARC study (Kogevinas et al., 1997) found an SMR of 1.0 (CI 0.7–1.5) for all workers, with 34 deaths from bladder cancer, and an SMR of 1.4 (CI 0.9–2.1) among workers exposed to TCDD or higher chlorinated dioxins, based on 24 deaths. Hertzman et al.'s (1997) study of Canadian sawmill workers reported an SIR of 1.0 (CI 0.8–1.2) based on 94 cases, and an SMR of 0.9 (CI 0.7–1.2) based on 33 cases.

Environmental Studies

In a study of rice growers, Gambini et al. (1997) found an SMR of 1.0 (CI 0.5–1.8) based on 12 deaths. A follow-up of BASF employees exposed to TCDD as a result of a 1953 chemical reactor accident (Ott and Zober, 1996) found two deaths due to "bladder or kidney" cancer, from a total of five cases (SIR = 1.4, CI 0.4–3.2). In the Bertazzi et al. (1997) follow-up of Seveso residents, 29 deaths were observed, with SMRs near 1.0 among both women and men in all exposure zones. A study of Swedish fishermen (Svensson et al., 1995) showed SIRs of 0.7 (CI 0.4–1.3) in a presumed elevated-exposure cohort and 0.9 (CI 0.7–1.1) in a comparison cohort, based on 65 cases. SMRs for the two cohorts were 1.3 (CI 0.4–3.1) and 1.0 (CI 0.6–1.6), respectively, based on 25 total deaths.

Vietnam Veterans' Studies

Clapp's (1997) update of his Massachusetts veteran cohort found an OR of 0.6 (CI 0.2–1.3) based on 80 cases. None of the other studies of U.S. Vietnam veterans (Dalager and Kang, 1997; Watanabe and Kang, 1996) published since the release of *Update 1996* reported bladder cancer outcomes. Crane and colleagues' (1997a) comparison of all Australian military veterans with the rest of the male Australian population reported 11 deaths due to bladder cancer: SMR = 1.1 (CI 0.6–2.0). A second study of the mortality experience of conscripted Australian veterans relative to military personnel who did not serve in the conflict reported a statistically significant RR of 0.6 based on one death among Vietnam veterans and two in the comparison population (Crane et al., 1997b).

Synthesis

Although there is no evidence that exposure to herbicides alone is related to bladder cancer, RRs in some of the largest cohorts tended to be greater than 1, weakening the committee's prior conclusion that there was positive evidence of *no* relationship. Coexposure to TCDD and a variety of known bladder carcinogens makes it very difficult to isolate any possible additional effect of herbicides, although little total effect was seen.

Conclusions

Strength of Evidence in Epidemiologic Studies

Based on an evaluation of all of the epidemiologic evidence, the committee felt that the previous conclusion of "limited/suggestive evidence of *no* association" between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and urinary bladder cancer should be

TABLE 7-22 Selected Epidemiologic Studies—Urinary Bladder Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Hertzman et al., 1997	British Columbia sawmill workers		
	Mortality	33	0.9 (0.7–1.2)
	Incidence	94	1.0 (0.8–1.2)
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	24	1.4 (0.9–2.1)
	Workers exposed to any phenoxy herbicide or chlorophenol	34	1.0 (0.7–1.5)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators—incidence	12	1.6 (0.8–2.8)
Bueno de Mesquita et al., 1993	Dutch production workers	1	1.2 (0.0–6.7)
Collins et al., 1993	Monsanto 2,4-D production workers	16	6.8 (3.9–11.1)
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farmers	300	0.6 ($p < .05$)
Fingerhut et al., 1991	NIOSH cohort	9	1.6 (0.7–3.0)
	20 year latency	4	1.9 (0.5–4.8)
Green, 1991	Herbicide sprayers in Ontario	1	1.0 (0.01–5.6)
Saracci et al., 1991	IARC cohort	13	0.8 (0.2–1.4)
Zober et al., 1990	BASF production workers	0	— (0.0–15.0)
Alavanja et al., 1989	USDA forest/soil conservationists	8	0.8 (0.3–1.6)
Henneberger et al., 1989	Mortality among paper and pulp workers	4	1.2 (0.3–3.2)
Alavanja et al., 1988	USDA agricultural extension agents	8	0.7 (0.4–1.4)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0–7.2)
Coggon et al., 1986	British MCPA production workers	8	0.9 (0.4–1.7)
Robinson et al., 1986	Paper and pulp workers	8	1.2 (0.6–2.6)
Lynge, 1985	Danish male production workers	11	0.8
Blair, 1983	Florida pesticide applicators	3	1.6
Burmeister, 1981	Farmers in Iowa	274	0.9 (NS)
ENVIRONMENTAL			
New Studies			
Gambini et al., 1997	Italian rice growers	12	1.0 (0.5–1.8)
Ott and Zober, 1996	BASF cleanup workers	2	1.4 (0.4–3.2)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast		1.3 (0.4–3.1)
	West coast		1.0 (0.6–1.6)
	Swedish fishermen incidence		
	East coast		0.7 (0.4–1.3)
	West coast		0.9 (0.7–1.1)
Studies reviewed in *VAO*			
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)

TABLE 7-22 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Lampi et al., 1992	Finnish community exposed to chlorophenols		1.0 (0.6–1.9)
VIETNAM VETERANS			
New Studies			
Clapp, 1997	Massachusetts Vietnam veterans	80	0.6 (0.2–1.3)
Crane et al., 1997a	Australian military veterans	11	1.1 (0.6–2.0)
Crane et al., 1997b	Australian national service veterans	1	0.6
Studies reviewed in *VAO*			
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	—

[a] Given when available.
[b] Many of the employees studied were also exposed to 4-aminobiphenyl, a known bladder carcinogen.

changed to "inadequate/insufficient evidence to determine whether an association exists."

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and bladder cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

RENAL CANCER

Background

Cancers of the kidney (ICD·9 189.0) and renal pelvis (ICD·9 189.1) are often grouped together in epidemiologic studies, although the diseases have different characteristics and may have different risk factors. The American Cancer Society estimates that 17,600 men and 12,300 women will be diagnosed with renal cancers (ICD·9 189.0, 189.1) in the United States in 1998 and that 7,100 men and 4,500 women will die from the disease (ACS, 1998). These figures represent a little more than 2 percent of all new cancer diagnoses and deaths.

Renal cancer is twice is common in men as in women. In the age groups that represent most Vietnam veterans, African-American men have a slightly higher

incidence than white men; African-American and white women have roughly the same rate of the disease. With the exception of Wilm's tumor (which is more likely to occur in children), renal cancer is more common in individuals older than 50 years of age.

Smoking is a well-established risk factor for renal cancer. Phenacetin-containing analgesic abuse has also been implicated. Individuals with certain rare syndromes—notably, von Hippel-Lindau syndrome and tuberous sclerosis—are at higher risk. Other potential factors include diet, weight, and occupational exposure to asbestos and cadmium.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Kidney and Renal Pelvis Cancer

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	12	12	22	22	22	34	31	31	37
females	6	6	7	11	11	10	15	16	18

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Studies of renal cancer have generally produced inconclusive results—in some cases because of small sample sizes, in other cases with SMRs or PMRs near unity. 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; Bueno de Mesquita et al., 1993; Asp et al., 1994); agricultural workers (Burmeister, 1981; Wiklund, 1983; Ronco et al., 1992; Blair et al., 1993); pesticide applicators (Blair, 1983); paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989); the Seveso population (Pesatori et al., 1992; Bertazzi et al., 1993); and Vietnam veterans (Anderson et al., 1986a,b; Breslin et al., 1988; Kogan and Clapp, 1985, 1988; Clapp et al., 1991). Alavanja et al. (1988, 1989) found excess mortality due to renal cancer in studies of USDA agricultural extension agents (PMR = 2.0, CI 1.2–3.3) and forest and soil conservationists (PMR = 2.1, CI 1.2–3.3). In subsequent case-control studies of these deaths, comparing ever versus never being an extension agent resulted in a RR of 1.7 (CI 0.9–3.3). The RRs for being a soil conservationist and a forest conservationist were 2.4 (CI 1.0–5.9) and 1.7 (CI 0.5–5.5), respectively.

A case-control study of occupational risk factors and renal cell carcinoma included 365 cases identified from the Denmark Cancer Registry and pathology records and 396 controls selected from the country's Central Population Registry (Mellemgaard et al., 1994). Based on 13 cases, the OR for men for herbicide exposure was 1.7 (CI 0.7–4.3). Based on three cases, the OR for herbicide exposure for women was 5.7 (CI 0.6–5.8).

A PMR study examining causes of death among veterans on the state of Michigan's Vietnam era bonus list (Visintainer et al., 1995) compared 3,364 Vietnam veterans with 5,229 age-matched veterans who served elsewhere. Based on 21 cases of renal cancer among Vietnam veterans, the PMR was 1.4 (CI 0.9–2.2).

Update of the Scientific Literature

Occupational Studies

As for other cancer types, the most important new study is the IARC combined cohorts (Kogevinas et al., 1997). This study found 26 cases of kidney cancer and an SMR of 1.6 (CI 1.1–2.4), for workers exposed to TCDD, although there was no trend by duration of exposure or time since exposure, and an SMR of 1.1 (CI 0.7–1.6) for all workers exposed to any phenoxy herbicide or chlorophenol. In a study of 770 pentachlorphenol workers (Ramlow, 1996), three deaths from kidney cancer were found among workers exposed to TCDD, yielding SMRs with broad CIs and high correlation between exposure to PCP and TCDD. Hertzman's study of Canadian sawmill workers reported an SMR of 1.1 (CI 0.9–1.5) and an SIR of 0.9 (CI 0.7–1.1).

Environmental Studies

Renal cancer totals were not reported in Gambini's study of rice growers (Gambini et al., 1997). In Bertazzi's continued Seveso follow-up (Bertazzi et al., 1997), genitourinary tract totals were reported for men, that apparently included bladder cancer, which was also reported separately. SMRs were equal to or less than unity in all zones where numbers were sufficient for analysis. In a study of employees exposed after a reactor accident (Ott and Zober, 1996), five cases of bladder and kidney cancer were observed, with an SIR of 1.4 (CI 0.4–3.2). In a study of Swedish fishermen with unknown exposure to TCDD (Svensson et al., 1995), neither SMRs nor SIRs exceeded unity, with 34 cases observed and 16 deaths. A study of Icelandic pesticide users reported an SIR of 0.52 for cancer of other urinary organs (besides testis and prostate) based on three cases (Zhong and Rafnsson, 1996).

Veteran's Studies

No genitourinary cancer results are reported in either study of Vietnam veterans by Watanabe and Kang (1995, 1996). Clapp's (1997) update of Massachusetts' veterans reported an OR of 1.0 (CI 0.4–2.3) for kidney cancer incidence, based on 55 cases. Crane and colleagues, comparing the mortality of all Australian military veterans of Vietnam with the rest of the male Australian population, reported an SMR of 1.2 (CI 0.8–1.9) based on 22 deaths during 1982–1994 (Crane et al.,

TABLE 7-23 Selected Epidemiologic Studies—Renal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	26	1.6 (1.1–2.4)
	Workers exposed to any phenoxy herbicide or chlorophenol		1.1 (0.7–1.6)
Studies reviewed in *Update 1996*			
Mellemgaard et al., 1994	Danish Cancer Registry patients		
	Occupational herbicide exposure among males	13	1.7 (0.7–4.3)
	Occupational herbicide exposure among females	3	5.7 (0.6–5.8)
Blair et al., 1993	U.S. farmers in 23 states		
	White males	522	1.1 (1.0–1.2)
	Nonwhite males	30	
	White females	6	
	Nonwhite females	6	
Studies reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farm workers	141	0.6 ($p < .05$)
Fingerhut et al., 1991	NIOSH cohort	8	1.4 (0.6–2.8)
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)
Alavanja et al., 1989	USDA forest conservationists		1.7 (0.5–5.5)
	Soil conservationists		2.4 (1.0–5.9)
Henneberger et al., 1989	Paper and pulp workers	3	1.5 (0.3–4.4)
Alavanja et al., 1988	USDA agricultural extension agents		1.7 (0.9–3.3)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0.0–6.2)
Robinson et al., 1986	Paper and pulp workers	6	1.2 (0.5–3.0)
Coggon et al., 1986	British MCPA production workers	5	1.0 (0.3–2.3)
Lynge, 1985	Danish male production workers	3	0.6
Wiklund, 1983	Swedish agricultural workers	775	0.8 (0.7–0.9)[b]
Blair, 1983	Florida pesticide applicators	1	0.5
Burmeister, 1981	Farmers in Iowa	178	1.1 (NS)
ENVIRONMENTAL			
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents		
	Males in zone R	10	0.9 (0.4–1.7)
	Females in zone R	7	1.2 (0.5–2.7)
Studies reviewed in *VAO*			
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)

TABLE 7-23 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	22	1.2 (0.8–1.9)
Crane et al., 1997b	Australian national service veterans	3	3.9
Studies reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans	21	1.4 (0.9–2.2)
Studies reviewed in *VAO*			
Breslin et al., 1988	Army Vietnam veterans	55	0.9 (0.5–1.5)
	Marine Vietnam veterans	13	0.9 (0.5–1.5)
Kogan and Clapp, 1988	Massachusetts Vietnam veterans	9	1.8 (1.0–3.5)
Anderson et al., 1986a	Wisconsin Vietnam veterans	1	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	2	—

NOTE: NS = not significant.
[a] Given when available.
[b] 99% CI.

1997a). A second study examining the mortality experience of conscripted Australian veterans relative to military personnel who did not serve in the conflict reported a statistically significant RR of 3.9 based on three deaths among Vietnam veterans and one in the comparison population between 1982 and 1994 (Crane et al., 1997b). Neither Australian study has exposure information.

Synthesis

Since *Update 1996*, only the study by Kogevinas and colleagues (1997) points to a possible association of herbicides with renal cancer. However, although this result is mildly suggestive, because of the marginal significance, lack of trend data, and heterogeneity of the cohorts it is not strong enough to outweigh the equivocal results from other studies.

Conclusions

Strength of Evidence in Epidemiologic Studies

In the judgment of the committee there is still inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and renal cancer.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and renal cancer is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

BRAIN TUMORS

Background

According to the American Cancer Society, approximately 9,800 men and 7,600 women will be diagnosed with new cases of brain and other nervous system cancers (ICD·9 191.0–191.9, 192.0–192.3, 192.8–192.9) in the United States in 1998, and 7,300 men and 6,000 women will die from them (ACS, 1998). These numbers represent approximately 1.5 percent of new cancer diagnoses and 2.5 percent of deaths.

For individuals in the United States age 45–59, brain cancer is slightly more common in males than females and slightly more common in whites than African Americans.

Exposure to ionizing radiation is an established risk factor for brain cancer. Several other potential factors have been examined, but the American Cancer Society notes that the majority of brain cancers are not associated with any known risk factors.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Brain Cancer

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
Males	7	7	4	10	11	5	14	14	10
Females	5	5	3	7	8	3	8	9	5

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

In *VAO* and *Update 1996*, the committee reviewed relevant reports on brain cancers, including 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; Alavanja et al., 1988; Musicco et al., 1988; Wigle et al., 1990; Morrison et al., 1992; Ronco et al., 1992; Blair et al., 1993; Dean, 1994); pesticide appliers (Blair et al., 1983; Swaen et al., 1992; Asp et al., 1994); paper and pulp workers (Robinson et al., 1986; Henneberger et al., 1989); the Seveso population (Bertazzi et al., 1989a,b; Pesatori et al., 1992; Bertazzi et al., 1993); and Vietnam veterans (Lawrence et al., 1985; Anderson et al., 1986a,b; Boyle et al., 1987; Breslin et al.,

1988; Thomas and Kang, 1990; Dalager et al., 1995a; Visintainer et al., 1995). The majority of the studies found no excess risk of central nervous system tumors.

Update of the Scientific Literature

In an update and expansion of the IARC cohort study, Kogevinas et al. (1997) examined cancer mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. These cohorts were assembled from 12 countries, drawn from national studies that followed the same core protocol jointly developed by the participants and coordinated by IARC.

Nonsignificantly decreased risk of death from brain cancer was observed among the group including all workers exposed to any phenoxy herbicide or chlorophenol (SMR = 0.7, CI 0.4–1.0, 22 deaths). When this group was divided into those exposed and unexposed to TCDD or higher chlorinated dioxins, the result was unchanged, and both groups had nonsignificantly decreased risks (exposed SMR = 0.6, CI 0.3–1.1, 12 deaths; unexposed SMR = 0.8, CI 0.4–1.5, 10 deaths). More detailed analysis by exposure variables such as duration and time since first exposure was not conducted for brain cancers.

Although the study included large numbers of workers who were likely to be exposed at levels substantially higher than general population exposures, lack of information about actual exposure limits the investigator's ability to examine exposure–response relationships within the cohort. In addition the inclusion of workers in the exposed group based on ever having worked in a job considered to involve exposure makes it impossible to distinguish heavily exposed workers from those with very minor exposures.

Becher et al. (1996) examined cancer mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population included workers who had a least one month of employment, resulting in a cohort consisting of 2,479 male workers. The cohort was assembled from four plants, and analysis was conducted on the total cohort divided into four subcohorts corresponding to each plant considered separately.

Based on production information and limited blood dioxin measurements, subcohorts I and II are supposed to have higher TCDD exposures than subcohorts III and IV. Of the four subcohorts, only group I had at least one observed or expected death for brain cancer. An SMR of 2.3 (CI 0.5–6.8) was reported, based on three observed cases.

Ramlow et al. (1996) examined mortality in a cohort of workers exposed to pentachlorophenol, as part of a larger study of Dow chemical manufacturing workers exposed to higher chlorinated dioxins. The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential PCDD exposure between 1937 and 1980.

In the study analysis, the U.S. white male death rates (five-year age and calendar specific) and the non-PCP and PCDD male Dow Michigan employees for 1940–1989

were both used as reference values to calculate expected deaths. Four exposure groups were developed for TCDD (1 unit = very low, 1–1.9 = low, 2–2.9 = medium, 3 = high). Calculation of SMRs with exposure lagged by 15 years using both the U.S. and the Dow referent populations found no significant excess mortality for brain cancer (one death observed, one expected). Brain cancer was not included in the more detailed analysis by the four categories of cumulative exposure.

Cancer mortality among a cohort of rice growers in northern Italy was investigated by Gambini et al. (1997). Using a set of registered farm owners consisting of 1,493 males who worked on farms from 1957 to 1992, they examined the cause of death for 958 subjects and compared this with expected numbers calculated from national rates. No direct exposure information was available, so employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides employed during the study period. A nonsignificant decrease in brain cancer mortality (SMR = 0.9, CI 0.2–2.3, 4 deaths) was reported. Brain cancer was not included in the more detailed analysis with stratification by age at death and duration of exposure (employment as a farmer).

Bertazzi et al. (1997) continued the follow-up of people environmentally exposed to TCDD in Seveso, Italy. The events that led to the exposure and the methods used to study this population have been fully described in earlier reports. This report updates the population after 15 years follow-up. Death from brain cancer was nonsignificantly elevated for men in zone R (SMR = 1.3, CI 0.7–2.3, 12 observed deaths) and for women in zone B (SMR = 3.2, CI 0.6–9.4, 3 deaths) and zone R (SMR = 1.1, CI 0.5–2.2, 8 deaths). More detailed investigation of subjects exposed in zone B did not include brain cancer.

Svensson et al. (1995) studied mortality and cancer incidence in two cohorts of Swedish fishermen. One group (2,896 men) resided on the east coast of Sweden and consumed fish from the Baltic Sea. These fatty fish (particularly salmon and herring) are reported to contain elevated levels of PCB, PCDD, and PCDF. The other group of fishermen (8,477) resided on the west coast of Sweden and were presumed to have a higher intake of lean (and less contaminated) fish, including cod and flat fish. This distinction in exposure by place of residence is reportedly confirmed by the finding that blood levels of dioxin-like compounds were two times higher among east coast than west coast fishermen; however, no data are provided to support this point. East coast fishermen were found to have nonsignificantly decreased mortality and incidence of brain cancer, whereas west coast fishermen had nonsignificantly decreased incidence and nonsignificantly increased mortality (SMR = 1.0, CI 0.6–1.7, 15 deaths observed) compared to Swedish national rates

In a comparison of mortality between Army Chemical Corps Vietnam and non-Vietnam veterans, Dalger and Kang (1997) reported that there was a nonsignificant excess of deaths from brain cancer among Vietnam veterans. The study compared 2,872 Vietnam veterans with 2,737 non-Vietnam veterans (all of whom served in Chemical Corps specialties). All study subjects served at least 18 months' active duty between 1965 and 1973, and vital status ascertainment was

complete for both groups. A nonsignificant increase in death for Vietnam veterans (RR = 1.1, CI 0.1–4.1, 2 observed deaths) and a nonsignificant decrease for non-Vietnam veterans (RR = 0.8, CI 0.0–4.2, 1 death observed) from brain cancer was found compared to U.S. general population rates. When Vietnam and non-Vietnam cohorts were compared directly, the crude rate ratio of brain cancer death was 1.89 (Vietnam versus non-Vietnam). Direct exposure information on the two cohorts was not available, and the presumption that the Vietnam veterans had potentially higher levels of dioxin exposure because of their duties involving Agent Orange and other dioxin-contaminated herbicides (compared to the non-Vietnam Chemical Corps veterans) has not been verified.

Crane et al. (1997a) examined the mortality experience of male Australian Vietnam veterans from 1980 to 1994. The cohort consists of 59,036 male veterans, who were followed from 22 to 32 years. There were 2,067 deaths recorded among this group from 1980 to 1994, and vital status was ascertained for 96.9 percent of the cohort. No excess mortality was observed for brain cancer in the overall military population (SMR = 1.1, CI 0.8–1.5, 39 deaths) or when analyzed separately by branch of service. Study authors have described the strengths and limitations of the Australian veterans cohort study, including virtually complete identification of the study population, a period of follow-up ranging from 22 to 32 years, and vital status ascertainment of 96.9 percent. Among the weaknesses of the study are the possibility of underascertainment of death and the uncertain quality of exposure assessment to a variety of risk factors, including smoking and alcohol consumption, as well as herbicide and dioxin exposure.

Synthesis

As in *VAO* and *Update 1996*, the studies reviewed in this report found a small number of cases of brain tumors, and the RRs associated with herbicide exposure are fairly evenly distributed around 1.0, with relatively narrow confidence intervals.

Conclusions

The committee has not changed the conclusion of the earlier reports that there is limited/suggestive evidence of no association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and brain tumors.

Strength of Evidence in Epidemiologic Studies

The evidence regarding association is drawn from occupational, environmental, and veteran studies in which subjects were exposed to a variety of herbicides and herbicide components. These studies reported a small number of cases of brain tumors, with the risks associated with herbicide exposure fairly evenly distributed around 1.0, and with relatively narrow confidence intervals.

TABLE 7-24 Selected Epidemiologic Studies—Brain Tumors

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	12	0.6 (0.3–1.1)
	Workers not exposed to TCDD (or higher chlorinated dioxins)	10	0.8 (0.4–1.5)
	Workers exposed to any phenoxy herbicide or chlorophenol	22	0.7 (0.4–1.0)
Gambini et al., 1997	Italian rice growers	4	0.9 (0.2–2.3)
Becher et al., 1996	German chemical production workers		
	Subcohort I	3	2.3 (0.5–6.8)
	Subcohort II	0	
	Subcohort III	0	
	Subcohort IV	0	
Ramlow et al., 1996	Pentachlorophenol production workers		
	0 year latency	1	
	15 year latency	1	
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	3	1.2 (0.3–3.6)
Dean, 1994	Irish farmers and farm workers		
	Males	195	
	Females	72	
Blair et al., 1993	U.S. farmers in 23 states		
	White males	447	1.2 (1.1–1.3)
	Nonwhite males	16	1.0 (0.6–1.6)
	White females	9	1.1 (0.5–2.1)
	Nonwhite females	1	0.4 (0.0–2.1)
Studies reviewed in *VAO*			
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
Swaen et al., 1992	Dutch herbicide applicators	3	3.2 (0.6–9.3)
Fingerhut et al., 1991	NIOSH cohort	5	0.7 (0.2–1.6)
Saracci et al., 1991	IARC cohort	6	0.4 (0.1–0.8)
Wigle et al., 1990	Saskatchewan farmers	96	1.0 (0.8–1.3)
Alavanja et al., 1989	USDA forest/soil conservationists	6	1.7 (0.6–3.7)
Henneberger et al., 1989	Paper and pulp workers	2	1.2 (0.1–4.2)
Alavanja et al., 1988	USDA agricultural extension agents		1.0 (0.4–2.4)
Bond et al., 1988	Dow 2,4-D production workers	0	— (0.0–4.1)
Musicco et al., 1988	Men and women in the Milan, Italy, area	61	1.6 (1.1–2.4)
Coggon et al., 1986	British MCPA production workers	11	1.2 (0.6–2.2)
Robinson et al., 1986	Paper and pulp workers	4	0.6 (0.2–2.1)
Lynge, 1985	Danish male production workers	4	0.7
Blair et al., 1983	Florida pesticide applicators	5	2.0
Burmeister, 1981	Farmers in Iowa	111	1.1 (NS)

TABLE 7-24 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	Males—zone A	0	
	Males—zone B	1	0.8 (0.0–4.2)
	Males—zone R	12	1.3 (0.7–2.3)
	Females—zone A	0	
	Females—zone B	3	3.2 (0.6–9.4)
	Females—zone R	8	1.1 (0.5–2.2)
Svensson et al., 1995	Swedish fishermen mortality		
	East coast	2	0.6 (0.1–2.1)
	West coast	15	1.0 (0.6–1.7)
	Swedish fishermen incidence		
	East coast	3	0.5 (0.1–1.4)
	West coast	24	0.9 (0.6–1.4)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso male and female residents—zones A and B	0	
	Male residents—zone R	6	0.6 (0.3–1.4)
	Female residents—zone R	6	1.4 (0.6–3.4)
Studies reviewed in *VAO*			
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)
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)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	39	1.1 (0.8–1.5)
Crane et al., 1997b	Australian national service veterans	13	1.4
Dalager and Kang, 1997	Army Chemical Corps veterans	2	1.9[b]
Studies reviewed in *Update 1996*			
Dalager et al., 1995a	Women Vietnam veterans	4	1.4 (0.4–3.7)
Visintainer et al., 1995	Michigan Vietnam veterans	36	1.1 (0.8–1.5)
Boyle et al., 1987	Vietnam Experience Study	3	
Studies reviewed in *VAO*			
Thomas and Kang, 1990	Army Chemical Corps Vietnam veterans	2	5.0
Breslin et al., 1988	Army Vietnam veterans	116	1.0 (0.3–3.2)
	Marine Vietnam veterans	25	1.1 (0.2–7.1)
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] Crude rate ratio of Vietnam to non-Vietnam veterans.

Biologic Plausibility

Although a possible association between these exposures and brain cancer is considered plausible given current knowledge of ways in which dioxin and herbicides affect human systems, the literature reviewed for this report does not support a change from the previous conclusion of limited/suggestive evidence of no association. A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and brain tumors is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

NON-HODGKIN'S LYMPHOMA

Background

Non-Hodgkin's lymphoma (NHL) (ICD·9 200.0–200.8, 202.0–202.2, 2028–202.9) is the more common of the two primary types of cancer of the lymphatic system. The American Cancer Society estimates that 31,100 men and 24,300 women will be diagnosed with this disease in the United States in 1998 and that 13,000 men and 11,900 women will die from it (ACS, 1998). Collectively, lymphomas (which also include Hodgkin's disease) are the fifth most common form of cancer in the United States and the sixth leading cause of cancer death.

NHL incidence is uniformly higher in males than females and, in most age groups, higher in whites than African Americans. In the cohorts that characterize most Vietnam veterans, rates increase with age for whites and vary inconsistently for African Americans.

The causes of NHL are poorly understood. Individuals with suppressed or compromised immune systems are known to be at higher risk, and some studies show increased incidence in individuals with HIV, human T-cell lymphotropic virus (HTLV), Epstein-Barr virus, and gastric *Helicobacter pylori* infections. A number of behavioral, occupational, and environmental risk factors have also been proposed (Blair et al., 1997).

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Non-Hodgkin's Lymphoma

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	24	24	33	30	31	27	36	37	31
females	12	12	9	18	19	16	26	27	14

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

The original *VAO* committee concluded that a positive association existed between exposure to herbicides and the development of NHL. A large, well-con-

ducted case-control study in Sweden by Hardell (1981) examined NHL and Hodgkin's disease together and found an OR of 6.0 (CI 3.7–9.7) for exposure to phenoxy acids or chlorophenols, based on 105 cases. These results were replicated in further investigations of the validity of exposure assessment and other potential biases (Hardell, 1981). Similar data by Persson et al. (1989) showed an 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 were generally positive for an association between NHL and herbicides or TCDD; however, only some were statistically significant. All of the studies of U.S. agricultural workers reviewed showed elevated RRs (although none were statistically significant), and two National Cancer Institute studies of farmers in Kansas and Nebraska (Hoar et al., 1986; Zahm et al., 1990) showed 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, were consistent with an association. 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)—indicated no increased risk. Thus, unlike most of the other cancers studied in *VAO*, where the data did not distinguish between the effects of herbicides and TCDD, these data suggested that the phenoxy herbicides (including 2,4-D) rather than TCDD are associated with NHL.

Occupational studies discussed in *Update 1996* included data demonstrating an increased but nonsignificant risk of NHL associated with manufacture of 2,4,5-T or TCDD (Kogevinas et al., 1995) and with herbicide manufacture (Bloemen et al., 1993; Bueno de Mesquita et al., 1993). Similarly, several studies of agricultural and forestry workers also reported increased risks of NHL in farmers using herbicides (Morrison et al., 1994), including the statistically significant increases reported in a large U.S. PCMR study (Blair et al., 1993) and a cancer mortality study from Ireland (Dean, 1994). Environmental studies included the Seveso cohort (Bertazzi et al., 1993), which showed nonsignificant increases in zones A and B for men. Finally, a study of Michigan veterans (Visintainer et al., 1995) showed that the PMR was significantly increased for NHL, although no data were available on herbicide exposure.

The conclusion of *Update 1996* was that the more recently published scientific literature continued to support the positive association between exposure to herbicides and NHL.

Update of the Scientific Literature

Occupational Studies

Production Workers Ramlow et al. (1996) evaluated mortality in a cohort of 770 workers with potential PCP exposure for 1940 through 1989, a subset from

a larger cohort of workers with potential exposure to higher chlorinated dioxins. They found an insignificant increase in SMR for lymphopoietic cancer of 1.3 (CI 0.4–3.1). Becher et al. (1996) have also shown a significant increase in SMR for NHL (SMR = 3.3, CI 1.2–7.1, *N* = 6) in a cohort of 2,479 workers in four plants in Germany with exposure to phenoxy herbicide and contaminants (dioxins and furans). A variety of herbicides was produced, including those known to have been contaminated with TCDD. Mortality from all neoplasms increased with latency and was highest in the largest plant where the highest blood levels of TCDD were recorded. A larger study of 21,863 workers from 12 countries was reported by Kogevinas et al. (1997). Subjects in this updated and expanded multinational study coordinated by IARC were followed from 1939 to 1992. Exposure was reconstructed using job records, company exposure questionnaires, and serum and adipose tissue dioxin levels. Among workers exposed to phenoxy herbicides contaminated with TCDD or higher chlorinated dioxins, mortality increased from all malignant neoplasms (SMR = 1.1, 95% CI 1.0–1.2, 710 deaths), as well as NHL (SMR = 1.4, CI 0.9–2.1, 24 deaths). Risks for all neoplasms and for lymphomas increased with time since first exposure. In workers exposed to phenoxy herbicides with minimal or no contamination by TCDD and higher chlorinated dioxins, mortality from all neoplasms (SMR = 1.0, CI 0.9–1.1, 398 deaths), and NHL (SMR = 1.0, 9 deaths), was similar to that expected.

Agricultural Workers In a study reported by Gambini et al. (1997), data were analyzed for a cohort of rice growers that contained 1,493 subjects and for a follow-up that was more than 99 percent complete with regard to both traced subjects and known causes of deaths. A total of 960 subjects (65 percent) died during the observation period (1957–1992). A nonsignificant risk (SMR = 1.3, CI 0.3–3.3) was found for NHL among workers with longer exposure during the period when phenoxy herbicide was in use (1950–1992). Two recent case-control studies (Amadori et al., 1995; Nanni et al., 1996) reported an OR of 1.8 (CI 1.2–2.6) for agricultural farmer-breeders at risk for development of NHL and chronic lymphocetic leukemia combined. A case-control study of NHL and myeloma incidence rates in Italy (Masala et al., 1996) found higher than expected rates of low-grade follicular lymphomas contributed to the excess NHL cases in agricultural areas (known to be exposed to phenoxyacetic acid herbicides), whereas intermediate histocytic lymphomas produced the excess among urban populations known to have higher than expected rates of exposure to organic solvents.

Tatham et al. (1997) examined the relationship between occupational exposures and three subgroups of NHL: small-cell diffuse lymphomas (*N* = 185), follicular lymphomas (*N* = 268), and large-cell diffuse lymphomas (*N* = 526). There were 1,659 controls available for comparison. After controlling for demographic variables and previously identified risk factors for NHL, a significant

positive association was observed for solvent exposure and small-cell diffuse lymphomas (OR = 1.6, CI 1.1–2.2) and a borderline significant association for meat packaging or processing and follicular lymphoma (OR = 1.6, CI 1.0–2.6). However, exposures to herbicides nonsignificantly elevated the risk of only follicular NHL (farming exposure: OR = 1.1, CI 0.8–1.5; non-farming exposure: OR = 1.3, CI 0.8–1.9). No variance from null was observed for the overall NHL cohort or for specific exposure to chlorophenoxy herbicides.

Lastly, a meta-analysis of several studies described above examining the association between NHL and employment as a farmer in the central United States was performed by Keller-Byrne et al. (1997) to test the observation of Blair et al. (1993) that this group is at excess risk of NHL. Six studies were selected for meta-analysis, and the estimated RR was 1.3 (CI = 1.2–1.6).

Environmental Studies

A single new study describing a small German cohort involved in a 1953 reactor accident was reported by Ott and Zober (1996). No cases of NHL have yet been reported among 243 workers followed until 1992, including those whose exposure to TCDD, based on analysis of blood lipids, indicated that they were among a higher-risk group (TCDD doses of >1 μg/kg body weight). The recently updated Seveso cohort analysis (Bertazzi et al., 1997) also failed to show any increase in NHL deaths among men or women with the exception of men in zone B (RR = 3.3, CI 0.4–11.9).

Vietnam Veteran Studies

In a report by Watanabe and Kang (1996), the mortality experience of 33,833 U.S. Army and Marine Corps Vietnam veterans who died during 1965–1988 was compared with that of 36,797 deceased non-Vietnam veterans using PMRs. Military service information was abstracted from military personnel records and cause of death information from death certificates. This study showed statistically increased risks of NHL among Marine (PMR 1.7, CI 1.2–2.2) but not among Army veterans. Similarly, the Australian Vietnam veterans' report (Crane et al., 1997a) reported the RR of death due to NHL to be 1.3 (CI 0.5–3.5) although this was not statistically significant.

Cytogenetic Studies

To further investigate the possible relationships between agricultural pesticide exposure and increased risk of NHL among farm workers in the north central United States, Garry et al. (1996) performed G-banded chromosome analyses of peripheral blood from workers classified according to primary types of pesticide exposure: herbicides (N = 20), insecticides (N = 18), fumigants (N = 23), and

occupationally unexposed controls (N = 33). Significantly increased rearrangement frequencies were demonstrated in fumigant and insecticide appliers compared to control subjects. At certain chromosome bands, significant excesses of breaks were observed in herbicide appliers, but no breaks were observed in controls. Some of these bands contained genes with potential implications for cancer risk, including oncogenes and genes involved in tumor suppression and apoptosis. Of particular interest with regard to lymphoma risk were the excess rearrangements and breaks involving band 14q32 in fumigant appliers and the excess breaks involving band 18q21 in herbicide appliers; translocations linking 14q32 and 18q21 are the most common rearrangements observed in NHL patients. The potential pathobiological relevance of these cytogenetic events warrants additional investigation at the molecular level.

Synthesis

The recent scientific literature continues to support the conclusion of a positive association between exposure to herbicides and non-Hodgkin's lymphoma and provides a biological rationale that includes the possible interaction between environmental toxins such as phenoxyacid herbicides and oncogene abnormalities of bcl-1 and bcl-2 found in follicular NHL, a tumor that is sharply increasing in incidence in Western countries.

Conclusions

Strength of Evidence in Epidemiologic Studies

Evidence continues to accumulate to conclude of a positive association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and non-Hodgkin's lymphoma. The strength of the evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components and is increasing in significance, including among studies of Vietnam veterans.

Biologic Plausibility

The biologic plausibility for an etiological cause of follicular NHL, at least, is also strong, given the increasing incidence of these tumors in Western countries and the demonstration of association of high exposure to herbicides and specific chromosome breaks related to NHL oncogenesis. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and NHL is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

(text continues on page 371)

TABLE 7-25 Selected Epidemiologic Studies—Non-Hodgkin's Lymphoma

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers		1.3 (0.3–3.3)
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)	24	1.4 (0.9–2.1)
	Workers exposed to any phenoxy herbicide or chlorophenol	9	1.0
Becher et al., 1996	German chemical production workers	6	3.3 (1.2–7.1)
Keller-Byrne et al., 1997	Farmers in the central United States		1.3 (1.2–1.6)
Nanni et al., 1996	Italian farming and animal-breeding workers	23[e]	1.8 (1.2–2.6)
Ramlow et al., 1996	Pentachlorophenol production workers	[f]	1.3 (0.4–3.1)
Amadori et al., 1995	Italian farming and animal-breeding workers	164	1.8 (1.2–2.6)
Studies reviewed in *Update 1996*			
Kogevinas et al., 1995	IARC cohort diagnosed with NHL		
	Exposed to 2,4,5-T		1.9 (0.7–4.8)
	Exposed to TCDD		1.9 (0.7–5.1)
Asp et al., 1994	Finnish herbicide applicators	1	0.4 (0.0–2.0)
Dean, 1994	Irish farmers and farm workers		
	Males	244[e]	
	Females	84[e]	
Hardell et al., 1994	Male residents of northern Sweden		
	Exposure to phenoxy herbicides	25	5.5 (2.7–11.0)
	Exposure to chlorophenols	35	4.8 (2.7–8.8)
Morrison et al., 1994	Farm operators in 3 Canadian provinces		
	All farm operators		0.8 (0.7–0.9)
	Highest quartile of herbicides sprayed	19	2.1 (1.1–3.9)
	Highest quartile of herbicides sprayed relative to no spraying	6	3.0 (1.1–8.1)
Blair et al., 1993	U.S. farmers in 23 states (white males)	843	1.2 (1.1–1.3)
Bloemen et al., 1993	Dow 2,4-D production workers	2	2.0 (0.2–7.1)
Bueno de Mesquita et al., 1993	Dutch production workers		
	Workers exposed to phenoxy herbicides	2	3.0 (0.4–10.8)
Lynge, 1993	Danish male production workers	10	1.7 (0.5–4.5)
Persson et al., 1993	Swedish NHL patients		
	Exposure to phenoxy herbicides		2.3 (0.7–7.2)
	Occupation as a lumberjack		6.0 (1.1–31.0)
Zahm et al., 1993	Females in eastern Nebraska farms		1.0 (0.7–1.4)
Kogevinas et al., 1992	IARC cohort		
	Workers exposed to any phenoxy herbicide or chlorophenol	11	1.0 (0.5–1.7)
Studies reviewed in *VAO*			
Hansen et al., 1992	Danish gardeners—men and women	8	2.0 (0.9–3.9)

continued

TABLE 7-25 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Ronco et al., 1992	Danish farm workers—self-employed and employees	147	1.0
	Italian farm workers—self-employed and employees	14	1.3
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)
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)
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)
Alavanja et al., 1989	USDA soil conservationists		1.8 (0.7–4.1)
	USDA forest conservationists		2.5 (1.0–6.3)
Corrao et al., 1989	Italian farmers licensed to apply pesticides		
	Licensed pesticide users and nonusers	45	1.4 (1.0–1.9)
	Farmers in arable land areas	31	1.8 (1.2–2.5)
LaVecchia et al., 1989	Residents of the Milan, Italy, area		
	Agricultural occupations		2.1 (1.3–3.4)
Persson et al., 1989	Orebro Hospital		
	Exposed to phenoxy acids	6	4.9 (1.0–27.0)
Wiklund et al., 1989b	Swedish pesticide applicators	27	1.1 (0.7–1.6)
Alavanja et al., 1988	USDA extension agents		1.2 (0.7–2.3)
Dubrow et al., 1988	Ohio residents	15	1.6 (0.8–3.4)
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)
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)
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)
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)
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)

TABLE 7-25 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Pearce et al., 1986b	Male residents of New Zealand		
	Agricultural sprayers	19[c]	1.5 (0.7–3.3)
Pearce et al., 1985	Male residents of New Zealand		
	Agricultural occupations, ages 20–64		1.4 (0.9–2.0)
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
Riihimiki et al., 1983	Finnish herbicide applicators	0	—
Wiklund, 1983	Swedish agricultural workers		1.1 (0.9–1.2)
Cantor, 1982	Wisconsin residents	175	1.2 (1.0–1.5)
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]
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	Males in zone B		3.3 (0.4–11.9)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents		
	Males in zone B	3	2.3 (0.7–7.4)
	Females in zone B	1	0.9 (0.1–6.4)
	Males in zone R	12	1.3 (0.7–2.5)
	Females in zone R	10	1.2 (0.6–2.3)
Studies reviewed in *VAO*			
Lampi et al., 1992	Finnish community exposed to chlorophenols		
	Compared to two uncontaminated municipalities		2.8 (1.4–5.6)
	Compared to cancer control region		2.1 (1.3–3.4)
Pesatori et al., 1992	Seveso residents		
	Males in zones A and B	3	
	Females in zones A and B	1	
	Males in zone R	13	
	Females in zone R	10	
Bertazzi et al., 1989b	Seveso residents		
	Females in zone B	2	
	Males in zone R	3	
	Females in zone R	4	

continued

TABLE 7-25 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans		1.3 (0.5–3.5)
Watanabe and Kang, 1996	Marine Vietnam veterans		1.7 (1.2–2.2)
Studies reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans	32	1.5 (1.0–2.1)
Studies reviewed in *VAO*			
Clapp et al., 1991	Massachusetts Vietnam veterans		1.2 (0.6–2.4)
Dalager et al., 1991	Vietnam veterans diagnosed with NHL	100	1.0 (0.7–1.8)
O'Brien et al., 1991	Army enlisted Vietnam veterans	7	1.8
Thomas et al., 1991	Women Vietnam veterans	3	1.3 (0.3–1.8)
Watanabe et al., 1991	Army Vietnam veterans compared to Vietnam-era Army 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
CDC, 1990	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)
Michalek et al., 1990	Air Force Ranch Hand veterans mortality	0	
Wolfe et al., 1990	Air Force Ranch Hand veterans morbidity	1	
Breslin et al., 1988	Army Vietnam veterans	108	0.8 (0.6–1.0)
	Marine Vietnam veterans	35	2.1 (1.2–3.8)
Garland et al., 1988	Navy enlisted personnel 1974–1983		0.7
Burt et al., 1987	Army combat Vietnam veterans	39	1.1 (0.7–1.5)
	Marine combat Vietnam veterans	17	3.2 (1.4–7.4)
	Army Vietnam veterans (service 1967–1969)	64	0.9 (0.7–1.3)
	Marine Vietnam veterans (service 1967–1969)	17	2.5 (1.1–5.8)
Fett et al., 1987b	Australian Vietnam veterans	4	1.8 (0.4–8.0)
Anderson et al., 1986a	Wisconsin Vietnam veterans		
	Wisconsin Vietnam veterans compared to Wisconsin nonveterans	13	0.7

TABLE 7-25 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
	Wisconsin Vietnam veterans compared to non-Vietnam-era veterans	13	0.6
	Wisconsin Vietnam veterans compared to Vietnam-era veterans	13	1.0
Anderson et al., 1986b	Wisconsin Vietnam veterans compared to general population	24	0.7
	Wisconsin Vietnam veterans compared to Wisconsin veterans	24	1.1
Holmes et al., 1986	West Virginia Vietnam veterans compared to West Virginia Vietnam-era veterans	2	1.1
Lawrence et al., 1985	New York Vietnam veterans	10	1.0 (0.4–2.2)

[a] Given when available.
[b] Includes both NHL and Hodgkin's disease.
[c] Only NHL other than lymphosarcoma and reticulosarcoma (ICD 202).
[d] NHL, 4 living cases and 3 deaths listed by Boyle et al., 1987.
[e] Includes NHL and chronic lymphocetic leukemia (CLL) combined.
[f] Includes all lymphomas combined.

HODGKIN'S DISEASE

Background

Hodgkin's disease (HD) (ICD·9 201.0–201.9) is distinct from NHL in its cell of origin, demographics, and genetics. According to American Cancer Society estimates, 3,700 men and 3,400 women will be diagnosed with the disease in the United States in 1998, and 700 men and an equal number of women will die from it (ACS, 1998).

HD is less common in individuals in the age groups that characterize most Vietnam veterans than in individuals both younger and older. For individuals older than 40, the incidence rate for males generally exceeds that for females and the rate for whites exceeds that for African Americans. However, the very small number of cases indicates that care should be exercised when interpreting the figures.

The potential infectious nature of HD has been a topic of discussion since its earliest description. Increased incidence in individuals with a history of infectious mononucleosis has been observed in some studies, and a link with Epstein-Barr virus has been proposed. In addition to the occupational associations discussed below, higher rates of the disease have been observed in individuals with suppressed or compromised immune systems.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Hodgkin's Disease

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	3.4	3.5	3.4	3.6	3.7	4.3	3.4	3.3	4.3
females	1.7	1.7	2.5	1.5	1.7	1.5	1.7	1.9	1.7

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

HD, also a malignant lymphoma, is a neoplastic disease characterized by painless, progressive enlargement of lymph nodes, spleen, and general lymphoid tissues. Fewer studies have been conducted of HD in relation to exposure to herbicides or TCDD than of NHL, but the pattern of results is consistent. The 60 HD cases in the study by Hardell et al. (1981) were examined later by Hardell and Bengtsson (1983), who found ORs 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 exposure. The study by Persson et al. (1989) of 54 HD cases showed a large, but not statistically significant, OR of 3.8 (CI 0.5–35.2) for exposure to phenoxy acids. Furthermore, nearly all of the 13 case-control and agricultural worker studies show an increased risk for HD, although only a few of these results are statistically significant for HD. 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 statistically significant.

Occupational studies have included those of Kogevinas (1993) showing no increase in HD among 13,898 production workers or sprayers and a negative U.S. PCMR study of farmers in 23 states (Blair, 1993). A smaller study among Finnish herbicide appliers (Asp et al., 1994) showed a nonsignificant increase in SIR, whereas another study (Persson et al., 1993) reported a significant increase in OR for HD among Swedish farmers exposed to phenoxy acid herbicides.

Neither environmental nor veteran studies provided any additional data to strengthen an association, primarily because of the very low incidence of HD among the Seveso cohorts (Bertazzi et al., 1993) and in selected veteran studies (Visintainer et al., 1995). *Update 1996* nonetheless concluded that the data continued to support a positive association between exposure to herbicides and HD, based primarily on occupational and environmental studies.

Update of the Scientific Literature

Occupational Studies

Two studies recently reported with positive (although nonsignificant) data for the association between TCDD (Becher et al., 1996) or PCP (Ramlow et al.,

1996) and NHL found no similar association for HD among manufacturing workers. An update of a very large occupational cohort by Kogevinas et al. (1997) also showed no association between HD and phenoxy herbicides or chlorophenols (SMR = 1.0, CI 0.5–1.8) but did show a nonsignificant increase of HD among workers exposed to TCDD or higher chlorinated hydrocarbons (SMR = 1.3, CI 0.6–2.5). Among agricultural workers, Gambini et al. (1997) described no increase in HD (SMR = 0.7, CI 0.1–3.6) among rice growers in northern Italy, again confounded by the extremely low incidence of this malignancy (one case observed among 221 cancer deaths). A prospective epidemiologic study conducted in a rural farming community in Michigan (Waterhouse et al., 1996) demonstrated a significant increase in the combined incidence of lymphopoietic neoplasms, namely NHL, HD, and chronic lymphocytic leukemia (CLL); the combined SIR was 1.4 (95% CI 1.0–1.9; $p = .03$) and the SIR for HD was 2.9 (CI 1.1–3.4).

Environmental Studies

The Seveso cohort analysis has recently been updated (Bertazzi et al., 1997) and remains one of the highest documented exposures to TCDD but is limited by the relatively small size of the population in zone A. It has now shown an increase in HD in zone B for men (RR = 3.3, CI 0.4–11.9) and for women (RR = 6.5, CI 0.7–23.5), but no increase for either sex in zone R.

Vietnam Veteran Studies

Watanabe and Kang (1996) compared the mortality experience of 33,833 U.S. Army and Marine Corps Vietnam veterans who died during 1965–1988 with that of 36,797 deceased non-Vietnam veterans using PMRs. Service information was abstracted from military personnel records, and cause of death information from death certificates. As with NHL, they demonstrated a significantly increased PMR (1.9, CI 1.2–2.7) among Marine, but not among Army, Vietnam veterans.

Synthesis

The data for HD are more limited than for NHL, due primarily to the lower incidence of this lymphoreticular tumor. Nonetheless, data drawn from agricultural, production, and environmental exposures and, more recently, from Vietnam veterans continues to support the conclusions of a positive association between herbicides and HD. Although not as clearly demonstrated as for NHL, biologic plausibility also exists for a positive association between TCDD and the development of HD due to their common lymphoreticular origin and association with common risk factors.

Conclusions

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and Hodgkin's disease. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

TABLE 7-26 Selected Epidemiologic Studies—Hodgkin's Disease

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers	1	0.7 (0.1–3.6)
Kogevinas et al., 1997	IARC cohort		1.0 (0.5–1.8)
Becher et al., 1996	German chemical production workers		NS
Ramlow et al., 1996	Pentachlorophenol production workers		NS
Waterhouse et al., 1996	Residents of Tecumseh, Michigan		2.9 (1.1–3.4)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	2	1.7 (0.2–6.0)
Blair et al., 1993	U.S. farmers in 23 states—white males	56	1.0 (0.8–1.3)
Kogevinas et al., 1993	IARC cohort—females	1	
Persson et al., 1993	Swedish NHL patients		
	Exposure to phenoxy herbicides	5	7.4 (1.4–40.0)[d]
Kogevinas et al., 1992	IARC cohort	3	0.6 (0.1–1.7)
Studies reviewed in *VAO*			
Eriksson et al., 1992	Swedish Cancer Registry patients		
	Male sawmill workers	10	2.2
	Male farmers	97	1.2
	Male forestry workers	35	1.2
	Male horticulture workers	11	1.2
Ronco et al., 1992	Danish and Italian farm workers		
	Male Danish farmers—self-employed	27	0.6
	Male Italian farmers—self-employed	10	2.9
	Male Italian farmers—employees	1	0.4
	Male Italian farmers—self-employed and employees	11	1.9
	Female Italian farmers—self-employed	1	1.9
Swaen et al., 1992	Dutch herbicide applicators	1	3.3
Fingerhut et al., 1991	NIOSH cohort	3	1.2 (0.3–3.5)
	20 years latency, 1+ years exposure	1	—
Green, 1991	Herbicide sprayers in Ontario	0	—
Saracci et al., 1991	IARC cohort	2	0.4 (0.1–1.4)
Zober et al., 1990	BASF production workers	0	—
Alavanja et al., 1989	USDA forest/soil conservationists	4	2.2 (0.6–5.6)

TABLE 7-26 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
LaVecchia et al., 1989	Residents of the Milan, Italy, area		
	Agricultural occupations		2.1(1.0–3.8)
	Chemical industry occupations		4.3 (1.4–10.2)
Persson et al., 1989	Orebro Hospital patients		
	Farming	6	1.2 (0.4–3.5)
	Exposed to phenoxy acids	4	3.8 (0.5–35.2)
Wiklund et al., 1989b	Swedish pesticide applicators	15	1.5 (0.8–2.4)
Alavanja et al., 1988	USDA agricultural extension agents		
	PMR analysis	6	2.7 (1.2–6.3)
	Case-control analysis	6	1.1 (0.3–3.5)
Bond et al., 1988	Dow workers with chloracne	1	
Dubrow et al., 1988	Ohio residents	3	2.7
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)
Hoar et al., 1986	Kansas residents		
	All farmers	71	0.8 (0.5–1.2)
	Farm use of herbicides (phenoxy acids and others)		0.9 (0.5–1.5)
	Farmers using herbicides >20 days/year	3	1.0 (0.2–4.1)
	Farmers using herbicides >15 years	10	1.2 (0.5–2.6)
Pearce et al., 1985	Male residents of New Zealand		
	Agricultural occupations, ages 20–64		1.0 (0.6–2.0)
Burmeister et al., 1983	Iowa residents		1.4
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)
	Exposed to low-grade chlorophenols	5	2.4 (0.9–6.5)
Riihimaki et al., 1983	Finnish herbicide applicators	0	—
Wiklund, 1983	Swedish agricultural workers	226	1.0 (0.9–1.2)[b]
Burmeister, 1981	Farmers in Iowa		1.2
Hardell et al., 1980	Umea Hospital patients		
	Exposed to phenoxy acids	41	4.8 (2.9–8.1)[c]
	Exposed to chlorophenols	50	4.3 (2.7–6.9)[c]
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	zone B—male	2	3.3 (0.4–11.9)
	zone B—female	2	6.5 (0.7–23.5)
	zone R—female	4	1.9 (0.5–4.90
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents		
	zone A—male	0	—
	zone A—female	0	—
	zone B—male	1	1.7 (0.2–12.8)
	zone B—female	1	2.1 (0.3–15.7)

continued

TABLE 7-26 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
	zone R—male	4	1.1 (0.4–3.1)
	zone R—female	3	1.0 (0.3–3.2)
VIETNAM VETERANS			
New Studies			
Watanabe and Kang, 1996	Marine and Army Vietnam veterans		1.9 (1.2–2.7)
Studies reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans	20	1.1 (0.7–1.8)
Studies reviewed in *VAO*			
Watanabe et al., 1991	Army Vietnam veterans compared to Vietnam-era Army 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, 1990	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)
Michalek et al., 1990	Air Force Ranch Hand veterans mortality	0	—
Wolfe et al., 1990			
Breslin et al., 1988	Army Vietnam veterans compared to Vietnam-era Army veterans	92	1.2 (0.7–1.9)
	Marine Vietnam veterans compared to Marine Vietnam-era veterans	22	1.3 (0.7–2.6)
Boyle et al., 1987	Vietnam Experience Study	0	—
Fett et al., 1987	Australian Vietnam veterans	0	—
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)
Anderson et al., 1986b	Wisconsin Vietnam veterans	4	—
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[c]	1.0 (0.4–2.2)

[a] Given when available.
[b] 99% CI.
[c] Includes both non-Hodgkin's lymphoma and Hodgkin's disease.
[d] 90% CI.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and Hodgkin's disease is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

MULTIPLE MYELOMA

Background

Multiple myeloma (MM) (ICD·9 203.0, 203.2–203.8) is characterized by proliferation of bone marrow stem cells that results in an excess of neoplastic plasma cells and the production of excess abnormal proteins, usually immunoglobulins. The American Cancer Society estimates that 7,200 men and 6,200 women will be diagnosed with this disease in 1998 and that 5,800 men and 5,500 women will die from it (ACS, 1998).

MM incidence is highly age dependent, with a relatively low rate in individuals under 40 and most cases occurring between 55 and 70 years of age. Rates for African Americans are about twice those for whites. Within racial groups, incidence in males is slightly higher than in females.

Increased incidence of MM has been observed in several occupational groups, including farmers and agricultural workers and those with workplace exposure to rubber, leather, paint, and petroleum (Riedel et al., 1991). Individuals with high exposure to ionizing radiation are also at greater risk. Evidence regarding other risk factors is mixed.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Multiple Myeloma

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
males	4	3	7	6	5	14	11	10	20
females	2	2	5	5	4	13	7	6	20

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.

Summary of *VAO* and *Update 1996*

Although multiple myeloma has been less extensively studied than the NHL, a consistent pattern of elevated risk was described in most studies evaluated for *VAO* and *Update 1996*. Several studies of agricultural and forestry workers provided information on MM risk in relation to herbicide or pesticide exposure. These studies demonstrated an OR or SMR greater than 1.0; several did so at a statistically significant level. The committee determined that the evidence for this association was limited/suggestive, because the individuals in the existing studies (mostly farmers) have, by nature of their occupation, probably been exposed to a

range of potentially carcinogenic agents other than herbicides and TCDD. MM, like NHL and HD, for which there is stronger epidemiologic evidence of an association, is derived from lymphoreticular cells, which adds to the biologic plausibility of an association. The committee concluded that there was continuing accumulation of limited/suggestive evidence of an association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and MM. The evidence regarding this association was drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Update of the Scientific Literature

Occupational Studies

Production Workers In an update and expansion of the IARC cohort study, Kogevinas et al. (1997) examined cancer mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. These workers were assembled from 12 countries, drawn from national studies that followed the same core protocol developed jointly by the participants and coordinated by IARC. Of the total study population, 21,863 (20,851 men and 1,012 women) were classified as exposed to phenoxy herbicides or chlorophenols based on individual job records and company exposure questionnaires; 4,160 were unexposed; and 592 were classified as unknown exposure status. The great majority of workers were considered exposed if they had ever worked in production or spraying of phenoxy herbicides or chlorophenols (four cohorts were exceptions, with minimum employment periods of 1 to 12 months). The period of follow-up also varied between cohorts; overall it extended from 1939 to 1992 (488,482 person-years at report [PYAR]). A total of 4.4 percent (970 workers) were lost to follow-up. Exposure information varied between cohorts, but in general, exposures were reconstructed from job records. The exposed workers were aggregated into five groups: main production, maintenance, other exposed jobs, unspecified tasks, and sprayers. Based on these categories and information on production processes and the composition of materials used, exposed workers were further classified into three categories: exposed to TCDD or higher chlorinated dioxins; unexposed to the same; and unknown exposure to the same.

Analysis was performed by calculating SMRs and 95% CI, using the World Health Organization mortality data bank to calculate national mortality rates by sex, age (five-year intervals), and calendar period (five year). Within-cohort analysis was also performed using Poisson regression adjusting for time since first exposure, duration of exposure, and employment status. MM mortality was nonsignificantly elevated (SMR = 1.3, CI 0.8–2.1, based on 17 deaths) among the group including all workers exposed to any phenoxy herbicide or chlorophenol. When this group was divided into groups exposed and unexposed to TCDD or

higher chlorinated dioxins, the TCDD-exposed group had a slightly lower risk (SMR = 1.2, CI 0.6–2.3) than the unexposed group (SMR = 1.6, CI 0.7–3.1), although neither achieved statistical significance. More detailed analysis by exposure variables such as duration and time of first exposure was not conducted for multiple myeloma.

Although the study includes large numbers of workers who were likely to be exposed at levels substantially higher than the general population, the lack of information available about actual exposures limits the investigator's ability to examine exposure–response relationships within the cohort. In addition, the inclusion of workers in the exposed group based on ever having worked in a job considered exposed makes it impossible to distinguish heavily exposed workers from those with very minor exposures.

Becher et al. (1996) examined cancer mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. These cohorts were also part of the IARC study described above. The population included workers who had at least one month of employment, resulting in a cohort consisting of 2,479 male workers. The cohort was assembled from four plants, and analysis was conducted on the total cohort divided into four subcohorts corresponding to each plant considered separately. The period of follow-up varied between plants, and 100 workers were lost to follow-up. The nature of chemical production varied substantially between plants and over time; some facilities synthesized and formulated a wide range of phenoxy herbicides and chlorophenols (subcohorts III and IV), whereas others produced primarily 2,4,5-T and/or 2,4,5-TCP (subcohorts I and II). SMRs and 95% CI were calculated using West German mortality rates by five-year age and calendar intervals. Cox regression was performed to evaluate the effect of smoking in the one subcohort for which smoking information was available. Each subcohort was analyzed separately since the exposure pattern was judged to be characteristic for each facility. Based on production information and limited blood dioxin measurements, subcohorts I and II are supposed to have higher TCDD exposures than subcohorts III and IV. Of the four subcohorts, only group I had at least one observed death from MM. In this group, 3 deaths were observed versus 0.6 expected for a SMR of 5.4 (CI 1.1–15.9).

Agricultural Workers Cancer mortality among a cohort of rice growers in northern Italy was investigated by Gambini et al. (1997). Using a set of registered farm owners consisting of 1,493 males who worked on farms from 1957 to 1992, they examined the cause of death for 958 subjects and compared this with expected numbers calculated from national rates. No direct exposure information was available, so employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides employed during the study period. Cancer mortality was evaluated for MM and observed and expected deaths were 0 and 1.9 for the overall cohort (SMR = 0.0, CI 0.0–2.0). Although the study population is

small, it does describe the experience of a cohort with good follow-up (99 percent) and long latency (37 percent of deaths observed beyond the age of 80). It is limited by crude exposure assessment, however, and the degree to which study subjects were actually exposed to phenoxy herbicides can not be established with any certainty.

Environmental Studies

Bertazzi et al. (1997) continued the follow-up of people environmentally exposed to TCDD in Seveso, Italy. The events that led to the exposure and the methods used to study this population have been fully described in earlier reports. This report updates the population after 15 years' follow-up. Death from multiple myeloma showed significant increases in zone B for women (RR = 6.6, CI = 1.8–16.8), but not for men. There were no increases in zones A and R for either sex.

Vietnam Veteran Studies

Recent veteran studies are limited because of the small number of MM deaths in the few analyses that have been conducted. The only reported data for MM for U.S. veterans come from the Watanabe and Kang study (1996), showing no increase among either Army or Marine veterans (SMR = 0.9 and 0.6, respectively). The Australian Vietnam veteran study (Crane et al., 1997a) found an SMR of 0.6 (CI 0.2–1.4) based on six deaths. A second study examining the mortality experience of conscripted Australian veterans relative to military personnel who did not serve in the conflict reported no MM deaths among Vietnam veterans and one in the comparison population between 1982 and 1994 (Crane et al., 1997b).

Synthesis

The low incidence of MM among the various cohorts that have been studied makes it difficult to draw firm conclusions from every study. Nonetheless, the consistent pattern of elevated risk appears to continue for the few reports evaluating myeloma cases discussed in this report. Two additional production studies and one subset of the Seveso cohort appear to be at increased risk, with SMRs or RRs greater than 1.0 reported. New studies of Vietnam veterans reported lower than expected MM mortality.

The committee has determined that the evidence for this association is limited/suggestive, because individuals in the existing studies (mostly farmers) have, by nature of their occupation, probably been exposed to a range of potentially carcinogenic agents other than herbicides and TCDD. MM—like NHL and HD, for which there is stronger epidemiologic evidence of an association—is derived from lymphoreticular cells, which adds to the biologic plausibility of an associa-

TABLE 7-27 Selected Epidemiologic Studies—Multiple Myeloma

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers	0	—
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher chlorinated dioxins)		1.2 (0.6–2.3)
	Workers not exposed to TCDD (or higher chlorinated dioxins)		1.6 (0.7–3.1)
	Workers exposed to any phenoxy herbicide or chlorophenol	17	1.3 (0.8–2.1)
Becher et al., 1996	German chemical production workers—Plant I	3	5.4 (1.1–15.9)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	3	2.6 (0.5–7.7)
Dean, 1994	Irish farmers and farm workers	170	1.0
Blair et al., 1993	U.S. farmers in 23 states		
	White males	413	1.2 (1.0–1.3)
	White females	14	1.8 (1.0–3.0)
	Nonwhite males	51	0.9 (0.7–1.2)
	Nonwhite females	11	1.1 (0.6–2.0)
	Farmers in central U.S. states		
	White males	233	1.2
	White females	12	2.6
Lynge, 1993	Danish production workers		
	Male	0	—
	Female	2	12.5 (1.5–45.1)
Semenciw et al., 1994	Farmers in Canadian prairie provinces	160	0.8 (0.7–1.0)
Brown et al., 1993	Iowa male users of pesticides or herbicides	111	1.2 (0.8–1.7)
Zahm et al., 1992	Eastern Nebraska users of herbicides		
	Male	8	0.6 (0.2–1.7)
	Female	10	2.3 (0.8–7.0)
	Eastern Nebraska users of insecticides		
	Male	11	0.6 (0.2–1.4)
	Female	21	2.8 (1.1–7.3)
Studies reviewed in *VAO*			
Eriksson and Karlsson, 1992	Residents of northern Sweden	20	2.2 (1.0–5.7)
Swaen et al., 1992	Dutch herbicide applicators	3	8.2 (1.6–23.8)
Fingerhut et al., 1991	NIOSH cohort	5	1.6 (0.5–3.9)
	20 years latency, 1+ years exposure	3	2.6 (0.5–7.7)
Saracci et al., 1991	IARC cohort	4	0.7 (0.2–1.8)
Alavanja et al., 1989	USDA forest/soil conservationists		1.3 (0.5–2.8)
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)

continued

TABLE 7-27 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
LaVecchia et al., 1989	Residents of the Milan, Italy, area		
	Agricultural employment		2.0 (1.1–3.5)
Morris et al., 1986	Residents of four SEER areas		2.9 (1.5–5.5)
Pearce et al., 1986	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)
Cantor and Blair, 1984	Wisconsin residents		
	Farmers in counties with highest herbicide usage		1.4 (0.8–2.3)
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 ($p < .05$)
Riihimaki et al., 1983	Finnish herbicide applicators	1	2.5 (0.3–14.0)
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	zone B—female		6.6 (1.8–16.8)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents		
	zone A—male	0	—
	zone A—female	0	—
	zone B—male	2	3.2 (0.8–13.3)
	zone B—female	2	5.3 (1.2–22.6)
	zone R—male	1	0.2 (0.0–1.6)
	zone R—female	2	0.6 (0.2–2.8)
Studies reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Males—zones A and B	2	2.7 (0.6–11.3)
	Female—zones A and B	2	4.4 (1.0–18.7)
	Males—zone R	1	0.2 (0.0–1.5)
	Females—zone R	3	0.9 (0.3–3.1)
VIETNAM VETERANS			
New Studies			
Crane et al., 1997a	Australian military veterans	6	0.6 (0.2–1.4)
Crane et al., 1997a	Australian military veterans	0	
Watanabe and Kang, 1996	Army Vietnam veterans		0.9
	Marine Vietnam veterans		0.6
Studies reviewed in *VAO*			
Breslin et al., 1988	Army Vietnam veterans		0.8 (0.2–2.5)
	Marine Vietnam veterans	2	0.5 (0.0–17.1)

[a] Given when available.

tion. The new data available on MM do not change the committee's earlier view that there is a limited/suggestive association between exposure to herbicides and multiple myeloma.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and multiple myeloma. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and multiple myeloma is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

LEUKEMIA

Background

There are four primary types 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): the acute and chronic forms of lymphocytic leukemia and the acute and chronic forms of myeloid (or granulocytic) leukemia. According to American Cancer Society estimates, 16,100 men and 12,600 women will be diagnosed with some form of the disease in the United States in 1998, and 12,000 men and 9,600 women will die from it (ACS, 1998). Collectively, leukemias are expected to account for slightly more than 2 percent of all new cancer diagnoses and nearly 4 percent of cancer deaths in 1998.

The different forms of leukemia have different patterns of incidence and, in some cases, different risk factors.

Acute lymphocytic leukemia (ALL) is a disease of the young and of individuals older than 70 years of age, and plays a rather small role in the age groups that characterize most Vietnam veterans. The lifetime incidence of ALL is slightly higher in whites than in African Americans and in males than females. Exposure to high doses of ionizing radiation is a known risk factor for this form of leukemia; evidence for other factors is inconsistent

Acute myeloid leukemia (AML) is the most common leukemia among adults—incidence increasing steadily with age for individuals older than 40. In

the Vietnam veteran age groups, AML accounts for roughly one out of every four leukemias in men and one out of three in women. Overall, this leukemia is slightly more common in males than females. White males have a higher incidence that white females; the lifetime incidence in African-American males and females is roughly equal. Risk factors associated with an increased risk of AML include high doses of ionizing radiation, occupational exposure to benzene, and some medications used in cancer chemotherapy (melphalan, for example). Genetic disorders including Fanconi's anemia and Down's syndrome are associated with an increased risk of AML, and tobacco smoking has been suggested as a risk factor.

Average Annual Cancer Incidence (per 100,000 individuals) in the United States[a]
Leukemias

	45–49 years of age			50–54 years of age			55–59 years of age		
	all races	white	black	all races	white	black	all races	white	black
All leukemias									
males	8	8	7	13	13	12	20	21	20
females	6	5	7	8	8	5	11	11	9
Acute lymphocytic leukemia									
males	0.6	0.7	[b]	0.4	0.5	[b]	1.2	1.3	[b]
females	0.6	0.6	0.6	0.5	0.5	0.5	0.3	0.3	0.4
Chronic lymphocytic leukemia									
males	1.0	0.9	1.1	2.3	2.3	2.4	4.8	4.8	5.1
females	0.5	0.5	0.2	1.1	1.0	1.1	2.1	2.2	0.7
Acute myeloid leukemia									
males	2.0	2.0	2.2	2.5	2.6	2.6	4.7	4.6	4.8
females	2.2	2.1	2.7	2.8	2.9	1.8	3.4	3.2	3.5
Chronic myeloid leukemia									
males	1.7	1.5	2.5	2.1	2.3	1.7	2.7	2.6	3.2
females	1.0	0.9	1.6	1.7	1.6	2.2	1.8	1.8	2.2
All other leukemias									
males	1.8	2.1	0.3	2.5	2.6	2.6	3.8	3.9	2.7
females	1.0	0.8	1.1	1.2	1.3	0.4	2.0	2.0	0.4

[a] SEER nine standard registries crude, age-specific rate, 1990–1994.
[b] Insufficient data to provide a meaningful incidence rate.
[c] Includes leukemic reticuloendotheliosis (hairy cell), plasma cell, monocytic, and acute and chronic erythremia and erythroleukemia.

Chronic lymphocytic leukemia (CLL) is the most common of the four primary types of leukemia for men. It is largely a disease of individuals older than 40, and incidence doubles every five years for individuals in the three age groups that characterize most Vietnam veterans. Over a lifetime, CLL is nearly twice as common in whites than African Americans and more common in men than women. Some occupational groups, notably farmers, appear to have a higher

incidence of CLL than would otherwise be expected. A family history of the disease and a compromised immune system are among additional suspected risk factors. Unlike the other primary forms of leukemia, exposure to ionizing radiation does not appear to be associated with increased incidence of CLL.

The incidence of chronic myeloid leukemia (CML) increases steadily with age for individuals over 30. Lifetime incidence is roughly equal in whites and African Americans and is slightly higher in males than females. For individuals in the age groups that characterize most Vietnam veterans, CML accounts for approximately one in five leukemias. CML is associated with an acquired chromosomal abnormality known as the "Philadelphia chromosome." Exposure to high doses of ionizing radiation is a known risk factor for this abnormality; other factors are under study.

Little is known about the risk factors associated with other forms of leukemia. However, two human retroviruses have been linked to human leukemias: HTLV-1 appears to cause adult T-cell leukemia or lymphoma, whereas the data linking HTLV-2 to hairy cell leukemia are less definitive.

Summary of *VAO* and *Update 1996*

The epidemiologic evidence for an association between exposure to herbicides and leukemia in *VAO* came primarily from studies of farmers and residents of Seveso, Italy. The observed overall RR for leukemia mortality and incidence in Seveso was elevated, but not significantly. The increase was significant, however, for cases who were in the most highly exposed zone and died five to ten years after the accident. A number of studies of farmers also showed 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 analysis. Also, 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.

The available data on Vietnam veterans are generally not conclusive, because exposure data were inadequate for the cohort being studied. Small sample sizes weaken the studies of the Ranch Hand or Chemical Corps veterans; therefore, excess risks were not likely to be detected.

Since no study has differentiated adequately between exposure solely either to herbicides or to TCDD, or demonstrated a dose–response for any subtype of leukemia, it is not possible to attribute any symptom or subtype of leukemia to result of exposure.

Update 1996 described a number of new studies, each with relatively small numbers of leukemia cases. Three studies of agricultural workers described in the update (Asp et al., 1994; Dean, 1994; Semenciw et al., 1994) described in *Update 1996* failed to show any elevation in chronic or acute leukemia risk, as did one production worker study (Kogevinas et al., 1993).

The U.S. PCMR study of farmers in 23 states (Blair et al., 1993) showed an overall significant increase in leukemia deaths (PCMR = 1.3, CI 1.2–1.4), as did the Seveso update for men in zone B only (Bertazzi et al., 1993). However, the single Vietnam veteran study of this period showed no increased leukemia risk (Visintainer et al., 1995).

For *Update 1996*, the committee concluded that there was inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides and leukemia. The updated studies did not affect the original conclusion of *VAO*.

Update of the Scientific Literature

Occupational Studies

Production Workers In an update and expansion of the IARC cohort study, Kogevinas et al. (1997) examined cancer mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. These cohorts were assembled from 12 countries, drawn from national studies that followed the same core protocol developed jointly by the participants and coordinated by IARC. Of the total study population, 21,863 (20,851 men and 1,012 women) were classified as exposed to phenoxy herbicides or chlorophenols based on individual job records and company exposure questionnaires; 4,160 were unexposed, and 592 were classified as unknown exposure status. The great majority of workers were considered exposed if they had ever worked in production or spraying of phenoxy herbicides or chlorophenols (four cohorts were exceptions, with minimum employment period of 1 to 12 months). The period of follow-up also varied between cohorts; overall it extended from 1939 to 1992 (488,482 PYAR). Overall, 4.4 percent (970 workers) were lost to follow-up. Exposure information varied between cohorts, but in general, exposures were reconstructed from job records.

No significant risk of death from leukemia was observed among the group including all workers exposed to any phenoxy herbicide or chlorophenol. When this group was divided into groups exposed and unexposed to TCDD or higher chlorinated dioxins, the result was unchanged. More detailed analysis by exposure variables such as duration and time since first exposure was not conducted for leukemias.

Becher et al. (1996) examined cancer mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population included workers who had a least one month of employment, resulting in a cohort consisting of 2,479 male workers. The cohort was assembled from four plants, and the analysis as conducted on the total cohort divided into four subcohorts corresponding to each plant considered separately. The period of follow-up varied between plants, and 100 workers were lost to follow-up. The nature of chemical production varied substantially between plants and over time; some

facilities synthesized and formulated a wide range of phenoxy herbicides and chlorophenols (subcohorts III and IV), whereas others produced primarily 2,4,5-T and/or 2,4,5-TCP (subcohorts I and II). SMRs and 95% CI were calculated using West German mortality rates by five-year age and calendar intervals. Cox regression was performed to evaluate the effect of smoking in the one subcohort for which smoking information was available. Each subcohort was analyzed separately since the exposure pattern was judged to be characteristic for each facility. Based on production information and limited blood dioxin measurements, subcohorts I and II were supposed to have higher TCDD exposures than subcohorts III and IV. Of the four subcohorts, only group I had at least one observed or expected death from leukemia. An SMR of 1.8 (CI 0.5–4.1) was reported, based on four observed cases.

Ramlow et al. (1996) examined mortality in a cohort of workers exposed to pentachlorophenol, as part of a larger study of Dow chemical manufacturing workers exposed to the higher chlorinated dioxins. The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential PCDD exposure between 1937 and 1980. From this cohort, 770 workers were identified who were considered to have potential PCP exposure based on work history records. Exposure to PCP was assessed using historical industrial hygiene and process data, resulting in a strategy for ranking jobs by exposure intensity based on a scale of 1 to 3. Exposure to PCDD was analyzed using the process described by Ott et al. (1987), in which semiquantitative, logarithmic exposure intensity scores ranging from 1 to 4 for TCDD and 0 to 2 for H/OCDD were assigned to each job title. Cumulative exposure indices for PCP and dioxin were calculated using these assigned scores. In the study analysis, the U.S. white male death rates (five-year age and calendar specific) and the death rates of non-PCP and PCDD male Dow Michigan employees for 1940 to 1989 were both used as reference values to calculate expected deaths. Leukemia deaths showed no increase (SMR = 1.0, CI 0.1–3.6) in this study. Calculation of SMRs with exposure lagged by 15 years using both the U.S. and the Dow referent populations also found no significant excess mortality for leukemia. Leukemia was not included in the more detailed analysis by the four categories of cumulative exposure.

Agricultural Workers Cancer mortality among a cohort of rice growers in northern Italy was investigated by Gambini et al. (1997). Using a set of registered farm owners consisting of 1,493 males who worked on farms from 1957 to 1992, they examined the cause of death for 958 subjects and compared this with expected numbers calculated from national rates. No direct exposure information was available, so employment on the farm was used as a surrogate for exposure to the range of phenoxy herbicides employed during the study period. A nonsignificant decrease in leukemia mortality (SMR = 0.6, CI 0.2–1.7) was reported. Leukemia was not included in the more detailed analysis with stratification by age at death and duration of exposure (employment as a farmer).

Waterhouse et al. (1996) conducted a survey of total and site-specific cancer incidence among 70,16 male and female adult from 1959 to 1987, and compared the observed number with the expected number, based on age-, sex-, race-, calendar-, and site-specific cancer incidence rates reported by the Connecticut tumor registry. Based on the results of this survey, a hypothesis was advanced concerning the potential risks of exposure to insecticides and herbicides. This was pursued by analyzing for each county in Michigan the comparative annual number of acres and the percentage of acreage treated with agricultural chemicals in 1978 and for 1982–1987. Finally, because of the availability of information on lifestyle risk factors that had been collected in the 1960s on all participants, a nested case-control study was implemented. A significantly increased risk for males and females combined was demonstrated in the incidence of lymphopoietic neoplasms, namely, NHL, HD, and CLL; the combined SIR was 1.4 (95% CI 1.00–1.9; $p = .03$). Comparison of the Tecumseh, Michigan, cohort (ranked highest in pesticide exposure) with all sites combined was not significantly different from the expected incidence in females (SIR = 1.0, CI 0.9–1.1) and was decreased by more than 10 percent in males (SIR = 0.9, CI 0.8–1.0). However, the SIR for NHL in females was significantly elevated (SIR = 1.9; CI 1.1–3.1; $p = .02$); the trend for increased risk of lymphoma and leukemia was also evident in males. In the nested case-control study based on risk factor information documented prior to diagnosis, the RR of a family history of lymphoma, leukemia, or MM was significantly increased among patients with lymphoproliferative neoplasms (OR = 3.8, CI 1.5–9.8; $p = .005$).

Finally, Amadori et al. (1995) conducted a population based case-control study in a highly agricultural area in the northeast of Italy to evaluate the association between farming and animal breeding and the risk of developing NHL and CLL. Occupational histories and other data were collected by personal interview of individuals diagnosed with NHL ($N = 164$), and CLL ($N = 23$) between 1988 and 1990, and 977 controls. Estimates of ORs for occupational variables were calculated, after adjustment for sex, age, altitude of municipality, first-degree familiarity, and previous herpes zoster infection. From analysis of the more frequent occupational categories, no occupation had a significantly high risk. When the two job titles of farmers only and farmer–breeders, who are also involved in animal breeding are classified within the extremely varied occupations of animal breeding, agriculture, or fishing, a high risk for NHL and CLL is seen in the farmer-breeders (OR = 1.8, 95% CI 1.2–2.6). Analyses according to histological type show that risks are concentrated in CLL and in low-grade NHL. No effect or trend by period at work or duration of employment in farming and animal breeding was found.

Environmental Studies

Bertazzi et al. (1997) continued the follow-up of people environmentally exposed to TCDD in Seveso, Italy. The events that led to the exposure and the

methods used to study this population have been fully described in earlier reports. This report updates the population after 15-years follow-up. There were no cases of leukemia in zone A. Among men in zone B, there were seven cases of leukemia (RR = 3.1, CI 1.4–6.4). Among women in zone B, there was also one case of leukemia (RR = 0.6, CI 0.0–3.1). In zone R, where residents had much lower potential exposure to TCDD than in zones A and B, there were 12 cases of leukemia in men (RR = 0.8, CI 0.4–1.4) and 12 cases in women (RR = 0.9, CI 0.4–1.5).

Vietnam Veteran Studies

In a comparison of mortality between Army Chemical Corp Vietnam and non-Vietnam veterans, Dalager and Kang (1997) reported that a nonsignificant excess of deaths from leukemia among Vietnam veterans. The study compared 2,872 Vietnam veterans with 2,737 non-Vietnam veterans (all of whom served in Chemical Corps specialties). All study subjects served at least 18 months' active duty between 1965 and 1973, and vital status ascertainment was complete for both groups. A nonsignificant increase in death for Vietnam veterans (RR = 1.0, CI 0.1–3.8, two observed deaths) and a nonsignificant decrease for non-Vietnam veterans (RR = 0.7, CI 0.4–1.0, one death observed) from leukemia was found compared to general U.S. population rates. When Vietnam and non-Vietnam cohorts were compared directly, the crude rate ratio of leukemia death was 1.9 (Vietnam to non-Vietnam veterans). Direct exposure information on the two cohorts was not available, and the presumption that Vietnam veterans had potentially higher levels of dioxin exposure because of their duties involving Agent Orange and other dioxin-contaminated herbicides (compared to the non-Vietnam Chemical Corps veterans) has not been verified. Very similar data have been reported for the Australian Vietnam veteran cohort (Crane et al., 1997b). Nonsignificant decreases in death due to leukemia for Vietnam veterans (SMR = 0.5, CI 0.1–3.0) and for nonveteran deaths (SMR = 0.7, CI 0.1–3.0) were found compared to general Australian population rates. When Vietnam and non-Vietnam cohorts were compared directly, the crude rate ratio of leukemia death was 0.6 (veterans to nonveterans). Again, direct exposure information for dioxin is not available, and the presumption of higher dioxin exposure for Australian Vietnam veterans has not been verified.

Synthesis

As in *VAO* and *Update 1996*, the studies reviewed in this report found a small number of cases of leukemia, and it is apparent that the risks associated with herbicide exposure are fairly evenly distributed around the null, with relatively narrow confidence intervals. Some data on agricultural workers suggest an increased risk for all hematopoietic neoplasms (including NHL and MM, as well

as CLL), and very limited data from a small subset of the Seveso cohort suggesting an increased leukemia risk. Biologic plausibility would suggest an association of risk for hematopoietic and lymphoreticular malignancies, as would the etiologic similarity of CLL and NHL; however, the overall incidence and small number of positive studies are inadequate to change the previous classification.

Conclusion

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and leukemia. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and leukemia is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

SUMMARY

Based on the occupational, environmental, and veteran studies reviewed, the committee has reached one of four standard conclusions about the strength of the evidence regarding association between an exposure to herbicides and/or TCDD and each of the cancers studied. As explained in Chapter 4, these distinctions 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 committee by the Secretary of Veterans Affairs in Public Law 102-4 and with accepted standards for scientific reviews, the distinctions between these standard conclusions are based on statistical association, not on causality. The committee used the same criteria to categorize diseases by the strength of the evidence as were used in *VAO* and *Update 1996*.

Health Outcomes with Sufficient Evidence of an Association

In *VAO* and *Update 1996*, the committee found sufficient evidence of an association between exposure to herbicides and/or TCDD and three cancers: soft-tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease. The scientific

TABLE 7-28 Selected Epidemiologic Studies—Leukemia

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Gambini et al., 1997	Italian rice growers		0.6 (0.2–1.7)
Kogevinas et al., 1997	IARC cohort	34	1.0 (0.7–1.4)
Becher et al., 1996	German chemical production workers		1.8 (0.5–4.1)
Ramlow et al., 1996	Pentachlorophenol production workers		1.0 (0.1–3.6)
Waterhouse et al., 1996	Residents of Tecumseh, Michigan		1.4 (1.0–1.9)
Amadori et al., 1995	Italian farming and animal-breeding workers		1.8 (1.2–2.6)
Studies reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators	2	—
Semenciw et al., 1994	Farmers in Canadian prairie provinces	357	0.9 (0.8–1.0)
Blair et al., 1993	U.S. farmers in 23 states	1,072	1.3 (1.2–1.4)
Kogevinas et al., 1993	Female herbicide spraying and production workers	1	—
Studies reviewed in *VAO*			
Bueno de Mesquita et al., 1993	Dutch production workers		
	Workers exposed to phenoxy herbicides	2	2.2 (0.3–7.9)
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
Fingerhut et al., 1991	U.S. chemical workers	6	0.7 (0.2–1.5)
Saracci et al., 1991	Chemical workers		
	Exposed	18	
	Probably exposed	0	—
	Nonexposed	3	0.9 (0.2–2.6)
	Unknown exposure	0	—
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)

continued

TABLE 7-28 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Risk (95% CI)[a]
Wigle et al., 1990	Saskatchewan farmers	138	0.9 (0.7–1.0)
Zober et al., 1990	BASF production workers		
	Second additional cohort	1	5.2 (0.4–63.1)
Alavanja et al., 1988	USDA agricultural extension agents		1.9 (1.0–3.5)
Bond et al., 1988	Dow workers with chloracne	2	3.6 (0.4–13.0)
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)
ENVIRONMENTAL			
New Studies			
Bertazzi et al., 1997	Seveso residents		
	Males—zone B	7	3.1 (1.4–6.4)
	Females—zone B	1	0.6 (0.0–3.1)
Studies reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents		
	Males—zone B	2	1.6 (0.4–6.5)
	Females—zone B	2	1.8 (0.4–7.3)
Studies reviewed in *VAO*			
Bertazzi et al., 1993	Seveso residents		
	Males—zones A, B, and R	4	2.1 (0.7–6.9)
	Females—zones A, B, and R	1	2.5 (0.2–27.0)
VIETNAM VETERANS			
New Studies			
Dalager and Kang, 1997	Army Chemical Corps veterans		1.0 (0.1–3.8)
Crane et al., 1997a	Australian military veterans		0.5 (0.1–3.0)
Studies reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans	30	1.0 (0.7–1.5)

[a] Given when available.

literature continues to support the classification of these three cancers in the category of sufficient evidence. Based on the literature, there are no additional cancers that satisfy the criteria necessary for this category.

For diseases 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 also regarded 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.

Health Outcomes with Limited/Suggestive Evidence of Association

In *VAO* and *Update 1996*, the committee found limited/suggestive evidence of an association between herbicide or dioxin exposure and the following cancers: larynx, lung, bronchus (trachea), prostate, and multiple myeloma. The scientific literature continues to support the classification of these diseases in the category of limited/suggestive evidence. Based on the literature, there are no additional cancers that satisfy the criteria necessary for this category.

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.

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

The scientific data for many of the cancers reviewed by the committee were inadequate or insufficient to determine whether an association exists. For these cancers, 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 category includes hepatobiliary cancers (cancers of the liver and intrahepatic bile duct), nasal and nasopharyngeal cancer, bone cancer, skin cancers (including basal cell carcinoma, squamous cell carcinoma, and nonmelanocytic skin cancers), breast cancer, cancers of the female reproductive system (including cervix, endometrium, and ovaries), testicular cancer, urinary bladder cancer, renal cancer (cancers of the kidney and renal pelvis), and leukemias.

Based on an evaluation of all the epidemiologic evidence, including studies published since the release of *Update 1996*, the committee felt that the previous conclusion of limited/suggestive evidence of *no* association between exposure to the herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and urinary bladder cancer should be changed to "inadequate/insufficient evidence to determine whether an association exists." Although there is no evidence that exposure to herbicides or dioxin is related to this cancer, RRs in some of the largest cohorts tended to be greater than one, weakening the committee's prior conclusion that there was positive evidence of *no* relationship. Coexposures to TCDD and a variety of known bladder carcinogens makes it very difficult to isolate the possible additional effect of herbicides, although little total effect was seen.

Health Outcomes with Limited/Suggestive Evidence of *No* Association

In *VAO* and *Update 1996*, the committee found a sufficient number and variety of well-designed studies to conclude that there is limited/suggestive evi-

dence of *no* association between a small group of cancers and exposure to TCDD or herbicides. This group includes gastrointestinal tumors (colon, rectal, stomach, and pancreatic) and brain tumors. The most recent scientific evidence continues to support the classification of such cancers in this category. Based on an evaluation of the whole of the scientific literature, there are no additional cancers that satisfy the criteria necessary for this category.

As noted above, the committee responsible for this report felt that the previous classification of urinary bladder cancer in this category should be changed to "inadequate/insufficient evidence to determine whether an association exists."

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. These studies 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.

Biologic Plausibility

Chapter 3 details the committee's evaluation of data from studies with animals and cells regarding the biologic plausibility of a connection between exposure to dioxin or herbicides and various forms of cancer. This section summarizes that evidence. Some of the preceding discussions of cancer outcomes include references to specific relevant papers.

A number of animal species, including strains of rats, mice, and hamsters, have been exposed to TCDD and examined for increases in cancer and tumor incidence. In these studies, TCDD was fed to animals, applied to their skin, injected under their skin, or injected into the abdominal cavity. This research indicates that TCDD can both cause cancers or tumors and enhance the incidence of certain cancers or tumors in the presence of known carcinogens. Increased cancer rates were observed at several different sites in the body, notably the thyroid gland, skin, and lungs. In studies in which liver cancer incidence was enhanced, other adverse changes in the liver were observed. Decreased rates of some cancers—including those of the uterus; pancreas; and pituitary and mammary glands—were also reported. The sites where effects were observed and the exposure levels needed to induce them varied considerably from species to species.

TCDD can act a promoter in the presence of other carcinogens. One study showed that when a single dose of a known carcinogen was applied to the skin of mice followed by multiple doses of TCDD over several months, more skin tumors were seen than would be expected from the single dose of carcinogen alone. Similar results were obtained in rat livers when a single dose of a liver carcinogen was followed by multiple doses of TCDD.

In female rats, enhanced liver tumor formation associated with TCDD exposure is dependent on the presence of intact ovaries, suggesting that complex hormonal interactions are involved in TCDD-induced carcinogenesis.

More generally, TCDD has a wide range of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. These effects may influence tumor formation.

Studies in animals indicate that most TCDD effects are mediated through the AhR, a protein in animal and human cells to which TCDD can bind. It is hypothesized that TCDD exposure induces binding of the AhR to DNA to alter the information obtained from DNA in a way that transforms normal into abnormal cells. Although structural differences in the AhR have been identified, this receptor operates in a similar manner in animals and humans.

As a tumor initiator and promoter, TCDD has been shown to significantly induce CYP1A1 mRNA levels and ethoxyresorufin O-deethylase (EROD) activity in several types of human cancer cells; that is, TCDD affects some forms of cellular metabolism at a very basic level. Experiments involving several strains of mice provide evidence that a functional AhR is required for TCDD induction of CYP1A1 and liver tumor promotion. However, CYP1A1 induction in various mice strains was not directly related to the degree of tumor-promoting capability, suggesting that other undefined genetic factors may play an important role. There is some evidence that certain cells have mechanisms that serve to regulate CYP1A1 tumor promotion.

There are great differences between the susceptibility of different experimental animals to TCDD-induced effects, and the sites at which tumors are induced also varies from species to species. Some mice strains, for example, are differentially responsive to TCDD liver induction due to differences in a protein called cytochrome P450, indicating a genetic susceptibility. Although expression of P450 is highly cell specific, it exhibits a surprisingly similar pattern of hormonal regulation. Studies conducted to compare the AhR in cultured fetal cells and adult liver tumors from TCDD-responsive and less-responsive mice indicate that the responsiveness of fetal cells is likely mediated by the AhR in these cells and is not due to a different allelic form of AhR ligand binding subunit in fetal versus adult cells.

Evidence has also begun to accumulate for non-AhR mediated effects. There is new evidence that the mechanism by which TCDD induces tumor promotion may involve oxygen radicals, since scavengers of hydroxyl radicals or antioxidants hinder the tumor-promoting effects of TCDD in transformed mice fibroblasts. In support of this, other studies have shown that TCDD induces changes in liver cells that lead to a release of oxygen radicals and subsequent oxidative DNA damage. This also suggests that TCDD tumor promotion may be due to interference with gap junctional intercellular communications.

Controversy exists about the TCDD exposure levels required to induce adverse health outcomes.

Limited information is available on health effects of exposure to the herbicides discussed in this report. 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 produced negative results. However, because some studies do not meet present-day standards for cancer bioassays, and some produced equivocal results, it is not possible to draw confident conclusions at this time.

2,4-D was administered to rats, mice, and dogs in their food, by injecting it under their skin, or placing it directly into their stomachs. All results were negative, except for one study that found an increased rate of brain tumors in male, but not female, rats receiving the highest dose. These tumors also occurred in the control group and thus may have occurred spontaneously and not as a result of 2,4-D exposure. In a recent mutagenicity study, 2,4-D induced significant numbers of mutations in at least one of the cell types tested, either spermatocytes or spermatogonia. Because these results differ from earlier studies, it was hypothesized that different germ cell stages and treatment regimens may account for the observed inconsistencies. Similar results were obtained in a 2,4,5-T mutagenicity study.

2,4,5-T has been administered to rats and mice in their food, in their drinking water, by injecting it under their skin, or by placing it directly into their stomachs. In a recent study, 2,4,5-T exposure increased the formation of DNA adducts by cytochrome P450-derived metabolites of benzo[*a*]pyrene. The latter effects are particularly interesting since they are strikingly similar to those elicited by dioxin.

Picloram has been tested in rats and mice in their food. Results of all of these studies were negative, with the exception of one study in which liver tumors appeared. These were attributed to the presence of a picloram contaminant, hexachlorobenzene.

A recent study indicates that cacodylic acid (also known as dimethylarsinic acid) may induce DNA modifications that sensitize it to free radical injury, whereas another study concluded that it is a promoter of urinary bladder, kidney, liver, and thyroid gland carcinogenesis in rats. In particular, cacodylic acid may promote rat urinary bladder carcinogenesis by stimulating cell proliferation in the urinary bladder epithelium. An exposure study in mice produced negative results.

The foregoing evidence suggests that a connection between TCDD or herbicide exposure and human health effects is, in general, biologically plausible. However, differences in sensitivity and susceptibility across individual animals, strains, and species; the lack of strong evidence of organ-specific effects across species; and differences in route, dose, duration, and timing of exposure complicate any more definitive conclusions about the presence or absence of a mechanism for the induction of site-specific cancers by TCDD.

Considerable uncertainty remains about how to apply this information to the evaluation of potential health effects of herbicides or dioxin exposure in Vietnam veterans. Scientists disagree over the extent to which information derived from

animals and cellular studies predicts human health outcomes and the extent to which the health effects resulting from high-dose exposure are comparable to those resulting from low-dose exposure. Research on biological mechanisms is burgeoning, and subsequent updates of this report may have more and better information on which to base conclusions.

Increased Risk of Disease Among Vietnam Veterans

Under the Agent Orange Act of 1991, the committee is asked to determine (to the extent that available scientific data permit meaningful determinations) the increased risk of the diseases it studies among those exposed to herbicides during their service in Vietnam. Chapter 1 presents the committee's general findings regarding this charge. Where more specific information about particular health outcomes is available, this information can be found in the preceding discussions of those diseases.

REFERENCES

American Cancer Society (ACS) Cancer Facts and Figures [WWW site]. 1998. URL http://www.cancer.org/statistics/cff98/graphicaldata.html (accessed March 12, 1998).

Air Force Health Study. 1996. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update 1996. Brooks AFB, TX: Epidemiologic Research Division. Armstrong Laboratory. AL/AO-TR-1996-0068. 31 pp.

Alavanja MC, Blair A, Merkle S, Teske J, Eaton B. 1988. Mortality among agricultural extension agents. American Journal of Industrial Medicine 14:167–176.

Alavanja MC, Merkle S, Teske J, Eaton B, Reed B. 1989. Mortality among forest and soil conservationists. Archives of Environmental Health 44:94–101.

Amadori D, Nanni O, Falcini F, Saragoni A, Tison V, Callea A, Scarpi E, Ricci M, Riva N, Buiatti E. 1995. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on job titles. Occupational and Environmental Medicine 52(6):374–379.

Anderson HA, Hanrahan LP, Jensen M, Laurin D, Yick W-Y, Wiegman P. 1986a. Wisconsin Vietnam Veteran Mortality Study: Proportionate Mortality Ratio Study Results. Madison: Wisconsin Division of Health.

Anderson HA, Hanrahan LP, Jensen M, Laurin D, Yick W-Y, Wiegman P. 1986b. Wisconsin Vietnam Veteran Mortality Study: Final Report. Madison: Wisconsin Division of Health.

Asp S, Riihimaki V, Hernberg S, Pukkala E. 1994. Mortality and cancer morbidity of Finnish chlorophenoxy herbicide applicators: an 18-year prospective follow-up. American Journal of Industrial Medicine 26:243–253.

Axelson O, Sundell L, Andersson K, Edling C, Hogstedt C, Kling H. 1980. Herbicide exposure and tumor mortality: an updated epidemiologic investigation on Swedish railroad workers. Scandinavian Journal of Work, Environment, and Health 6:73–79.

Becher H, Flesch-Janys D, Kauppinen T, et al. 1996. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins [see comments]. Cancer Causes Control. 7:312–321.

Bender AP, Parker DL, Johnson RA, Scharber WK, Williams AN, Marbury MC, Mandel JS. 1989. Minnesota highway maintenance worker study: cancer mortality. American Journal of Industrial Medicine 15:545–556.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989a. Mortality in an area contaminated by TCDD following an industrial incident. Medicina Del Lavoro 80:316–329.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989b. Ten-year mortality study of the population involved in the Seveso incident in 1976. American Journal of Epidemiology 129:1187–1200.

Bertazzi A, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C. 1993. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin [see comments]. Epidemiology 4:398–406.

Bertazzi PA, Zochetti C, Guercilena S, Consonni D, Tironi A, Landi MT, Pesatori AC. 1997. Dioxin Exposure and Cancer Risk: A 15-Year Mortality Study after the "Seveso Accident," Epidemiology 8(6):646–652.

Blair A and Kazerouni N. 1997. Reactive chemicals and cancer. Cancer Causes Control 8(3):473–490.

Blair A, Grauman DJ, Lubin JH, Fraumeni JF Jr. 1983. Lung cancer and other causes of death among licensed pesticide applicators. Journal of the National Cancer Institute 71:31–37.

Blair A, Mustafa D, Heineman EF. 1993. Cancer and other causes of death among male and female farmers from twenty-three states. American Journal of Industrial Medicine 23:729–742.

Blair A, Zahm SH, Cantor KP, Ward MH. 1997. Occupational and environmental risk factors for chronic lymphocytic leukemia and non-Hodgkin's lymphoma. In: Marti GE, Vogt RF, Zenger VE, eds. Proceedings of the USPHS Workshop on Laboratory and Epidemiologic Approaches to Determining the Role of Environmental Exposures as Risk Factrors for B-Cell Chronic Lymphocytic and Other B-Cell Lymphoproliferative Disorders. U.S. Department of Health and Human Services, Public Health Service.

Bloemen LJ, Mandel JS, Bond GG, Pollock AF, Vitek RP, Cook RR. 1993. An update of mortality among chemical workers potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. Journal of Occupational Medicine 35:1208–1212.

Boffetta P, Stellman SD, Garfinkel L. 1989. A case-control study of multiple myeloma nested in the American Cancer Society prospective study. International Journal of Cancer 43:554–559.

Bois FY, Eskenazi B. 1994. Possible risk of endometriosis for Seveso, Italy, residents: an assessment of exposure to dioxin [see comments]. Environmental Health Perspectives 102(5):476–477.

Bond GG, Ott MG, Brenner FE, Cook RR. 1983. Medical and morbidity surveillance findings among employees potentially exposed to TCDD. British Journal of Industrial Medicine 40:318–324.

Bond GG, Wetterstroem NH, Roush GJ, McLaren EA, Lipps TE, Cook RR. 1988. Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. British Journal of Industrial Medicine 45:98–105.

Bosl GJ, Motzer RJ. 1997. Testicular germ-cell cancer. New England Journal of Medicine 337(4): 242–253.

Boyle C, Decoufle P, Delaney RJ, DeStefano F, Flock ML, Hunter MI, Joesoef MR, Karon JM, Kirk ML, Layde PM, McGee DL, Moyer LA, Pollock DA, Rhodes P, Scally MJ, Worth RM. 1987. Postservice Mortality Among Vietnam Veterans. Atlanta: Centers for Disease Control. CEH 86-0076. 143 pp.

Breslin P, Kang H, Lee Y, Burt V, Shepard BM. 1988. Proportionate mortality study of U.S. Army and U.S. Marine Corps veterans of the Vietnam War. Journal of Occupational Medicine 30:412–419.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Research 50:6585–6591.

Brown LM, Burmeister LF, Everett GD, Blair A. 1993. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes and Control 4:153–156.

Bueno de Mesquita HB, Doornbos G, van der Kuip DA, Kogevinas M, Winkelmann R. 1993. Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in the Netherlands. American Journal of Industrial Medicine 23:289–300.

Bullman TA, Watanabe KK, Kang HK. 1994. Risk of testicular cancer associated with surrogate measures of Agent Orange exposure among Vietnam veterans on the Agent Orange Registry. Annals of Epidemiology 4:11–16.

Burmeister LF, Everett GD, Van Lier SF, Isacson P. 1983. Selected cancer mortality and farm practices in Iowa. American Journal of Epidemiology 118:72–77.

Burmeister LF. 1981. Cancer mortality in Iowa farmers: 1971–1978. Journal of the National Cancer Institute 66:461–464.

Cantor KP. 1982. Farming and mortality from non-Hodgkin's lymphoma: a case-control study. International Journal of Cancer 29:239–247.

Cantor KP, Blair A. 1984. Farming and mortality from multiple myeloma: a case-control study with the use of death certificates. Journal of the National Cancer Institute 72:251–255.

Centers for Disease Control. 1988. Health status of Vietnam veterans. II. Physical health. Journal of the American Medical Association 259:2708–2714.

Centers for Disease Control. 1990. The association of selected cancers with service in the U.S. military in Vietnam. III. Hodgkin's disease, nasal cancer, nasopharyngeal cancer, and primary liver cancer. The Selected Cancers Cooperative Study Group [see comments]. Archives of Internal Medicine 150:2495–2505.

Clapp RW, Cupples LA, Colton T, Ozonoff DM. 1991. Cancer surveillance of veterans in Massachusetts, 1982–1988. International Journal of Epidemiology 20:7–12.

Clapp RW. 1997. Update of cancer surveillance of veterans in Massachusetts, USA. International Journal of Epidemiology 26(3):679–681.

Coggon D, Pannett B, Winter PD, Acheson ED, Bonsall J. 1986. Mortality of workers exposed to 2-methyl-4-chlorophenoxyacetic acid. Scandinavian Journal of Work, Environment, and Health 12:448–454.

Coggon D, Pannett B, Winter P. 1991. Mortality and incidence of cancer at four factories making phenoxy herbicides. British Journal of Industrial Medicine 48:173–178.

Collins JJ, Strauss ME, Levinskas GJ, Conner PR. 1993. The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a trichlorophenol process accident. Epidemiology 4:7–13.

Cordier S, Le TB, Verger P, Bard D, Le CD, Larouze B, Dazza MC, Hoang TQ, Abenhaim L. 1993. Viral infections and chemical exposures as risk factors for hepatocellular carcinoma in Vietnam. International Journal of Cancer 55:196–201.

Corrao G, Caller M, Carle F, Russo R, Bosia S, Piccioni P. 1989. Cancer risk in a cohort of licensed pesticide users. Scandinavian Journal of Work, Environment, and Health 15:203–209.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997a. Mortality of Vietnam Veterans: The Veteran Cohort Study: A Report of the 1996 Retrospective Cohort Study of Australian Vietnam Veterans. Canberra: Department of Veterans' Affairs.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997b. Mortality of National Service Vietnam Veterans: A Report of the 1996 Retrospective Cohort Study of Australian Vietnam Veterans. Canberra: Department of Veterans' Affairs.

Dalager NA, Kang HK. 1997. Mortality among Army Chemical Corps Vietnam veterans. American Journal of Industrial Medicine 31:719–726.

Dalager NA, Kang HK, Thomas TL. 1995a. Cancer mortality patterns among women who served in the military: the Vietnam experience. Journal of Occupational and Environmental Medicine 37:298–305.

Dalager NA, Kang HK, Burt VL, Weatherbee L. 1991. Non-Hodgkin's lymphoma among Vietnam veterans. Journal of Occupational Medicine 33:774–779.

Dalager NA, Kang HK, Burt VL, Weatherbee L. 1995b. Hodgkin's disease and Vietnam service [see comments]. Annals of Epidemiology 5:400–406.

Dean G. 1994. Deaths from primary brain cancers, lymphatic and haematopoietic cancers in agricultural workers in the Republic of Ireland. Journal of Epidemiology and Community Health 48:364–368.

Demers PA, Boffetta P, Kogevinas M, Blair A, Miller BA, Robinson CF, Roscoe RJ, Winter PD, Colin D, Matos E, et al. 1995. Pooled reanalysis of cancer mortality among five cohorts of workers in wood-related industries. Scandinavian Journal of Work, Environment and Health 21(3):179–190.

Donna A, Betta P-G, Robutti F, Crosignani P, Berrino F, Bellingeri D. 1984. Ovarian mesothelial tumors and herbicides: a case-control study. Carcinogenesis 5:941–942.

Dubrow R, Paulson JO, Indian RW. 1988. Farming and malignant lymphoma in Hancock County, Ohio. British Journal of Industrial Medicine 45:25–28.

Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. 1981. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. British Journal of Industrial Medicine 38:27–33.

Eriksson M, Hardell L, Adami HO. 1990. Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. Journal of the National Cancer Institute 82:486–490.

Eriksson M, Karlsson M. 1992. Occupational and other environmental factors and multiple myeloma: a population based case-control study. British Journal of Industrial Medicine 49:95–103.

Fett MJ, Nairn JR, Cobbin DM, Adena MA. 1987. Mortality among Australian conscripts of the Vietnam conflict era. II. Causes of death. American Journal of Epidemiology 125:878–884.

Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. 1991. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New England Journal of Medicine 324:212–218.

Flesch-Janys D, Berger J, Gurn P, et al. 1995. Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. American Journal of Epidemiology 142:1165–1175.

Gallagher RP, Bajdik CD, Fincham S, Hill GB, Keefe AR, Coldman A, McLean DI. 1996. Chemical exposures, medical history, and risk of squamous and basal cell carcinoma of the skin. Cancer Epidemiology, Biomarkers and Prevention 5(6):419–424.

Gambini GF, Mantovani C, Pira E, Piolatto PG, Negri E. 1997. Cancer mortality among rice growers in Novara Province, Northern Italy. American Journal of Industrial Medicine 31:435–441.

Gann PH. 1997. Interpreting recent trends in prostate cancer incidence and mortality. Epidemiology 8(2):117–120.

Garry VF, Kelly JT, Sprafka JM, Edwards S, Griffith J. 1994. Survey of health and use characterization of pesticide appliers in Minnesota. Archives of Environmental Health 49:337–343.

Garry VF, Tarone RE, Long L, Griffith J, Kelly JT, Burroughs B. 1996. Pesticide appliers with mixed pesticide exposure: G-banded analysis and possible relationship to non-Hodgkin's lymphoma, Cancer Epidemiology, Biomarkers and Prevention 5(1):11–16.

Gonzalez FJ, Fernandez-Salguero P, Ward JM. 1996. The role of the aryl hydrocarbon receptor in animal development, physiological homeostasis and toxicity of TCDD. Journal of Toxicological Sciences 21:273–277.

Green LM. 1991. A cohort mortality study of forestry workers exposed to phenoxy acid herbicides. British Journal of Industrial Medicine 48:234–238.

Hansen ES, Hasle H, Lander F. 1992. A cohort study on cancer incidence among Danish gardeners. American Journal of Industrial Medicine 21:651–660.

Hardell L, Bengtsson NO. 1983. Epidemiological study of socioeconomic factors and clinical findings in Hodgkin's disease, and reanalysis of previous data regarding chemical exposure. British Journal of Cancer 48:217–225.

Hardell L, Eriksson M. 1988. The association between soft tissue sarcomas and exposure to phenoxyacetic acids: a new case-referent study. Cancer 62:652–656.

Hardell L, Sandstrom A. 1979. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. British Journal of Cancer 39:711–717.

Hardell L, Eriksson M, Lenner P. 1980. Malignant lymphoma and exposure to chemical substances, especially organic solvents, chlorophenols and phenoxy acids. Lakartidningen 77:208–210.

Hardell L, Eriksson M, Lenner P, Lundgren E. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. British Journal of Cancer 43:169–176.

Hardell L, Johansson B, Axelson O. 1982. Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid or chlorophenol exposure. American Journal of Industrial Medicine 3:247–257.

Hardell L, Bengtsson NO, Jonsson U, Eriksson S, Larsson LG. 1984. Aetiological aspects on primary liver cancer with special regard to alcohol, organic solvents and acute intermittent porphyria: an epidemiological investigation. British Journal of Cancer 50:389–397.

Hardell L. 1981. Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scandinavian Journal of Work, Environment, and Health 7:119–130.

Hayes HM, Tarone RE, Casey HW, Huxsoll DL. 1990. Excess of seminomas observed in Vietnam service U.S. military working dogs. Journal of the National Cancer Institute 82:1042–1046.

Hayes HM, Tarone RE, Casey HW, Jennings PB Jr, Hildebrandt PK, Reardon MJ. 1994. U.S. military working dogs with Vietnam service: definition and characteristics of the cohort. Military Medicine 159(11):669–675.

Hayes HM, Tarone RE, Cantor KP. 1995a. On the association between canine malignant lymphoma and opportunity for exposure to 2,4-dichlorophenoxyacetic acid. Environmental Research 70:119–125.

Hayes HM, Tarone RE, Casey HW. 1995b. A cohort study of the effects of Vietnam service on testicular pathology of U.S. military working dogs. Military Medicine 160(5):248–255.

Hayes RB. 1997. The carcinogenicity of metals in humans. Cancer Causes Control 8(3):371–385.

Henneberger PK, Ferris BG Jr, Monson RR. 1989. Mortality among pulp and paper workers in Berlin, New Hampshire. British Journal of Industrial Medicine 46:658–664.

Hertzman C, Teschke K, Ostry A, Hershler R, Dimich-Ward H, Kelly S, Spinelli JJ, Gallagher RP, McBride M, Marion SA. 1997. Mortality and cancer incidence among sawmill workers exposed to chlorophenate wood preservatives. American Journal of Public Health 87(1): 71–79.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. Journal of the American Medical Association 256:1141–1147.

Hoffman RE, Stehr-Green PA, Webb KB, Evans RG, Knutsen AP, Schramm WF, Staake JL, Gibson BB, Steinberg KK. 1986. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Journal of the American Medical Association 255:2031–2038.

Holmes AP, Bailey C, Baron RC, Bosanac E, Brough J, Conroy C, Haddy L. 1986. West Virginia Department of Health Vietnam-era Veterans Mortality Study: Preliminary Report. Charlestown: West Virginia Health Department.

Kauppinen TP, Pannett B, Marlow DA, Kogevinas M. 1994. Retrospective assessment of exposure through modeling in a study on cancer risks among workers exposed to phenoxy herbicides, chlorophenols and dioxins. Scandinavian Journal of Work, Environment, and Health 20:262–271.

Keller-Byrne JE, Khuder SA, Schaub EA, McAfee O. 1997. A meta-analysis of non-Hodgkin's lymphoma among farmers in the central United States. American Journal of Industrial Medicine 31(4):442–444.

Kogan MD, Clapp RW. 1985. Mortality Among Vietnam Veterans in Massachusetts, 1972–1983. Massachusetts Office of the Commissioner of Veterans Services, Agent Orange Program.

Kogan MD, Clapp RW. 1988. Soft tissue sarcoma mortality among Vietnam veterans in Massachusetts, 1972 to 1983. International Journal of Epidemiology 17:39–43.

Kogevinas M, Saracci R, Bertazzi PA, Bueno de Mesquita BH, Coggon D, Green LM, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Osman J, Pearce N, Winkelmann R. 1992. Cancer mortality from soft-tissue sarcoma and malignant lymphomas in an international cohort of workers exposed to chlorophenoxy herbicides and chlorophenols. Chemosphere 25:1071–1076.

Kogevinas M, Saracci R, Winkelmann R, Johnson ES, Bertazzi PA, Bueno de Mesquita BH, Kauppinen T, Littorin M, Lynge E, Neuberger M. 1993. Cancer incidence and mortality in women occupationally exposed to chlorophenoxy herbicides, chlorophenols, and dioxins. Cancer Causes Control 4:547–553.

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Bas B, Coggon D, Green L, Johnson E, Littorin M, Lynge E, Marlow DA, Mathews JD, Neuberger M, Benn T, Pannett B, Pearce N, Saracci R. 1995. Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols and dioxins: two nested case-control studies. Epidemiology 6:396–402.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno de Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Pearce N, Saracci R. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study, American Journal of Epidemiology 145(12):1061–1075.

Lampi P, Hakulinen T, Luostarinen T, Pukkala E, Teppo L. 1992. Cancer incidence following chlorophenol exposure in a community in southern Finland. Archives of Environmental Health 47:167–175.

LaVecchia C, Negri E, D'Avanzo B, Franceschi S. 1989. Occupation and lymphoid neoplasms. British Journal of Cancer 60:385–358.

Lawrence CE, Reilly AA, Quickenton P, Greenwald P, Page WF, Kuntz AJ. 1985. Mortality patterns of New York State Vietnam veterans. American Journal of Public Health 75:277–279.

Lynge E. 1985. A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. British Journal of Cancer 52:259–270.

Lynge E. 1993. Cancer in phenoxy herbicide manufacturing workers in Denmark, 1947–87—an update. Cancer Causes and Control 4:261–272.

Mack TM. 1995. Sarcomas and other malignancies of soft tissue, retroperitoneum, peritoneum, pleura, heart, mediastinum, and spleen. Cancer 75:211–244.

Mahan CM, Bullman TA, Kang HK, Selvin S. 1997. A case-control study of lung cancer among Vietnam veterans. Journal of Occupational and Environmental Medicine 39(8):740–747.

Mahaney FX. 1990. Military working dogs may be a sentinel for human cancer. Journal of the National Cancer Institute 82(12):1002–1003.

Manz A, Berger J, Dwyer JH, Flesch-Janys D, Nagel S, Waltsgott H. 1991. Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 338:959–964.

Masala G, Di Lollo S, Picoco C, Crosignani P, Demicheli V, Fontana A, Funto I, Miligi L, Nanni O, Papucci A, Ramazzotti V, Rodella S, Stagnaro E, Tumino R, Vigano C, Vindigni C, Seniori Costantini A, Vineis P. 1996. Incidence rates of leukemias, lymphomas and myelomas in Italy: geographic distribution and NHL histotypes. International Journal of Cancer 68(2):156–159.

McDuffie HH, Klaassen DJ, Dosman JA. 1990. Is pesticide use related to the risk of primary lung cancer in Saskatchewan? Journal of Occupational Medicine 32:996–1002.

McKinney WP, McIntire DD, Carmody TJ, Joseph A. 1997. Comparing the smoking behavior of veterans and nonveterans. Public Health Reports 112(3):212–217.

Mellemgaard A, Engholm G, McLaughlin JK, Olsen JH. 1994. Occupational risk factors for renal-cell carcinoma in Denmark. Scandinavian Journal of Work, Environment, and Health 20:160–165.

Michalek JE, Wolfe WH, Miner JC. 1990. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. II. Mortality. Journal of the American Medical Association 264:1832–1836.

Miller BA, Kolonel LN, Bernstein L, Young, Jr. JL, Swanson GM, West D, Key CR, Liff JM, Glover CS, Alexander GA, et al. (eds). 1996. Racial/Ethnic Patterns of Cancer in the United States 1988–1992, National Cancer Institute. NIH Pub. No. 96-4104. Bethesda, MD.

Molinari R, Grande C, Zucali R, Pilotti S, Della Torre G. 1995. Tumours of the nasopharynx. In: Peckham M, Pinedo HM, Veronesi U, eds. Oxford Textbook of Oncology. Oxford, UK: Oxford University Press.

Morris PD, Koepsell TD, Daling JR, Taylor JW, Lyon JL, Swanson GM, Child M, Weiss NS. 1986. Toxic substance exposure and multiple myeloma: a case-control study. Journal of the National Cancer Institute 76:987–994.

Morrison H, Semenciw RM, Morison D, Magwood S, Mao Y. 1992. Brain cancer and farming in western Canada. Neuroepidemiology 11:267–276.

Morrison H, Savitz D, Semenciw RM, Hulka B, Mao Y, Morison D, Wigle D. 1993. Farming and prostate cancer mortality. American Journal of Epidemiology 137:270–280.

Morrison HI, Semenciw RM, Wilkins K, Mao Y, Wigle DT. 1994. Non-Hodgkin's lymphoma and agricultural practices in the prairie provinces of Canada. Scandinavian Journal of Work, Environment, and Health 20:42–47.

Moses M, Lilis R, Crow KD, Thornton J, Fischbein A, Anderson HA, Selikoff IJ. 1984. Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. American Journal of Industrial Medicine 5:161–182.

Mueller N. 1995. Overview: viral agents and cancer. Environmental Health Perspectives 103(Suppl 8):259–261.

Musicco M, Sant M, Molinari S, Filippini G, Gatta G, Berrino F. 1988. A case-control study of brain gliomas and occupational exposure to chemical carcinogens: the risks to farmers. American Journal of Epidemiology 128:778–785.

Nanni O, Amadori D, Lugaresi C, Falcini F, Scarpi E, Saragoni A, Buiatti E. 1996. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on a priori exposure matrices. Occupational and Environmental Medicine 53(10):652–657.

National Cancer Institute, Surveillance, Epidemiology, and End Results (SEER) Program, Cancer Query System on the Web, 1973–1994 [WWW search engine]. 1998. URL http://www-seer.ims.nci.nih.gov/ScientificSystems/Canques7394/ (accessed March 9, 1998).

National Toxicology Program. 1982a. Carcinogenesis Bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (CAS No. 1746-01-6) in Osborne-Mendel Rats and B6C3F1 Mice (Gavage Study). Research Triangle Park, N.C.: NTP. NTP-80-31; NIH/PUB-82-1765.PC A09/MF A01.

National Toxicology Program. 1982b. Carcinogenesis Bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (CAS No. 1746-01-6) in Swiss Webster Mice (Dermal Study). Research Triangle Park, N.C.: NTP. NTP-80-31; NIH/PUB-82-1765.PC A09/MF A01.

Olsson H, Brandt L. 1988. Risk of non-Hodgkin's lymphoma among men occupationally exposed to organic solvents. Scandinavian Journal of Work, Environment, and Health 14:246–251.

O'Toole BI, Marshall RP, Grayson DA, et al. 1996. The Australian Vietnam Veterans Health Study: II. self-reported health of veterans compared with the Australian population. International Journal of Epidemiology 25(2):319–330.

Ott MG, and Zober A. 1996. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occuptional and Environmental Medicine 53:606–612.

Ott MG, Olson RA, Cook RR, Bond GG. 1987. Cohort mortality study of chemical workers with potential exposure to the higher chlorinated dioxins. Journal of Occupational Medicine 29:422–429.

Pearce NE, Sheppard RA, Smith AH, Teague CA. 1987. Non-Hodgkin's lymphoma and farming: an expanded case-control study. International Journal of Cancer 39:155–161.

Pearce NE, Smith AH, Fisher DO. 1985. Malignant lymphoma and multiple myeloma linked with agricultural occupations in a New Zealand cancer registry-based study. American Journal of Epidemiology 121:225–237.

Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA. 1986. Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment: a case-control study. British Journal of Industrial Medicine 43:75–83.

Percy C, Ries GL, Van Holten VD. 1990. The accuracy of liver cancer as the underlying cause of death on death certificates. Public Health Reports 105:361–368.

Persson B, Dahlander A-M, Fredriksson M, Brage HN, Ohlson C-G, Axelson O. 1989. Malignant lymphomas and occupational exposures. British Journal of Industrial Medicine 46:516–520.

Persson B, Fredriksson M, Olsen K, Boeryd B, Axelson O. 1993. Some occupational exposures as risk factors for malignant lymphomas. Cancer 72:1773–1778.

Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C, Bertazzi PA. 1992. Cancer morbidity in the Seveso area, 1976–1986. Chemosphere 25:209–212.

Ramlow JM, Spadacene NW, Hoag SR, Stafford BA, Cartmill JB, Lerner PJ. 1996. Mortality in a cohort of pentachlorophenol manufacturing workers, 1940–1989. American Journal of Industrial Medicine 30:180–194.

Riedel D, Pottern LM, Blattner WA. 1991. Etiology and epidemiology of multiple myeloma. In: Wiernick PH, Camellos G, Kyle RA, Schiffer CA, eds. Neoplastic Disease of the Blood and Blood Forming Organs. New York: Churchill Livingstone.

Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. 1993. Endometriosis in rhesus monkeys *(Macaca mulatta)* following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundamental and Applied Toxicology 21:433–441.

Ries LAG, Kosary CL, Hankey BF, Miller BA, Harras A, Edwards BK (eds). 1997. SEER Cancer Statistics Review, 1973–1994, National Cancer Institute. NIH Pub. No. 97-2789. Bethesda, MD.

Riihimaki V, Asp S, Hernberg S. 1982. Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland: first report of an ongoing prospective cohort study. Scandinavian Journal of Work, Environment, and Health 8:37–42.

Robinson CF, Waxweiler RJ, Fowler DP. 1986. Mortality among production workers in pulp and paper mills. Scandinavian Journal of Work, Environment, and Health 12:552–560.

Ronco G, Costa G, Lynge E. 1992. Cancer risk among Danish and Italian farmers. British Journal of Industrial Medicine 49:220–225.

Saracci R, Kogevinas M, Bertazzi PA, Bueno De Mesquita BH, Coggon D, Green LM, Kauppinen T, L'Abbe KA, Littorin M, Lynge E, Mathews JD, Neuberger M, Osman J, Pearce N, Winkelmann R. 1991. Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. Lancet 338:1027–1032.

Semenciw RM, Morrison HI, Morison D, Mao Y. 1994. Leukemia mortality and farming in the prairie provinces of Canada. Canadian Journal of Public Health 85:208–211.

Smith JG, Christophers AJ. 1992. Phenoxy herbicides and chlorophenols: a case control study on soft tissue sarcoma and malignant lymphoma. British Journal of Cancer 65:442–448.

Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR, Miller AB, Potter JD, Speizer FE, Willett WC, Wolk A, Hunter DJ. 1998. Alcohol and breast cancer in women: a pooled analysis of cohort studies. Journal of the American Medical Association 279(7):535–540.

Solet D, Zoloth SR, Sullivan C, Jewett J, Michaels DM. 1989. Patterns of mortality in pulp and paper workers. Journal of Occupational Medicine 31:627–630.

Stehr PA, Stein G, Webb K, Schramm W, Gedney WB, Donnell HD, Ayres S, Falk H, Sampson E, Smith SJ. 1986. A pilot epidemiologic study of possible health effects associated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin contaminations in Missouri. Archives of Environmental Health 41:16–22.

Stehr-Green P, Hoffman R, Webb K, Evans RG, Knusten A, Schramm W, Staake J, Gibson B, Steinberg K. 1987. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Chemosphere 16:2089–2094.

Suskind RR, Hertzberg VS. 1984. Human health effects of 2,4,5-T and its toxic contaminants. Journal of the American Medical Association 251:2372–2380.

Svensson BG, Mikoczy Z, Stromberg U, Hagmar L. 1995. Mortality and cancer incidence among swedish fishermen with a high dietary intake of persistent organochlorine compounds. Scandinavian Journal of Work, Environmental and Health 21(2):106–115.

Swaen GMH, van Vliet C, Slangen JJM, Sturmans F. 1992. Cancer mortality among licensed herbicide applicators. Scandinavian Journal of Work, Environment, and Health 18:201–204.

Tarone RE, Hayes HM, Hoover RN, Rosenthal JF, Brown LM, Pottern LM, Javadpour N, O'Connell KJ, Stutzman RE. 1991. Service in Vietnam and risk of testicular cancer. Journal of the National Cancer Institute 83:1497–1499.

Tatham L, Tolbert P, Kjeldsberg C. 1997. Occupational risk factors for subgroups of non-Hodgkin'slymphoma. Epidemiology 8(5):551–558.

Thiess AM, Frentzel-Beyme R, Link R. 1982. Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF AG on November 17, 1953. American Journal of Industrial Medicine 3:179–189.

Thomas TL, Kang HK. 1990. Mortality and morbidity among Army Chemical Corps Vietnam veterans: a preliminary report. American Journal of Industrial Medicine 18:665–673.

Thomas TL, Kang H, Dalager N. 1991. Mortality among women Vietnam veterans, 1973–1987. American Journal of Epidemiology 134:973–980.

Thomas TL. 1987. Mortality among flavour and fragrance chemical plant workers in the United States. British Journal of Industrial Medicine 44:733–737.

U.S. Bureau of the Census. Statistical Abstract of the United States: 1997 (117th edition). 1997. Washington, DC.

Vineis P, Faggiano F, Tedeschi M, Ciccone G. 1991. Incidence rates of lymphomas and soft-tissue sarcomas and environmental measurements of phenoxy herbicides. Journal of the National Cancer Institute 83:362–363.

Visintainer PF, Barone M, McGee H, Peterson EL. 1995. Proportionate mortality study of Vietnam-era veterans of Michigan. Journal of Occupational and Environmental Medicine 37/4

Watanabe KK, Kang HK. 1995. Military service in Vietnam and the risk of death from trauma and selected cancers. Annals of Epidemiology 5:407–412.

Watanabe KK, Kang HK. 1996. Mortality patterns among Vietnam veterans: a 24-year retrospective analysis. Journal of Occupational and Environmental Medicine 38(3):272–278.

Watanabe KK, Kang HK, Thomas TL. 1991. Mortality among Vietnam veterans: with methodological considerations. Journal of Occupational Medicine 33:780–785.

Waterhouse D, Carman WJ, Schottenfeld D, Gridley G, McLean S. 1996. Cancer incidence in the rural community of Tecumseh, Michigan: a pattern of increased lymphopoietic neoplasms. Cancer 77(4):763–770.

Wigle DT, Semenciw RB, Wilkins K, Riedel D, Ritter L, Morrison HI, Mao Y. 1990. Mortality study of Canadian male farm operators: non-Hodgkin's lymphoma mortality and agricultural practices in Saskatchewan. Journal of the National Cancer Institute 82:575–582.

Wiklund K. 1983. Swedish agricultural workers: a group with a decreased risk of cancer. Cancer 51:566–568.

Wiklund K, Dich J, Holm L-E. 1989. Risk of soft tissue sarcoma, Hodgkin's disease and non-Hodgkin lymphoma among Swedish licensed pesticide applicators. Chemosphere 18:395–400.

Wiklund K, Dich J, Holm L-E, Eklund G. 1989. Risk of cancer in pesticide applicators in Swedish agriculture. British Journal of Industrial Medicine 46:809–814.

Wiklund K, Lindefors BM, Holm L-E. 1988. Risk of malignant lymphoma in Swedish agricultural and forestry workers. British Journal of Industrial Medicine 45:19–24.

Wolfe WH, Michalek JE, Miner JC, Rahe A, Silva J, Thomas WF, Grubbs WD, Lustik MB, Karrison TG, Roegner RH, Williams DE. 1990. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. I. Physical health. Journal of the American Medical Association 264:1824–1831.

Woods JS, Polissar L, Severson RK, Heuser LS, Kulander BG. 1987. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxy herbicide and chlorinated phenol exposure in western Washington. Journal of the National Cancer Institute 78:899–910.

Zack JA, Suskind RR. 1980. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. Journal of Occupational Medicine 22:11–14.

Zahm SH, Fraumeni JF Jr. 1997. The epidemiology of soft tissue sarcoma. Seminars in Oncology 24(5):504–514.

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349–356.

Zhong Y, Rafnsson V. 1996. Cancer incidence among Icelandic pesticide users, International Journal of Epidemiology 25(6):1117–1124.

Zhu K, Levine RS, Brann EA, Gnepp DR, Baum MK. 1995. A population-based case-control study of the relationship between cigarette smoking and nasopharyngeal cancer (United States). Cancer Causes Control 6(6):507–512.

Zober A, Messerer P, Huber P. 1990. Thirty-four-year mortality follow-up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. International Archives of Occupational and Environmental Health 62:139–157.

8

Latency and Cancer Risk

One of the topics of special interest to the Department of Veterans Affairs (DVA) is the potential effect of herbicide exposure on cancer latency. The term "latency" is used in a variety of ways to denote the effect of the timing of exposure on the subsequent risk of disease. The importance of latency effects as well as other time-related factors, such as age at exposure, in determining cancer risk has long been recognized (Armenian, 1987). The following are some important practical questions at the heart of the investigation of these time-related factors: (1) How long does it take after exposure to detect an increase in disease risk? (2) How long do the effects of exposure last? (3) How does the effect of exposure vary with the age at which it was received? (4) Does a given carcinogen act at an early or a late stage of the carcinogenic process?

Often, because of either poor exposure assessment or the desire to report a simple summary measure of association, measures of exposure such as ever/never exposed or cumulative exposure are used to summarize exposure histories. Although such measures can be useful for detecting whether or not there is an association between exposure and disease, it is well known that the timing of exposure often plays an important role in determining when and by how much the eventual disease risk is increased (or decreased) by the exposure.

In response to the DVA's request to explore the latency issues related to Agent Orange, in this chapter the committee (1) presents a methodology to address the four questions listed above concerning the timing of herbicide exposure and the risk of cancer; (2) reviews the literature on herbicide exposure and cancers classified in the sufficient and limited/suggestive categories for results describing how the relative risks vary with time since exposure began or ended;

and (3) discusses the relevance of these data for cancer risk among Vietnam veterans.

ANALYSIS OF LATENCY IN EPIDEMIOLOGIC STUDIES

To discuss latency issues, we need to establish what is meant by the "effect of exposure over time." First, for purposes of epidemiologic research and quantification, we are interested in the rate of disease among exposed individuals compared to the rate that would be expected if the subjects had not been exposed, which is discussed more fully below. Thus, we are interested in the relative or excess rate of disease as the measure of comparison. Because diseases such as cancer may take a long time to develop (i.e., years or even decades) an analysis of the effects of exposure must consider the "latency period," or time between the exposure and the measurement of disease. The effect of any exposure on a population, whether measured as *relative* or *excess* rates, may change with "time since exposure." Typically, after exposure to a carcinogen, no excess cancer rate will be observed for a time. Then there will be a rise in the excess until it reaches a peak, at which point it may fall back down. For exposures of short duration, time since exposure is in many cases easy to define. This situation holds for environmental exposures from industrial accidents, particularly if the exposure involves chemicals that are not retained in the body tissues. If the exposure occurred over a long period of time (a protracted exposure), as with production workers and pesticide applicators, the time since exposure is more difficult to quantify, since there were many exposure times. It is important to note that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, TCDD, or dioxin) and other chlorinated herbicides are retained in some body tissues for a long time (e.g., decades), so that even after external exposure ceases, internal exposure continues. Thus, even a brief exposure such as occurred in Seveso, Italy, can involve protracted exposure of many organs of the body. Conceptually, we think of the effect of exposure at a particular time in the past as the resulting change in risk today that is ascribable to that exposure. Although this may be overly simplified, the effect of an entire exposure history can usefully be thought of as the sum of the effects from exposure at each time point in the past.

To adequately study the effects of protracted exposure, detailed exposure histories for each study subject, including the dates at which the individual was exposed and, ideally, the level of exposure, are needed. Appropriate statistical methods have been developed for investigating the effects of exposure accrued as a function of time since exposure (Thomas, 1983, 1988; Breslow and Day, 1987), but these have not been used to analyze of any of the cancer studies reviewed here.

In general, the ability to investigate the issue of timing of exposure in a given data set will depend on the quality of the exposure measure, the quality of the timing of exposure information, the number of people developing the disease,

and the variation of exposure over time within the study group. These aspects of study quality are of course important in evaluating any epidemiologic study. Special problems arise in the evaluation of time-related factors (Enterline and Henderson, 1973; Peto, 1985; Thomas, 1987).

Need to Control for the Effects of Aging

Progression along the scale of time since exposure is paralleled by increasing age. Since the rate of most cancers increases dramatically with age, for any given study group the expected number of cancers per unit of time will increase with time since exposure, simply because the study group is aging. Thus, an examination of the absolute number of cases versus time since exposure would be misleading. We would expect to see an increase even if there were no change related to the exposure. Thus, it is standard epidemiologic practice to "normalize" the comparison by reference to an appropriate unexposed group. "Appropriate" in this context means a group with the same age structure and otherwise having similar risk of disease as the study group; thus we are measuring the effects of exposure *over and above* those changes in cancer rates that would be expected if the study group were simply aging without having been exposed.

Correlations Among Various Time Factors

It is important to keep in mind that duration of exposure, time since exposure, age of exposure, and exposure level itself may well be correlated with each other, so that an observed pattern for one of these factors could actually be due to correlation with one or more of the others. Stratifying the analysis or modeling both effects is commonly needed to disentangle the confounding of time-related variables. Realistically, however, most data sets do not have sufficient data to explore these issues, and for the most part, these considerations merely serve as a cautionary note in the interpretation of results. The following hypothetical situations help to illustrate how various time-related factors are intertwined. For illustration, we the exposure of interest is referred to as "the agent."

- Two people are born in 1945. Both are exposed continuously to the agent for 10 years. One person begins exposure at age 20 (continuing to age 30); the other begins at age 30 (continuing to age 40). In 1995, at age 50, the first person has 30 years since first exposure, whereas the second has only 20 years. Thus, the *age at first exposure* and the *time since first exposure*, for a given duration of exposure, are linked.
- One person begins exposure to the agent in 1970; a second starts in 1975. They are the same age at first exposure and exposure continues until 1985. When evaluated in 1995, the first person has both a longer *duration of exposure* (15 years versus 10 years) and a longer *time since first exposure* (25 years versus 20

years), which illustrates the link between these two factors for a given age at first exposure.

- Two people are born in 1945. One begins exposure at age 20; the other, at age 30. Exposure stops for both at age 40. The first person has a younger *age at first exposure*, a longer *duration of exposure*, and a longer *time since first exposure*, showing that all three factors may potentially be linked.
- Two people are exposed to the same concentration of the agent. The person with the longer *duration of exposure* will, by definition, have a higher cumulative exposure.

Although it is possible to construct counterexamples to the above, these are fairly typical examples of what happens in many occupational settings. They are presented primarily to illustrate that examining the effects of one time-related factor may be difficult without information about the others.

Although it can be difficult to disentangle these interrelated effects, it is not impossible. Many occupational studies, for instance, have shown that the strongest effects of industrial chemicals on cancer occur 10 to 20 years after exposure begins, after age and calendar time have been controlled. Several radiation-related cancers including leukemia, gastrointestinal cancers, and breast cancer show age at exposure to be a strong determinant of risk (NAS, 1990).

Mortality and Incidence Studies for Examining Latency

If an agent is carcinogenic, it may increase the chance of cancer occurring, or it may accelerate development of the cancer so that it occurs at a younger age than it otherwise would have. The agent may also influence the likelihood that the cancer results in death or may shorten the time between occurrence of the disease and death caused by that disease. Which of these processes occurs may depend not only on the agent, but also on the site of cancer. For example, lung cancer tends to be fatal in a very high percentage of those who develop it, and death usually comes swiftly. For this site, therefore, a study of mortality is unlikely to provide different results from a study of incidence. In contrast, prostate cancer is fatal in a fairly small proportion of cases (incidence rates are five or more times higher than mortality rates) (Merrill and Brawley, 1997). For this reason, a study of prostate cancer mortality would be less likely to detect the effect of a carcinogenic agent than would a study of prostate cancer incidence, unless the agent increased the severity of disease. On the other hand, since prostate cancer is so common and occurs with an increasing frequency as men age, any study of prostate cancer incidence should examine whether those exposed to the agent of interest develop the cancer at an earlier age than those not exposed. This type of analysis could be accomplished using age-specific rates. Caution would have to be exercised in interpreting incidence studies because of the recent introduction of prostate-specific-antigen (PSA), a marker for prostate tumors that are not clinically detectable,

as a screening tool. Differences across subpopulations in the extent to which PSA is used could confound results (Gann, 1997).

In the investigation of latency, changes in relative risks with time since exposure will occur later for mortality studies than for incidence studies, by an amount of time approximately equal to the average time from occurrence of the cancer to death. If the agent has no effect on the probability of death or the age at death from the cancer, then mortality studies will result in a pattern of relative risks "shifted to the right" of the pattern that would have been observed in the corresponding incidence studies. In other words, the pattern with time since exposure will be similar, but the latency period will be longer. As a result, at any given point in the follow-up period a study of mortality will record fewer events than a study of incidence and consequently will have lower statistical power, even if the exposed and unexposed cases have the same prognosis. The problem of the mortality studies with lower statistical power is magnified for cancer sites that have long survival times or tend not to be fatal (e.g., prostate cancer). Most of the herbicide studies that reported latency results were mortality studies.

Measurement Errors That Are Time Related

In epidemiologic studies, a common problem in data quality involves errors in the assignment of exposure. These errors can occur when exposed individuals are erroneously categorized as unexposed, or when unexposed individuals are categorized as exposed. These errors can also occur when determining how large an exposure an individual received: high exposures may be assessed as lower than they really are, and vice versa. These errors can be classified in several ways:

1. One way of classifying errors considers whether they are related to the true exposure: either the errors are independent of true exposure (if highly exposed individuals are just as likely to be erroneously assessed as those who truly had low exposures) or they can depend on true exposure (e.g., if those receiving low exposure are not well assessed, but those at high exposure levels are assessed correctly).
2. Another way to categorize errors is whether they are random or systematic: random errors are just as likely to overestimate as to underestimate true exposure. By contrast, systematic errors occur when there is a tendency for the measured exposure to be lower or higher than the true exposure.
3. A third way to categorize errors is according to whether they are more likely or less likely—as opposed to equally likely—to occur in the nondiseased than in the diseased population (this problem arises more frequently in case-control studies than in cohort studies).

In general, most types of errors will distort the evidence, sometimes causing the analysis to show a weaker effect than is actually occurring and other times

showing an effect that is not real, or a stronger effect than the true one. In certain situations, namely when the misclassification of exposure status is unrelated to a person's ultimate disease status and neither time-related factors nor levels of exposure are examined (exposure is simply considered either present or absent), then the distortion will usually result in observing a weaker effect than truly exists. In an analysis evaluating how time-related factors such as duration of exposure or cumulative exposure influence risk, the effect of misclassification is difficult to predict. Finally it should be pointed out that errors can occur not just in exposure assessment, but also in ascertainment of the outcome. For example, the classification of diseases changes over time as new techniques for diagnosis are developed. The recent development of and widespread screening for PSA have seemingly "indicated" a large increase in the incidence of early, localized prostate cancer. In reality, we are simply moving our time of diagnosis to an earlier stage in the development of the cancer.

FOUR QUESTIONS ADDRESSED BY THE COMMITTEE

For each question outlined in the introduction to this chapter, the committee discusses the measures it is seeking in the reports of study results, how these measures are examined to address the particular question, the types of data a study would need to be informative about this question, and problems associated with the measures chosen by the committee.

How Long Does It Take After Exposure to Detect an Increase in Disease Risk?

Measures of Interest

Relative risks for specific intervals of time since exposure are the appropriate measures of interest for this question. One must examine the pattern of relative risks, looking for the earliest indication of an increase in risk relative to the unexposed comparison group. For protracted exposures, it is customary to examine the relative risks by time since first exposure, because the earliest detectable increase in relative risk may be a manifestation of the earliest exposure. In fact, relative risks for specific times since first exposure are often the only measures of latency reported for studies of protracted exposure to herbicides.

Data Requirements

The critical data item for this measure is the date of first exposure for each subject. With this date, the investigator can determine the time that each subject spent in each time since first exposure category. If full exposure histories are available, more sophisticated analyses are possible.

Potential Problems with this Approach

The "earliest indication of an increase in relative risk" is difficult to measure and will be refined as more data are collected. First, it is likely that latency periods vary among individuals; as a result, changes in risk in a population occur continuously rather than suddenly jumping from "normal" to "above normal." Actual changes in relative risk probably would occur earlier than indicated by the analysis, but because of limitations in study designs, this increase might not be detectable. In other words, the degree to which an increased risk is statistically detectable depends on the size of the particular data set, as well as the magnitude of both the background level of risk (which in turn depends on the age of distribution of study subjects) and the relative increase in risk (which in turn depends on the exposure level received, variation in susceptibilities, length of follow-up in the study, and true distribution of latency periods among the exposed population). It should be noted that if the latency periods are highly variable among individuals, an analysis by time since first exposure may be somewhat insensitive, because the increased risk will appear slight and will occur quite gradually. Additionally, if the effect of time since exposure is modified by the intensity of exposure or the age at exposure, these other factors would have to be accounted for in the analysis. For example, the latency might be longer for a low-level exposure than for a higher one, in which case a study that examined only time since first exposure might encounter greater variability in latency periods and hence have less ability to assess how long it takes to observe an effect of exposure. Such effect modification would also limit the generalizability of results from one study to a different population or to another exposure scenario.

Limitations in the Data Available

Studies that examined changes in risk by time since first exposure used different categories of time, so that results are not always directly comparable. In many of the studies, when specific cancer sites were examined by time since first exposure, the number of deaths in each category became quite small, leading to less stable estimates. In most studies, the analyses by time since first exposure did not adjust for other time-related factors such as duration of exposure, intensity of exposure, or age at start of exposure, so the apparent effect of time since first exposure could very well be confounded by these other factors. In the case of Australian veterans who served in Vietnam, latency analyses examined the time since start of service, which may not have corresponded to the time since first exposure; for some veterans, the time since first exposure would have been shorter than this surrogate variable.

While recognizing these limitations in its evaluations, the committee has determined the earliest increases in relative risk reported in the literature. These are, of course, subject to change as more information becomes available.

How Long Does the Effect of Exposure Last?

Measure of Interest

Relative risks for specific intervals of time since last exposure are used to address this question. The pattern of relative risks is examined for the latest indication that the relative risk is greater than one.

Data Requirements

Dates of each start and stop of exposure are required to answer this question. These are needed to classify the subjects' time spent in each time-since-exposure category. If full exposure histories are available, more sophisticated analyses are possible. However, if the critical issue is "time since exposure stopped," multiple starts and stops will be difficult to analyze.

Potential Problems with this Approach

If exposure is protracted, time since exposure must be analyzed in the proper time-dependent fashion (Clayton and Hills, 1993). Tight adjustment for age is also necessary. To achieve adequate power and precision, a study group must have a sufficient number of subjects with long times since exposure ended. If exposure has been protracted, then much longer time periods of follow-up are needed than for addressing the previous question.

Limitations in the Data Available

Most of the studies reporting latency data for cohorts with protracted exposure examined only the time since first exposure (not time since last exposure). The study of Vietnam era veterans examined changes in mortality by years since they last served in Vietnam. For the Seveso cohort, the time since exposure ended is, for most subjects, quite close to the time since the accident occurred. Hence, all other factors being equal, these two studies would be the most amenable to answering the question of how long the effect of exposure lasts.

How Does the Effect of Exposure Vary with the Age at Which It Was Received?

Measures of Interest

Relative risks for exposure beginning at various ages are the critical measures needed to address this question. One must examine the pattern of relative risks associated with exposure beginning at various ages and com-

pare the patterns of relative risks by time since exposure across age at exposure categories.

Data Requirements

Dates of exposure and date of birth are the critical data required to construct these measures. These are needed to classify subjects as exposed or unexposed in each age category. The date of birth of study participants is generally known in epidemiologic studies. If information about level of exposure is available, it would be used in preference to the simple exposed/unexposed categorization. For the relative risks stratified by time since exposure, the data requirements include those described above.

Potential Problems with this Approach

The problems with this approach parallel those for the previous questions. A large study with long follow-up is more likely than a small study to detect differential age effects. Sample size becomes a practical problem, since analysis within age groups requires more data than pooling all age groups in exposure categories. To examine the time since exposure within an age group, comments about the investigation of relative risk by time since exposure apply here as well.

Limitations in the Data Available

The committee found no studies that report the results needed to address this question, namely, changes in cancer risk by age at exposure (or age at first exposure) to herbicides.

Does the Exposure Appear to Act at an Early or a Late Stage of the Carcinogenic Process?

Measures of Interest

The key statistical measures needed to address this question are the relative risks by age at exposure, the time since exposure began, and the time since exposure ended or, alternatively, the parameters in one of several models of carcinogenesis. In the multistage model of carcinogenesis, a healthy cell is presumed to go through a series of stages before becoming a cancer cell (Armitage and Doll, 1961; Chu, 1987). This model predicts specific patterns of relative risks by age and time since exposure, depending on whether the agent acts at an early or late stage of the carcinogenic process (Whittemore, 1977; Thomas, 1988). Further, the parameters in the multistage model or other mechanistic models, such as the two-event "initiator-promoter" model of Moolgavkar and Venzon (1979), may be estimated from cohort data to distinguish early- and late-stage effects.

Data Requirements

To construct these measures, complete exposure histories and the date of birth are required. The study group must include subjects with protracted exposures, and there must be variation with respect to exposure histories. A sizable study cohort is needed.

Potential Problems with this Approach

Large studies with high-quality data on exposure history are needed. Even when such data are available, it is difficult to distinguish early- from late-stage effects, possibly because many carcinogens have effects at more than one stage in the carcinogenic process or because the differences in susceptibility and latency period among individuals mask such effects.

Limitations in the Data Available

No studies in the published literature attempted to conduct analyses to determine the stage(s) at which exposure to herbicides such as TCDD exerts an effect.

REVIEW OF THE SCIENTIFIC LITERATURE

For purposes of this discussion, the review of the literature on herbicide exposure and cancer was focused on cancers in the categories of "sufficient" and "limited/suggestive" evidence of association, as found in both *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (henceforth called *VAO*) (IOM, 1994) and *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996)—that is, those cancers for which there was some evidence of an association. These are soft-tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, prostate cancer, respiratory cancer, and multiple myeloma. Although *VAO*, *Update 1996*, and Chapter 7 of this report review the entire relevant literature on herbicide exposure, this chapter discusses only those articles that provide results the committee believes reflect, with reasonable accuracy, the timing of herbicide exposure, and that studied a sufficient number of cases to make some judgment about the patterns of relative risks reported. In addition, the discussion is restricted to those cancer sites for which the data were at least limited/suggestive of an association with the herbicides used in Vietnam.

Limitations of the Literature Review Approach

In Chapter 4, the committee considers the problem of using a literature review to determine whether an association exists between herbicides and disease. The committee concludes that for overall questions of association between

exposure and disease, the published literature would adequately report results, whether "positive" or "negative" with respect to association, from the studies that have been carried out to date. Thus, there should be little "publication bias" (the tendency for positive results to be published more frequently than negative) in the literature for association.

In a specific investigation of timing issues based on a review of the literature, the same question of publication bias has to be addressed. That is, is it more likely that results of investigations of timing issues will be published depending on the outcome of these investigations? Unlike measures of association (particularly relative risk) that are universally reported, results of investigations of timing issues are not reported routinely. Indeed, although it is not possible to determine the reasons that timing is or is not reported, it is quite plausible that negative results (i.e., no differential effect of timing) are reported less frequently than positive results. One likely scenario is that if no association is found between exposure and disease, then either timing issues were not investigated or they were investigated and only "interesting" results (i.e., large changes over time intervals) were reported; so-called "uninteresting" results (no association over all time intervals) were not reported. Thus, the committee recognizes that there is a potential for its review to reflect publication bias.

Overview of the Findings

Update 1996 reported results on the timing of exposure in relation to two cancer sites: respiratory and prostate, with considerably more information about the former than the latter. However, even for these cancers, the reports of some potentially informative studies did not include latency results, which suggests a potential for publication bias, although one cannot always know whether researchers did an analysis and failed to report it because the results were uninteresting or simply did not conduct the analyses. Also, both prostate and respiratory cancers are in the "limited/suggestive" evidence category, indicating the committee's belief that the evidence for association between herbicide exposure and these cancers is not conclusive. This view has not changed after the investigation of latency issues.

Since *Update 1996* several more reports provide latency information on lung cancer. New data have also appeared regarding prostate cancer. With regard to the outcomes, several studies also report latency analyses for all cancer deaths combined, and there are now data on non-Hodgkin's lymphoma, multiple myeloma, lymphatic and hematopoietic cancers combined, and a few other sites. However, only those cancer sites for which at least two studies have provided latency analyses are discussed in this chapter. In addition, for any particular site, individual studies that do not have an overall excess of cancer at that site are not evaluated for the patterns of risk with respect to latency.

Studies using the proportionate mortality ratio (PMR)—that is, those that

enumerated only the deaths, not the number of individuals at risk—are ignored for the purpose of evaluating latency. This decision is based on the inherent weakness of PMR studies, in which the results in terms of one outcome are influenced by the results of another. (If an exposure causes an increase in both a more common outcome and a rarer one, the increase in mortality for the rarer outcome could be obscured in a PMR study.)

Table 8-1 summarizes the newly published papers that provide data on latency periods, the time since exposure categories they used, and the cancer sites they reported. Respiratory cancer, prostate cancer, and non-Hodgkin's lymphoma are discussed below, because these are the only sites that either were reviewed in *Update 1996* or have more than one new study (not based on PMRs) that reports analyses by different latencies.

RESPIRATORY CANCER

Background

There is a substantial body of literature that explores issues of timing of exposure and respiratory cancer, because of its relatively high incidence and because numerous carcinogenic agents have been identified. Some of the studies are summarized here to provide a background for the examination of these issues.

Gamma Rays. In an investigation of latency issues for radiation exposure in atomic bomb survivors, it was found that the relative risk of respiratory cancer began to rise 5 to 10 years after exposure and reached a plateau about 15 years after exposure. Thirty years after exposure there was no evidence of a decrease in relative risk (Land, 1987). In addition, the effects of age at exposure are quite pronounced for some sites (NAS, 1990) (e.g., leukemia, digestive cancers, and breast cancer).

Radon Daughters. For miners exposed to radon daughters (radon decay products), the relative risk of lung cancer was seen to peak 5 to 10 years after first exposure, then slowly decline, although the risk still appears to be elevated even 30 years after exposure (Lubin et al., 1994; Thomas et al., 1994). In addition, the effect of exposure varies with age at exposure: a given exposure level results in a lower relative risk in older workers than it does in younger workers.

Smoking. Analyses of lung cancer indicate that the relative risks begin to rise substantially after about 20 years from the initiation of cigarette smoking. Among ex-smokers, the relative risk declines to about 50 percent of that of smokers by 12 years after cessation but then remains fairly constant (and elevated relative to those who never smoked). Among continuing smokers, for the same cumulative amount smoked, the relative risk declines with age at the start of smoking.

TABLE 8-1 New Studies with Latency Data

Reference	Measures of Association	Latency Periods*	Population	Cancer Outcomes
Michalek et al., 1998	SMR 20+	0–20	Ranch Hands	Respiratory (other sites presented for <20 or 20+ years, but not for both)
Bertazzi et al., 1997	RR (Mortality)	0–15	Seveso residents	All cancers Digestive cancer Rectal cancer (males only) Lymphatic and hematopoietic Stomach (females only) Leukemia (males only) Multiple myeloma (females only)
Crane et al., 1997	SMR	<10 11–15 16–20 21–25 >25	Australian Vietnam veterans	All cancers Lung cancer
Kogevinas et al., 1997	SMR	0–9 >20	IARC cohort 10–19	All neoplasms (140–208) Lung cancer NHL Soft tissue sarcoma
Becher et al., 1997	SMR	0– <10 10–<20 20+	German production workers NHL	All neoplasms (140–208) Buccal cavity/pharynx Lung All lymphatic and hematopoietic
Watanabe and Kang, 1996	PMR	0–10 11–15 >16	U.S. Vietnam-era veterans	All cancers (170–174, 185–209) Pancreas Larynx Lung Connective tissue Skin Prostate Testis NHL Hodgkin's disease Multiple myeloma

*Refers to years since start of exposure, with the exception of the study by Watanabe and Kang (1996), who examined time since last year in Vietnam, and Michalek et al. (1998), who did not specify what the latency represented.

Arsenic. In a cohort of workers from a copper smelter in Montana, relative risks were observed to increase with time after exposure, reaching a maximum between 15 and 20 years after exposure, after which they slowly declined (Breslow and Day, 1987). There was little change in relative risk with age at first exposure.

Asbestos. In a cohort of workers exposed only briefly to high levels of asbestos during World War II, the relative risk rose sharply between 5 and 10 years after exposure, after which it remained constant up to 40 years after exposure (U.S. EPA, 1986). The relative risks are independent of age at exposure.

Nickel. In a cohort study of nickel refiners in England and Wales, the relative risk for lung cancer peaks less than 20 years after exposure, then decreases sharply. After 50 years, however, the risk is still elevated, except in the low-exposure group. The relative risks are more or less constant across age at first exposure (Kaldor et al., 1986). It is interesting to note that in contrast to these results, the same author reported quite a different pattern of relative risks for nasal sinus cancer. It was found that the relative risks for nasal sinus cancer continued to increase slowly with time since exposure, but increased markedly with age at first exposure.

Thus, for all of these exposures, increases in relative risk either reached a plateau or peaked within 20 years after exposure. This indicates that the first detectable increases occurred somewhat earlier than this. The pattern of relative risks after reaching the peak and the pattern with age at exposure vary greatly across the agents, probably reflecting different mechanisms of action.

Review of the Scientific Literature

Since respiratory cancer is fairly common, the committee has focused on studies with at least 10 cases. Five studies have reported timing effects related to herbicide exposure and respiratory cancer. The National Institute for Occupational Safety and Health (NIOSH) study of chemical production workers gives the most detailed account of timing effects and exposure to TCDD (Fingerhut et al., 1991). Standardized mortality ratios (SMRs) for lung cancer were 0.8, 1.0, and 1.2 for 0–9, 10–19, and 20+ years since first exposure to TCDD, based on a total of 85 cases. SMRs for time since first exposure are further stratified by duration of exposure, as reproduced in Table 8.2. An association between TCDD exposure and respiratory cancer is not observed in years 0–9 after first exposure. Effects begin to be observed in the second decade after exposure began among those with at least 5 years of exposure, and they have not disappeared 20 or more years after exposure. The latency may be longer for those with shorter durations of exposure.

Data from Seveso provided by Bertazzi et al. (1989a,b) and summarized in Tables 8-3A and 8-3B indicate that respiratory cancer mortality was not in-

TABLE 8-2 NIOSH Study: Respiratory Cancer Relative Mortality by Time Since First Exposure and Duration of Exposure to TCDD

Time Since First Exposure	Duration of Exposure to TCDD (years)									
	<1		1–4		5–14		15+		Overall	
	Obs	SMR	Obs	SMR	Obs	SMR	Obs	SMR	Obs	SMR
0–9 years	3	0.8	3	1.0	1	0.8	0	0.0	7	0.8
10–19 years	6	0.7	5	0.8	9	1.8	1	1.4	21	1.0
≥20 years	17	1.0	17	1.3	14	1.5	9	1.6	57	1.2
Total	26	0.9	25	1.1	24	1.5	10	1.5	85	1.1

SOURCE: Fingerhut et al., 1991, Table 4.

TABLE 8-3a Seveso Study: Lung Cancer Mortality Ratios in Men by Calendar Period

	RR		
Time Since Exposure	Zone A	Zone B	Zone R
0–5 years	0.0	1.1	0.7
6–10 years	2.0	1.8	0.9
11–15 years*	1.0	1.0	1.0

SOURCE: Bertazzi et al., 1997, Table 3, and Bertazzi et al., 1989b, Tables 4, 5, and 7.
* Relative risks have been calculated using data from the two published reports.

TABLE 8-3b Seveso Study: Lung Cancer Mortality in Males for 15-Year Follow-Up

	Observed	Expected	RR
Zone A	4	4.2	1.0
Zone B	34	27.6	1.2
Zone R	178	194.4	0.9

SOURCE: Bertazzi et al., 1997, Table 3.

creased among those in the exposed areas during the period from 0 to 5 years after the accident but was increased in years 6–10 for the most proximate residential areas. In the 15-year follow-up of the Seveso cohort, no additional data are presented on latency for respiratory cancer (Bertazzi et al., 1997), but given the results from several publications, the committee has calculated the relative risk for years 11–15 as 1.0 in all three zones.

In an 18-year follow-up of Finnish herbicide applicators, Asp et al. (1994) give the SMRs for respiratory cancer relative to the Finnish male age- and calendar-year-specific rates in such a way that SMRs could be calculated by time since first exposure for 0–9, 10–15, and >15 years. These data show that there is no clear pattern according to time since first exposure, but there is also no overall association with respiratory cancer, probably because the exposures were on average only four weeks' duration.

In a report on four occupational cohorts involved in phenoxy herbicide and chlorophenol manufacturing in Germany, with 47 lung cancer deaths and an overall SMR of 1.4, Becher et al. (1996) showed the highest relative risk in the first decade (SMR = 1.80), declining thereafter (SMR = 1.38 between 10 and 20 years after exposure, and 1.35 thereafter). These data are presented in Table 8-4. The same cohorts were included in the much larger International Agency for Research on Cancer (IARC) multicohort occupational study (Kogevinas et al., 1997), which similarly found the highest relative risks in the first 10 years: SMRs

TABLE 8-4 German Phenoxy Herbicide and Chlorophenol Manufacturing Workers Study: Lung Cancer Observed and Expected Deaths and SMRs for Men by Time Since First Exposure

Time Since Exposure	Observed	Expected	SMR
<10 years	8	4.4	1.8
10 to <20 years	14	10.1	1.4
≥20 years	25	18.4	1.4

SOURCE: Becher et al., 1996, Table 4.

for 0–9, 10–19, and 20 or more years were 1.2, 1.0, and 1.2, respectively, based on 34, 64, and 127 lung cancer deaths. The IARC results are shown in Table 8-5.

The study of Ranch Hands (Michalek et al., 1998) does examine latency for several cancer sites but does not define whether this involves time since first service, since last service, since start of service in Vietnam, or since last service in Vietnam. This group of veterans experienced fewer respiratory cancer deaths than expected in the first 20 years (3 observed, 5.6 expected) and a slight excess after 20 years (9 observed, 7.2 expected).

A report on the Australian veterans who served in Vietnam provides additional information on the time since first year of service (Crane et al., 1997). Since the first year of service may have been earlier than the first year in Vietnam, or the first year of exposure, any latency observed in these data would be longer than the actual latency. The pattern of SMRs for lung cancer deaths during 1980–1994 (no lung cancers were observed before 1980) was as follows: 2.5, 0.9, 1.3, 1.3, and 1.1 for the periods <10, 11–15, 16–20, 21–25, and >25 years respectively since the start of service. Note, however, that the SMR of 2.5 in the early period is based on only 3 lung cancer deaths, whereas the remaining periods had 17, 60, 95, and 35 lung cancer deaths, respectively. These results can be found in Table 8-6.

TABLE 8-5 IARC International Study of Workers Exposed to TCDD or Higher Chlorinated Dioxins: Lung Cancer Observed and Expected Deaths and SMRs for Men by Time Since First Exposure

Time Since First Exposure	Observed	Expected	SMR
0–9 years	34	27.9	1.2
10–19 years	64	61.5	1.0
>20 years	127	110.4	1.2

SOURCE: Kogevinas et al., 1997, Table 5.

TABLE 8-6 Australian Vietnam Veterans Study: Lung Cancer Observed and Expected Deaths and SMRs for Men by Time Since Start of Military Service

Time Since Start of Service	Observed	Expected	SMR
<10 years	3	1.2	2.5
11–15 years	17	19.7	0.9
16–20 years	60	45.9	1.3
21–25 years	95	73.0	1.3
>25 years	35	32.2	1.1

SOURCE: Crane et al., 1997, Table E-19.

Conclusions

Perhaps because respiratory cancers are the most common type of cancer in all of the cohort studies, there is more latency information available for this site than for any other. However, based on the review of the evidence in *VAO*, *Update 1996*, and Chapter 7 of this report, respiratory cancer is in the "limited/suggestive" evidence category, indicating that the committee believes the evidence for association between herbicide exposure and these cancers is not conclusive. Although an investigation of latency effects could result in a change in the categorization of evidence, in this case it did not. The fact that the committee reviewed the literature for latency effects does not imply an *a priori* belief on the part of the committee that the association is definitive.

How Long Does It Take After Exposure to Detect an Increase in Disease Risk?

If the association between TCDD exposure and respiratory cancer is causal, then the evidence in the literature suggests that the time between exposure to TCDD and an increased risk of respiratory cancer may be less than 10 years. Although the NIOSH study (Fingerhut et al., 1991) does not begin to show an effect until 10 years after exposure, the Seveso cohort (Bertazzi et al., 1989a,b, 1997) data show an increased occurrence of death from respiratory cancer beginning 6–10 years after initiation of an exposure, and the IARC cohort (Kogevinas et al., 1997) demonstrates the highest increase in the first decade. Australian Vietnam veterans (Crane et al., 1997) also showed an elevated risk of lung cancer mortality in the first decade, but this finding is based on a small number of deaths. The latest report on Ranch Hands (Michalek et al., 1998) shows a reduced risk in the first 20 years since exposure.

The committee also finds evidence in the literature that the time between exposure and the detection of respiratory cancer probably depends on the magnitude of exposure. This evidence is seen in the Fingerhut et al. (1991) study, which was the only analysis that presented a cross-classification of time since first expo-

sure with duration of exposure. With latency depending on the level of exposure, one would not necessarily expect to see the same pattern for time since exposure in all studies. Nor would one expect the pattern of risk over time since exposure to be the same for Vietnam veterans as it was for those exposed in manufacturing plants or through accidental environmental releases of the same chemicals.

When exposure is not protracted, a pattern with time since exposure could be due to confounding by another exposure that has a similar trend among the exposed, but no such trend among the unexposed. When exposure is protracted, as in two of the occupational cohorts, an even more complex pattern would have to occur for confounding to explain the results. Although one can hypothesize that a particular pattern of risk with latency could be due to confounding, evidence for differential confounding by years-since-first-exposure may be difficult to find, particularly for cohorts with a protracted exposure.

How Long Do the Effects of Exposure Last?

If there is, in fact, a causal association between TCDD exposure and respiratory cancer, the literature suggests that the risk can be elevated beginning at least as early as 6 years after exposure, but the literature is less clear on how long the effect lasts. In the NIOSH study (Fingerhut et al., 1991), risks were most elevated 20 or more years after exposure began, even for those with only 1–4 years of exposure (i.e., 16–19 years after exposure ended). The SMR in the IARC study (Kogevinas et al., 1997) for workers exposed to TCDD or higher chlorinated dioxins dropped to 1.0 from 10 to 19 years after first exposure, and rose to 1.2 for 20 or more years since first exposure (95 percent confidence interval [95% CI] 1.0–1.4), but no analyses are presented by years since last exposure. The most recent follow-up of the Seveso cohort (Bertazzi et al., 1997) did not provide any data on lung cancer latency. The Ranch Hands (Michalek et al., 1998) showed an SMR of 1.3 for 20 or more years of latency, which, depending on how latency was defined, could represent approximately a few years shorter time since service ended. Among Australian veterans (Crane et al., 1997), SMRs were 1.3 for 16–25 years after service began and 1.3 for 25 or more years after service began, but again this analysis was not for years since service ended or since leaving Vietnam. Given the scant data, the committee cannot determine how long it takes before the relative risks return to one. The lack of conclusive data on timing parallels the lack of definitive data on whether exposures to TCDD and other herbicides are causally associated with respiratory cancer.

How Does the Effect of Exposure Vary with the Age at Which It Was Received?

None of the available studies provides information on the variation of the effect of exposure with age.

Does the Carcinogen Appear to Act at an Early or a Late Stage of the Carcinogenic Process?

None of the available studies address this issue.

PROSTATE CANCER

Background

There do not appear to be environmental exposures other than herbicides associated with prostate cancer for which latency issues have been investigated. Although there are new data on prostate cancer since *Update 1996*, there are few new data on latency for prostate cancer.

Review of the Scientific Literature

The NIOSH study of chemical production workers exposed to TCDD (Fingerhut et al., 1991) reports SMRs for prostate cancer for the entire cohort, as well as for 20+ years since first exposure, by the duration of exposure: short = <1 and long = 1+ years. The presentation of results in their paper did not allow a comparison of SMRs for <20 and 20+ years since first exposure within duration-of-exposure categories. Based on the material presented, SMRs were calculated according to years since first exposure; these are listed in Table 8-7. No difference in SMRs was observed for time since first exposure. The wide categories of time since exposure limit the degree to which any inferences can be drawn about latency.

If the exposure to TCDD after the Seveso accident was of relatively short duration, the time since the accident is essentially the same as the time since exposure. The mortality studies by Bertazzi et al. (1989a,b) provide results relevant to the timing of exposure for prostate cancer mortality. By combining the data from earlier reports with those published in 1997 (Bertazzi et al., 1997), relative risks were calculated for the 11–15 years since the accident. If a low rate of immigration is assumed, the calendar-period relative risks will approximate

TABLE 8-7 NIOSH Production Workers Study: Prostate Cancer Observed and Expected Numbers of Deaths and SMRs by Time Since First Exposure to TCDD

Time Since First Exposure	Observed	Expected	SMR
<20 years	6	5.0	1.20
≥20 years	11	8.9	1.23

SOURCE: Derived from Fingerhut et al., 1991, Table 2.

those for three categories of time since exposure. The relative risks are summarized in Tables 8-8A and 8-8B. There are no cases in zone A, 6 cases in zone B, and 39 in zone R. In zones B and R, there is a decrease in the relative risk with calendar period, although the small number of cases and the fact that this is a mortality rather than an incidence study preclude strong statements about the actual pattern of relative risks.

A recent update of the Ranch Hand study reported prostate cancer for >20 years since the start of exposure but not for <20 years (Michalek et al., 1998). Hence it provides no information about how time since exposure might be related to prostate cancer risk.

Conclusions

The committee's review of the literature yielded only two sets of articles (Fingerhut et al., 1991; Bertazzi et al., 1989a,b, 1997) on prostate cancer that presented latency-related results and a sufficient number of cases for statistical analysis. Prostate cancer is in the category of limited/suggestive evidence, so it is important to keep in mind that the committee believes the evidence for an association between herbicide exposure and prostate cancer is not conclusive. Although the investigation of latency effects could result in a change in the categorization of evidence, in this case it did not. The fact that the committee reviewed the literature for latency effects does not imply an *a priori* belief on the part of the

TABLE 8-8a Seveso Study: Prostate Cancer Relative Mortality in Men by Calendar Period

	RR		
Time Since Exposure	Zone A	Zone B	Zone R
0–5 years	no cases	2.8	1.9
6–10 years	no cases	1.5	1.2
11–15 years	no cases	0.9	1.0

SOURCE: Bertazzi et al., 1997, Table 3, and Bertazzi et al., 1989b, Tables 4, 5, and 7.

TABLE 8-8b Seveso Study: Prostate Cancer Relative Mortality in Men for 15-Year Follow-Up

	Observed	Expected	RR
Zone A	0	0.7	0.0
Zone B	6	4.8	1.2
Zone R	39	33.0	1.2

SOURCE: Bertazzi et al., 1997, Table 3.

committee that the association is definitive. A further caveat is the concern that evidence based on mortality studies may have no relation to the latency period that might apply to the incidence of prostate cancer.

How Long Does It Take After Exposure to Detect an Increase in Disease Risk?

The limited data from the NIOSH study (Fingerhut et al., 1991) are uninformative; they provide no information about any pattern of relative risk in the 0–20 years since exposure began and show no difference between the first 20 years and the subsequent years since exposure. The Seveso studies (Bertazzi et al., 1989a,b, 1997) suggest that the relative risk for prostate cancer mortality is higher in the early period after exposure begins and declines 11–15 years after exposure. As for respiratory cancer, the extrapolation of latency across studies is very uncertain, since it can vary according to the exposure level and other factors such as age at exposure.

How Long Do the Effects of Exposure Last?

The available evidence is limited to the results from the Seveso cohort (Bertazzi et al., 1989a,b, 1997). Since external exposure was for a brief period, the findings are the same as for time since first exposure, with evidence suggesting little risk if any, between 10 and 15 years after the end of exposure.

How Does the Effect of Exposure Vary with the Age at Which It Was Received?

None of the studies provides information on the variation of the effect of exposure with age.

Does the Carcinogen Appear to Act at an Early or a Late Stage of the Carcinogenic Process?

None of the studies address this issue.

NON-HODGKIN'S LYMPHOMA

Background

In *Update 1996*, non-Hodgkin's lymphoma (NHL) was not reviewed for time-related factors because of the lack of published data. Three recent reports provide latency data on non-Hodgkin's lymphoma in relation to herbicide exposures, but one of these relied on PMRs (Watanabe and Kang, 1996).

Review of the Scientific Literature

Two occupational cohort studies address the latency issue for non-Hodgkin's lymphoma. The first is a report from four cohorts in Germany, with a total of six deaths, shown in Table 8-9 (Becher et al., 1996). None were observed in the 10 years since first exposure, 2 were observed in the second decade, and 4 in the third decade or later, for SMRs of 0, 3.6, and 4.3, respectively. Only the latter is significantly elevated. These data are included in the large IARC cohort of herbicide manufacturing workers (Kogevinas et al., 1997), which observed 2, 8, and 14 deaths from this disease, yielding SMRs of 0.6 (95% CI 0.1–2.3), 1.5 (95% CI 0.6–2.9), and 1.6 (95% CI 0.9–2.7). These data are presented in Table 8-10.

Conclusions

The committee's review of the literature yielded only a few papers with data on non-Hodgkin's lymphoma that provided latency-related results and sufficient numbers of cases for statistical analysis. Non-Hodgkin's lymphoma is in the category of having sufficient evidence of an association with exposures to the herbicides used in Vietnam, which means that the committee finds strong evidence of an association and is convinced that the association is not due to bias or confounding from other factors.

TABLE 8-9 German Phenoxy Herbicide and Chlorophenol Manufacturing Workers Study: Non-Hodgkin's Lymphoma Observed and Expected Deaths and SMRs for Men by Time Since First Exposure

Time Since First Exposure	Observed	Expected	SMR
<10 years	0	0.4	0
10 to <20 years	2	0.6	3.6
>20 years	4	0.9	4.3

SOURCE: Becher et al., 1996, Table 4.

TABLE 8-10 IARC International Study of Workers Exposed to TCDD or Higher Chlorinated Dioxins: Non-Hodgkin's Lymphoma Observed and Expected Deaths and SMRs for Men by Time Since First Exposure

Time Since First Exposure	Observed	Expected	SMR
0–9 years	2	3.2	0.6
11–19 years	8	5.5	1.5
>20 years	14	8.6	1.6

SOURCE: Kogevinas et al., 1997, Table 5.

How Long Does It Take After Exposure to Detect an Increase in Disease Risk?

The data suggest that the increase in risk is not immediate. Occupational cohorts do not begin to show an excess of this type of cancer until the second decade after initial exposure.

How Long Do the Effects of Exposure Last?

The available evidence suggests that the effect of herbicide exposure on the risk of non-Hodgkin's lymphoma lasts for more than 20 years. No data are available to examine latencies of 30 or more years.

How Does the Effect of Exposure Vary with the Age at Which It Was Received?

None of the studies provides information on the variation of the effect of exposure with age.

Does the Carcinogen Appear to Act at an Early or a Late Stage of the Carcinogenic Process?

None of the studies address this issue.

RELEVANCE OF LATENCY IN ASSESSING THE EFFECT OF HERBICIDES ON CANCER RISK IN VIETNAM VETERANS

One of the committee's tasks was to assess the likelihood that exposure to herbicides used in Vietnam resulted in or will result in increased risk of disease in Vietnam veterans. Currently, any such inference would have to be based on extrapolation from the findings about disease experience of other groups exposed to TCDD or herbicides generally. Given that we know when the potential exposure to TCDD and other herbicides used in Vietnam began and ended, it would appear reasonable to examine time-related factors for those who served in Vietnam, but to date, no adequate analyses of time-related factors for cancer occurrence in Vietnam veterans have been published. The extrapolation from other types of studies is problematic for several reasons. Brief exposures, such as occurred in Seveso, and chronic occupational exposures may not apply to Vietnam veterans because of the different exposure situation. For example, there is evidence in the literature (e.g., for respiratory cancer) that latency can vary not only among individuals, but also according to other aspects of the exposure scenario, such as the magnitude of exposure. Thus, if high exposures in an occupational setting result in a certain pattern of relative risks with time since first

exposure, this pattern may not hold for lower-level exposures like those that occurred in Vietnam. Similarly, direct evidence was not presented to evaluate the impact of age at exposure to herbicides. It is possible that the age at which exposure was received could influence the pattern of latency observed (e.g., exposures incurred at younger ages could be more potent, but the impact might not be seen for a longer time period; conversely, exposures at older ages might be more harmful, particularly in the short run). Unfortunately, the data are not available to evaluate the hypothesis that age at exposure is important. A major limitation of the analyses discussed in this chapter is the failure of most studies to conduct analyses of latency that also controlled for factors such as duration of exposure, age, and calendar time of exposure (or analyses of age at exposure that controlled for time since exposure), particularly for occupational cohorts with protracted exposure periods.

Another consideration is the long retention time of TCDD and other highly chlorinated herbicides. Since body burdens from any exposure, no matter how brief, result in continuing exposure of internal organs, the concept of time since exposure ended has a different meaning than for chemical agents that are excreted quickly.

A third issue concerns the distinction between morbidity and mortality. As discussed earlier in this chapter, the latency between exposure and death is composed of two parts: latency until disease is detected and time between disease occurrence and death. For diseases with low survival, such as respiratory cancer, the time between disease occurrence and death is generally short, and therefore, a study focusing on mortality will give a good approximation of the latency period. However, for diseases that are not always fatal or that have a long survival time, such as prostate cancer, it is preferable to examine incidence rather than mortality. Thus, further data on the incidence of prostate cancer would be of great help, since relatively few men with prostate cancer die from it.

Overall, the data on latency do not alter the committee's conclusions with regard to the categories of evidence for individual cancer sites, but they do provide some information on how long the effects of herbicide exposures last. The evidence suggests that if respiratory cancer does result from exposures to the herbicides used in Vietnam, the greatest relative risk for lung cancer may be in the first decade after exposure, but until further follow-up has been carried out for some of the cohorts, it will not be possible to put an upper limit on the length of time these herbicides could exert their effect. For prostate cancer, the published data are largely uninformative, and conclusions must await more definitive studies, preferably using incidence rather than mortality. For non-Hodgkin's lymphoma, effects are seen in the second decade after exposure begins and continue to be observed more than 20 years after external exposure ends. Because of the long retention times of TCDD, internal exposures can continue long after external exposures cease.

REFERENCES

Armenian HK. 1987. Incubation periods in cancer epidemiology. Journal of Chronic Diseases 40:9–16.

Armitage P, Doll R. 1961. Stochastic models for carcinogenesis. In Neyman J ed. Proceedings of the 4th Berkeley Symposium on Mathematical Statistics and Probability. Berkeley: University of California Press.

Asp S, Riihimaki V, Hernberg S, Pukkala E. 1994. Mortality and cancer morbidity of Finnish chlorophenoxy herbicide applicators: an 18-year prospective follow-up. American Journal of Industrial Medicine 26:243–253.

Becher H, Flesch-Janys D, Kauppinen T, Kogevinas M, Steindorf K, Manz A, Wahrendorf J. 1996. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. Cancer Causes and Control 7:312–321.

Bertazzi PA, Zochetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989a. Mortality in an area contaminated by TCDD following an industrial incident. Medicina Del Lavoro 80:316–329.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989b. Ten-year mortality study of the population involved in the Seveso incident in 1976. American Journal of Epidemiology 129:1187–1200.

Bertazzi PA, Zochetti C, Guercilena S, Consonni D, Tironi A, Landi MT, Pesatori AC. 1997. Dioxin exposure and cancer risk: a 15-year mortality study after the "Seveso accident." Epidemiology 8:646–652.

Breslow NE, Day NE. 1987. Statistical Methods in Cancer Research, Vol II: The Design and Analysis of Cohort Studies. Lyon: International Agency for Research on Cancer. Oxford University Press.

Chu K. 1987. A non-mathematical view of mathematical models of cancer. Journal of Chronic Diseases 40:163–170.

Clayton D, Hills M. 1993. Statistical Models in Epidemiology. New York: Oxford University Press.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997. Mortality of Vietnam Veterans: The Veteran Cohort Study: A Report of the 1996 Retrospective Cohort Study of Australian Vietnam Veterans. Canberra: Department of Veterans' Affairs.

Enterline P, Henderson V. 1973. Type of asbestos and respiratory cancer in the asbestos industry. Archives of Environmental Health 27:312–317.

Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. 1991. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New England Journal of Medicine 324:212–218.

Gann PH. 1997. Interpreting recent trends in prostate cancer incidence and mortality. Epidemiology 8:117–120.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Kaldor J, Peto J, Easton D, Doll R, Hermon C, Morgan L. 1986. Models for respiratory cancer in nickel refinery workers. Journal of the National Cancer Institute 77:841–848.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Pearce N, Saracci R. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. American Journal of Epidemiology 145:1061–1075.

Land C. 1987. Temporal distributions of risk for radiation-induced cancers. Journal of Chronic Diseases 40:45–58.

Lubin J, Boice J, Edling C, Hornung R, Howe G, Kunz E, Kusiak R, Morrison H, Radford E, Samet J, Tirmarche M, Woodward A, Xiang Y, Pierce D. 1994. Radon and Lung Cancer Risk: A Joint Analysis of 11 Underground Miners Studies. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda.

Merrill RM, Brawley OW. 1997. Prostate cancer incidence and mortality rates among white and black men. Epidemiology 8:126–131.

Michalek JE, Ketchum NS, Akhtar FZ. 1998. Post-service mortality of Air Force veterans occupationally exposed to herbicides in Vietnam: 15 year follow-up. American Journal of Epidemiology 148(8):786–792.

Moolgavkar S, Venzon D. 1979. Two-event models for carcinogenesis: incidence curves for childhood and adult tumors. Mathematical Biosciences 47:55–77.

National Academy of Sciences (NAS), National Research Council, Committee on the Biological Effects of Ionizing Radiation (BEIR V). 1990. Health Effect of Exposures to Low Levels of Ionizing Radiation. Washington, DC: National Academy Press

Peto J. 1985. Some problems in dose-response estimation in cancer epidemiology. In Voug V, Butler G, Hoel D, Peakall D, eds. Methods for Estimating Risk of Chemical Injury: Human and Non-Human Biota and Ecosystems. New York: Wiley.

Thomas DC, Pogoda J, Langholz B, Mack W. 1994. Temporal modifiers of the radon-smoking interaction. Health Physics 66:257–262.

Thomas DC. 1983. Statistical methods for analyzing effects of temporal patterns of exposure on cancer risks. Scandinavian Journal of Work, Environment, and Health 9:353–366.

Thomas DC. 1987. Pitfalls in the analysis of exposure–time–reponse relationships. Journal of Chronic Disease 40:70–78.

Thomas DC. 1988. Models for exposure–time–response relationships with applications to cancer epidemiology. Annual Review of Public Health 9:451–482.

U.S. Environmental Protection Agency (EPA). 1986. Airborne Asbestos Health Assessment Update. Research Triangle Park, NC: Environmental Criteria and Assessment Office.

Watanabe KK and Kang HK. 1996. Mortality patterns among Vietnam veterans: a 24-year retrospective analysis. Journal of Occupational and Environmental Medicine 38:272–278.

Whittemore AS. 1977. The age distribution of human cancer for carcinogenic exposures of varying intensity. American Journal of Epidemiology 106:418–432.

9

Reproductive Effects

INTRODUCTION

This chapter summarizes published scientific literature on exposure to herbicides and adverse reproductive and developmental effects. The literature discussed includes papers published since *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996). Both *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (henceforth called *VAO*) (IOM, 1994) and *Update 1996* included a number of environmental, occupational, and Vietnam veteran studies that evaluated herbicide and dioxin exposure and the risk of adverse reproductive outcomes, including spontaneous abortion, birth defects, stillbirths, neonatal and infant mortality, low birthweight, and semen quality and infertility. The reports concluded that the evidence at that time was inadequate or insufficient to determine whether an association exists between exposure to herbicides and most of the above reproductive and developmental outcomes. *Update 1996* concluded that there was limited/suggestive evidence for an association between herbicides and spina bifida.

The primary emphasis of *VAO, Update 1996*, and this report is on the potential adverse reproductive and developmental effects of herbicide exposure on males, because the vast majority of Vietnam veterans are men. Nevertheless, a brief discussion of the epidemiologic findings pertaining to female exposure is warranted because of the Department of Veterans Affairs' current study of female Vietnam veterans, their reproductive history, and the health of their children. A number of studies have evaluated the potential association between herbicide exposure in women and the risk of adverse reproductive outcomes,

including spontaneous abortion, stillbirth, preterm delivery, and birth defects (Hemminki et al., 1980; McDonald et al., 1987; Ahlborg et al., 1989; Savitz et al., 1989; Fenster and Coye, 1990; Restrepo et al., 1990; Goulet and Theriault, 1991; Correa-Villasenor et al., 1991; Lin et al., 1994; Nurminen et al., 1995; Blatter and Roeleveld, 1996; Blatter et al., 1996). In addition, recent studies have investigated maternal dioxin exposure and neurological development among offspring (Koopman-Esseboom et al., 1996). Another study found no association between potential Agent Orange exposure and risk of gestational trophoblastic disease among women living in Vietnam (Ha et al., 1996). The quality and results of these studies have been mixed. A major limitation of nearly all the studies is the determination of specific exposures. Many studies have defined exposure based solely on employment in agricultural occupations. Exposure to specific chemicals and other agents in these agricultural settings is usually not ascertained. Further, problems such as incomplete ascertainment of the outcome of interest, selection of inappropriate or no control groups, and failure to account for confounding factors have limited some of this work. Improvements in study design, especially exposure assessment, should allow for a more definitive evaluation of the relationship between herbicide exposure and adverse reproductive outcomes among women.

The remainder of this chapter discusses the following specific categories of reproductive effects: birth defects, fertility, stillbirth, neonatal and infant death, and low birthweight and preterm birth. For most outcomes, a brief summary of the scientific evidence in *VAO* and *Update 1996* is presented, followed by a review of the recent scientific literature.

BIRTH DEFECTS

Background

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. Major birth defects are seen in approximately 2 to 3 percent of live births. 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), if one assumes 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). The cause of most birth defects is

unknown. In addition to genetic factors, a number of other factors and exposures including medications, environmental, occupational, and lifestyle have been implicated in the etiology of some birth defects (Kalter and Warkany, 1983). Most of the etiologic research has focused on maternal and fetal exposures. Paternal exposures could exert an effect through direct genetic damage to the male germ cell that is transmitted to the offspring and is expressed as a birth defect; through seminal fluid transfer of chemicals, with subsequent fetal exposure; or via indirect exposure from household contamination. There is limited animal evidence that some chemicals are associated with an increase in birth defects after paternal exposure, although the relative importance of male-mediated developmental toxicity is not established (Olshan and Faustman, 1993). The previous reports and this update have limited their reviews to studies of paternal exposures.

Summary of *VAO* and *Update 1996*

There have been several occupational and environmental studies of parental herbicide exposure. The results have been inconsistent, with some studies suggesting an increased risk of a variety of specific birth defects and others reporting no association. In addition, some studies conducted in Vietnam have indicated an association between birth defects and herbicide spraying. Several problems have limited these studies for the evaluation of specific birth defects: relatively small sample sizes; failure to document reported birth defects; use of ecologic exposure measures; inability to isolate specific pesticides and exposure of specific parents; and uncontrolled confounding.

Because of the importance of conclusions regarding spina bifida in *Update* 1996, the relevant studies are summarized in this section. Table 9.1 is a summary of the studies that have reported results specifically for neural tube defects (typically anencephaly and/or spina bifida), including studies in *VAO, Update 1996,* and more recent publications reviewed in this report. Results of the analysis of birth defects among the offspring of Ranch Hands and other Vietnam veterans suggested the possibility of an association between dioxin exposure and risk of neural tube defects. Unfortunately, some studies, particularly the occupational and environmental studies (e.g., Seveso), do not have results specific for individual birth defects, usually because of the small number of cases. Several studies of veterans appear to show an elevated relative risk for neural tube defects such as anencephaly and/or spina bifida in the offspring of veterans which may be related to either service in Vietnam or estimated exposure to herbicides or dioxin. Many of the estimates are imprecise, chance cannot be ruled out, and the specific parent exposed is unclear. Nonetheless, the pattern of association warrants further evaluation. The Centers for Disease Control and Prevention (CDC) Birth Defects Study (Erickson et al., 1984a,b), the CDC Vietnam Experience Study (VES) (CDC, 1989), and the Ranch Hand Study (Wolfe et al., 1995) are of the highest

TABLE 9-1 Selected Epidemiologic Studies—Neural Tube Defects

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Blatter et al., 1997	Offspring of Dutch farmers—spina bifida		
	Pesticides use (moderate or heavy exposure)	9	1.7 (0.7–4.0)
	Herbicides use (moderate or heavy exposure)	7	1.6 (0.6–4.0)
Kristensen et al., 1997	Offspring of Norwegian farmers—spina bifida		
	Tractor spraying equipment	28	1.6 (0.9–2.7)
	Tractor spraying equipment and orchards/greenhouses	5	2.8 (1.1–7.1)
Dimich-Ward et al., 1996	Sawmill Workers		
	Spina bifida or anencephaly	22[a]	2.4 (1.1–5.3)
	Spina bifida	18[a]	1.8 (0.8–4.1)
Garry et al., 1996	Private Pesticide Appliers		
	Central nervous system defects	6	1.1 (0.5–2.4)
ENVIRONMENTAL[b]			
Studies Reviewed in *VAO*			
Stockbauer et al., 1988	TCDD soil contamination in Missouri		
	Central nervous system defects	3	3.0 (0.3–35.9)
Hanify et al., 1981	Spraying of 2,4,5-T in New Zealand		
	Anencephaly	10	1.4 (0.6–3.3)
	Spina bifida	13	1.1 (0.6–2.3)
VIETNAM VETERANS			
Studies Reviewed in *Update 1996*			
Wolfe et al., 1995	Follow-up of Air Force Ranch Hands		
	Neural tube defects among Ranch Hands[c]	4	
	Neural tube defects among comparison	0	
Studies Reviewed in *VAO*			
CDC, 1989	Vietnam Experience Study		
	Spina bifida among Vietnam veterans	9	1.7 (0.6–5.0)
	Spina bifida among non-Vietnam veterans	5	
	Anencephaly among Vietnam veterans	3	
	Anencephaly among non-Vietnam veterans	0	
Erickson et al., 1984a,b	Birth Defects Study		
	Vietnam veteran: spina bifida	19	1.1 (0.6–1.7)
	Vietnam veteran: anencephaly	12	0.9 (0.5–1.7)
	EOI-5: spina bifida	19[d]	2.7 (1.2–6.2)
	EOI-5: anencephaly	7[d]	0.7 (0.2–2.8)

TABLE 9-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
Australia Department of Veteran Affairs Health Studies, 1983	Australian Vietnam veterans—Neural tube defects	16	0.9

[a]Number of workers with maximal index of exposure (upper three quartiles) for any job held up to three months prior to conception.

[b]Either or both parents potentially exposed.

[c]Four neural tube defects among Ranch Hand offspring include 2 spina bifida (high dioxin level), 1 spina bifida (low dioxin), and 1 anencephaly (low dioxin). Denominator for Ranch Hand group is 792 live-born infants and 981 for comparison group.

[d]Number of Vietnam veterans fathering a child with a neural tube defect given any exposure opportunity index (EOI) score based upon interview.

overall quality. The CDC VES cohort study found more Vietnam veterans than non-Vietnam veterans reported that their children had a central nervous system anomaly (odds ratio [OR] = 2.3, 95 percent confidence interval [95% CI] 1.2–4.5) (CDC, 1988). The odds ratio for spina bifida was 1.7 (CI 0.6–5.0). A substudy was conducted as an attempt to validate the reported cerebrospinal defects (spina bifida, anencephaly, hydrocephalus) by examination of hospital records. A difference was detected, but its interpretation was limited by differential participation between veteran groups and failure to validate negative reports (i.e., veterans who did not report children having a birth defect). Thus, the issue of recall bias remains a major concern with this study.

The CDC General Birth Defects Study utilized the population-based birth defects registry system in the metropolitan Atlanta area (Erickson et al., 1984a,b). There was no association between overall Vietnam veteran status and the risk of spina bifida (OR = 1.1, CI 0.6–1.7) or anencephaly (OR = 0.9, CI 0.5–1.7). However, the exposure opportunity index (EOI) based on interview data was associated with an increased risk of spina bifida; for the highest estimated level of exposure (EOI-5, based on interview data), the OR was 2.7 (CI 1.2–6.2). There was no similar pattern of association for anencephaly. This study has a number of strengths, including the use of a population-based birth defects registry system and adjustment for a number of potentially confounding factors. Two study limitations include the relatively low response proportions among both case and control subjects (approximately 56 percent) and the lag between birth and interview for some cases and controls.

The analysis of birth defects from the Air Force Health Study (AFHS) of Operation Ranch Hand veterans and their children was published in 1995 (Wolfe

et al., 1995). Of the 872 Ranch Hands, 419 fathered 792 live-born infants during their service in Vietnam or until January 1990. Of the 1,036 comparison veterans, 531 fathered 981 live-born infants during this period. Birth defects were validated by a medical records review. In considering all birth defects combined, there was a slightly higher proportion of defects among Ranch Hand children than among comparison children (22.3 percent versus 20.8 percent). No general pattern of increasing risk with increasing dioxin levels was found. However, neural tube defects (spina bifida, anencephaly) were in excess among offspring of Ranch Hands, with 4 total (rate of 5 per 1,000), in contrast to none among the comparison infants ($p = 0.04$). The four cases were distributed as two spina bifida in the high-dioxin-level category, one anencephaly and one spina bifida in the low-dioxin category (exact p for spina bifida = 0.09).

Thus, all three epidemiologic studies (Ranch Hand, VES, CDC Birth Defects Study) (Wolfe et al., 1995; CDC, 1988; Erickson et al., 1984a,b) suggest an association between herbicide exposure and an increased risk of spina bifida in offspring. Although the studies were judged to be of relatively high quality, they suffer from methodologic limitations, including possible recall bias, nonresponse bias, small sample size, and misclassification of exposure. In addition, the failure to find a similar association with anencephaly, an embryologically related defect, is of concern.

Update of Scientific Literature

Dimich-Ward et al. (1996) conducted a nested case-control analysis of birth defects among offspring of fathers employed in British Columbia sawmills. The cohort included 9,512 fathers who had worked at least one year in sawmills where chlorophenate wood preservatives (anti-sap stain fungicides) had been used. Teschke et al. (1994) summarized the concentrations of various dioxin congeners in a variety of product formulations used in the British Columbia sawmill industry. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was not detected in the samples. However, varying levels of related congeners, specifically hexachlorodibenzodioxins, heptachlorodibenzodioxins, and octachlorodibenzodioxins were found (2–330 ppm [parts per million], as a proportion of 100 percent active ingredient). Births (1952–1988) to these men were identified by linkage with the British Columbia (BC) live and stillbirth records. Further linkage with the BC Health Surveillance Registry identified cases of birth defects. The registry system is population based and uses multiple sources of identification. A case-control analysis was conducted, matching 5 controls (non-defect births) per case on year of birth and gender. Covariates included mother's and father's age. Exposure to chlorophenates for specific time periods was assessed by a team of industrial hygienists based on job title. Continuous estimates of cumulative hours of chlorophenate exposure were calculated for time windows relative to conception and pregnancy. Estimates of maximal exposure were determined for the most ex-

posed job in each time period. A total of 19,675 newborns were fathered by the 9,512 workers. There were a total of 942 birth defects among the offspring (4.8 percent). Several birth defects were associated with estimated chlorophenate exposure including cataracts, anencephaly or spina bifida, and anomalies of genital organs. Relative risk measures were calculated using a comparison of the 75th with the 25th percentile of estimated exposure. Exposure measures included MAX1—maximal exposure index in the three months prior to conception, and CUM2—estimated cumulative exposure in the three months prior to conception. Odds ratios were 2.4 (CI 1.1–5.3) for spina bifida or anenecephalus (N = 22; MAX1) and 1.8 (CI 0.8–4.1) for spina bifida alone (N = 18; MAX1). For cataracts, odds ratios of 2.3 (CI 0.7–2.9; N = 11; MAX1) and 5.7 (CI 1.4–22.6; CUM2) were reported. The difference in the odds ratios between the combined analysis of spina bifida and anencephaly (OR = 2.4) and spina bifida only (OR = 1.8) suggests some association with anencephaly alone, although no specific results were presented. Weaker associations were found for genital organ anomalies (OR = 1.3, CI 0.9–1.5 for CUM2). No other defect groups showed an association with the exposure indices. The study has a number of strengths including the use of a well-defined cohort, linkage with a population-based registry system, a careful time period-specific exposure assessment by a team of industrial hygienists, and analysis of exposure by specific time windows relative to conception and pregnancy. Limitations include the lack of direct individual exposure measurements, the inability to separate effects of potential chlorophenates and dioxins, and the use of broad defect groups based on anatomic systems that were of relatively small size. In addition, only the mother's and the father's ages were adjusted for in the analysis. Nonetheless, this unique cohort provides useful information on the relationship between a specific industry and related exposures and the risk of birth defects in offspring.

A series of analyses were conducted using data on birth defects among the offspring of male pesticide applicators in Minnesota (Garry et al., 1996). In addition, analyses of the relationship between birth defect rates and county-specific agricultural data were performed. Information on private state-licensed pesticide applicators registered with the Minnesota Department of Agriculture in 1991 (N = 34,772) was linked with live birth data for the state of Minnesota (1989–1992). Birth defect data were contained in these birth files. Pesticide data for units or clusters of Minnesota counties with similar geologic features and crops were employed to obtain use data for 12 specific herbicides (including 2,4-dichlorophenoxyacetic acid [2,4-D]). Overall, the pesticide applicators had a higher prevalence rate of birth defects than the general population (maternal age-adjusted OR = 1.4, CI 1.2–1.7) as well as higher rates of circulatory/respiratory (OR = 1.7, CI 1.04–2.8), gastrointestinal (OR = 1.7; CI 0.8–3.8), urogenital (OR = 1.7, CI 1.1–2.6), musculoskeletal/integumental (maternal age >30 OR = 2.5, CI 1.6–4.0), and other defects (maternal age >35 OR = 2.9, CI 1.6–5.3). No increase was seen for central nervous system defects (OR = 1.1, CI 0.5–2.4). The investi-

gators also reported higher rates of birth defects among the general population residing in predominantly agricultural regions of Minnesota compared to the rates in forest/urban regions. Higher rates were found for defects of the central nervous system, circulatory/respiratory, gastrointestinal, and urogenital systems. An additional analysis was conducted to evaluate specific pesticide use. Based on pounds of active ingredient per county, data for low- and high-use categories were defined for 12 specific pesticides and comparisons of the birth defect rates were made within each region. The authors reported that the most consistent associations were found for 2,4-D and MCPA (2-methyl-4-chlorophenoxyacetic acid). The overall rates were presented after combining the data for both herbicides and combining all defects into a "major" defects category (central nervous system, circulatory/respiratory, urogenital, musculoskeletal/integumental). The overall rate ratio, comparing high- and low-use regions, was 1.9 (CI 1.7–2.1) for major defects and 1.5 (CI 1.4–1.6) for all defects. No results for specific defect groups were presented. It was also noted that six of the seven counties with the greatest use of chlorophenoxy herbicides also used fungicides most frequently.

The results of the study suggest a higher rate of birth defects among offspring of pesticide applicators than among the general population and a higher rate in regions with a greater use of chlorophenoxy herbicides. The study had the advantage of evaluating the risk of birth defects among the offspring of a unique occupational group—pesticide applicators—with a high potential for exposure to a variety of pesticides. County pesticide use data also provided interesting information for a regional ecologic analysis. The study had several limitations including the use of birth vital statistics to ascertain birth defects, use of a small number of confounders, lumping of birth defects into broad system groups, ecologic rather than individual-level pesticide data, and inability to separate the possible effects of multiple pesticides.

Blatter et al. (1997) conducted a multicenter case-control study of paternal occupation and risk of spina bifida in offspring. Live-born cases of spina bifida were identified by medical records review at seven hospitals and two rehabilitation centers in the Netherlands (1980–1992). Controls were children who were born healthy but developed trauma capitis or meningitis during early childhood and were diagnosed at three of the hospitals where cases were identified (N = 456). Birth registries were used to identify another group of controls (N = 1,894). Case and control parents were initially mailed a questionnaire to collect data on occupational histories and potentially confounding factors. A follow-up telephone interview was conducted for fathers that had an occupation with potential chemical or physical exposure. This second interview included items on the frequency of tasks and exposures and the use of protective gear. Agricultural workers were included in this second interview. Estimation of exposure level was based on responses to a questionnaire, a detailed follow-up phone interview, and the judgment of industrial hygienists. Exposure was analyzed for the period from three months prior to the estimated conception date to one month after. Re-

sponses to the initial questionnaire included 77 percent of cases and 68 percent of controls. The final analysis sample, including the second interview, totaled 222 cases and 764 controls. Data were collected on a number of potentially confounding factors including medication use, maternal diabetes, parity, family history of neural tube defects, and parental smoking and alcohol consumption. Overall, the prevalence of paternal pesticide use did not differ between cases and controls (11 case fathers, 35 control fathers; OR = 0.9, CI 0.4–1.9). However, an association was found for estimated moderate or high exposure to pesticides (73 percent of cases, 35 percent of controls; OR = 1.7, CI 0.7–4.0). The association was slightly reduced after adjustment for maternal agricultural employment (OR = 1.6). It was also noted that more case fathers used a backpack sprayer than control fathers (45 percent versus 21 percent). Calculation of the unadjusted odds ratio from data presented in the paper (Blatter et al., 1997, Table III) shows moderate association with moderate or heavy exposure to herbicides specifically (OR = 1.6, CI 0.6–4.0; 7 exposed cases and 15 exposed controls). The study had a number of strengths such as a relatively large number of cases, a two-stage interview to elicit more specific information on occupational exposures and work practices, and adjustment for multiple confounders, including maternal employment in agriculture. Study limitations included the use of only live-born cases, the lag between exposure and interview (2 to 15 years), response proportions, the lack of data on folic acid use, and the absence of separate analyses for each type of control group (hospital, population) to evaluate potential selection bias. In addition, although exposure determination and assessment were better than in some other earlier studies, they were still incomplete. For example, no consideration was given to potential pesticide exposure in workers outside of the agricultural industry. Further, no analyses were presented on specific pesticides, especially herbicides of interest such as 2,4-D, probably because of the small number of exposed subjects.

A recent study of birth defects among the offspring of Norwegian farmers noted several associations, including spina bifida (Kristensen et al., 1997). The investigators created a cohort of farming families by linking several Norwegian national registries. Farm holders born after 1924 were identified from the computerized files of national agricultural censuses held in 1969, 1979, and 1989, and horticultural censuses in 1974 and 1985. Linkages with the Central Population Register and Medical Birth Registry identified a total of 192,417 births in 1967–1991 to farm holders. A comparison group consisted of 61,351 births to mothers residing in agricultural municipalities who were known to not be farm holders. Birth defects were identified from the Medical Birth Registry, a national registry of all births of 16 completed weeks' gestation with up to three birth defects recorded. In addition, data were available on potential confounding factors including maternal age, birth order, parental consanguinity, geographic location, and maternal chronic disease. Exposure information for each farm was obtained from the agricultural censuses. Exposure variables used in the analysis were

based on type of farming (animal husbandry, grain farming, and orchard and greenhouse farming) and indicators of use (amount of money spent on pesticides, tractor pesticide-spraying equipment, and amount of phosphorus and nitrogen in fertilizers). Exposure information was derived from the census closest to the time of birth. The sensitive period for exposure was considered to be three months before the estimated date of conception.

Overall, the prevalence rate of all birth defects among farmers was 217.7 per 10,000 births compared to 231.1 among non-farmer births (adjusted OR = 1.0, CI 0.9–1.1). Except for a few birth defects, there was no general association with farming. However, when specific exposure variables were evaluated, several associations were noted, including spina bifida and tractor spraying equipment (28 exposed cases; OR = 1.6, CI 0.9–2.7) and the combination of tractor spraying equipment and orchards/greenhouses (5 exposed cases; OR = 2.8, CI 1.1–7.1); hydrocephaly (tractor spraying equipment and orchards/greenhouses; 5 exposed cases; OR = 3.5, CI 1.3–9.1); cryptorchism (pesticide purchase and field vegetables; 19 exposed cases; OR = 2.3, CI 1.3–4.0); hypospadias (tractor spraying equipment and grain; 40 exposed cases; OR = 1.5 CI, 1.0–2.3); and limb reduction defects (pesticide purchase and grain; 16 exposed cases; OR = 2.5, CI 1.1–5.9). There was no positive association found with anencephaly (tractor spraying equipment; OR = 0.7, CI 0.4–1.2). No results were presented for spina bifida and other exposure combinations. The authors note that the finding for spina bifida is supported by the fact that more extensive use of pesticides at higher levels occurs in orchards/greenhouses in Norway and that higher ORs were found for births conceived in April–June. The study was truly population based (all of Norway), and some data on potential exposures and confounders were available. Nonetheless, exposure indices were indirect and based on an agricultural or horticultural census taken by the government at approximately 10-year intervals, hence not usually in the same year as conception and pregnancy, and exposure level of specific parents is uncertain. With respect to neural tube defects, the association appeared to be limited to spina bifida, not anencephaly.

Synthesis

The previous literature was generally inconsistent with regard to paternal occupational and environmental herbicide exposure and risk of birth defects in offspring. Several previous studies of veterans showed a suggestive association with spina bifida, although a number of methodologic issues limit interpretation. The occupational studies of Dimich-Ward et al. (1996), Blatter et al. (1997), and Kristensen et al. (1997) provide some additional support for the association of herbicide exposure with this specific birth defect, although concerns remain, including the control of confounding, exposure determination, statistical imprecision, and isolation of exposure to specific herbicides and TCDD. Association with other birth defects in the studies of veterans and agricultural occupations is inconsistent.

Conclusions

Strength of Evidence in Epidemiologic Studies

There are no changes from *Update 1996*. In *Update 1996*, there was limited/suggestive evidence of an association between exposure to the herbicides considered in this report and spina bifida. There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides and other birth defects.

Biologic Plausibility

Laboratory studies, using adult male animals, of the potential male-mediated developmental toxicity of TCDD and herbicides, specifically with regard to birth defects, are too limited to permit conclusions. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and reproductive and developmental disorders is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

Increased Risk of Disease Among Vietnam Veterans

Since there are some data suggesting that the highest risks occur in those veterans estimated to have had exposure to Agent Orange (e.g., Ranch Hands), it therefore follows that there is limited/suggestive evidence for an increased risk of spina bifida among offspring of Vietnam veterans. A more thorough discussion of the issue of increased risk of disease among Vietnam veterans is contained in Chapter 1.

FERTILITY

Background

Male reproductive function is a complex system under the control of several components whose proper coordination is important for normal fertility. There are several components or end points related to male fertility, including reproductive hormones and sperm parameters. Only a brief description of male reproductive hormones is given here; more detailed reviews can be found elsewhere (Yen and Jaffe, 1991; Knobil et al., 1994). The reproductive neuroendocrine axis involves the central nervous system, the anterior pituitary gland, and the testis. The hypothalamus integrates neural inputs from the central and peripheral nervous systems and regulates gonadotropins (luteinizing hormone and follicle-stimulating hormone). Both of these hormones are necessary for normal spermatogenesis. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are secreted in episodic bursts by the anterior pituitary gland into the circulation. LH interacts

with receptors on the Leydig cells, which leads to increased testosterone synthesis. FSH and testosterone from the Leydig cells interact with the Sertoli cells in the seminiferous tubule epithelium to regulate spermatogenesis. Several agents, such as lead and dibromochloropropane, have been shown to affect the neuroendocrine system and spermatogenesis (Bonde and Giwercman, 1995; Tas et al., 1996).

Summary of *VAO* and *Update 1996*

Only one occupational epidemiologic study was available for assessing the association between herbicide exposure and altered sperm parameters (sperm count, motility, morphology). This study of 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 (Lerda and Rizzi, 1991). The CDC VES (CDC, 1989) did not find any association between service in Vietnam and alterations in FSH, LH, and testosterone, although the Vietnam veterans had a lower average sperm concentration and a lower average proportion of morphologically normal sperm than non-Vietnam veterans. Researchers from the National Institute for Occupational Safety and Health (NIOSH) conducted a cross-sectional study to evaluate the relationship between serum dioxin and serum testosterone and gonadotropins in men previously occupationally exposed to dioxin and in a referent group (Egeland et al., 1994). The exposed group consisted of men who were either current or former employees at 2 of the 12 plants that are part of the NIOSH cohort study of dioxin-exposed workers. Results of the linear regression analysis indicated that current serum dioxin was related to FSH, LH, and testosterone levels. Serum dioxin was positively related to FSH (regression coefficient, $b = 0.04$ pg/g lipid) and LH ($b = 0.03$) and inversely related to testosterone ($b = –0.02$). The magnitude of the increases or decreases in hormones was rather small, compared to the normal range for these hormones in humans. In a categorical analysis, there was an association found between high LH and current serum dioxin (2nd dioxin quartile OR = 1.9; 3rd OR = 2.5; 4th OR = 1.9; p for trend = 0.03). For FSH, a pattern of increasing risk with increasing serum dioxin was also found, but the test for trend was not statistically significant ($p = 0.10$). The adjusted odds ratios for low testosterone were more elevated (2nd dioxin quartile = 3.9; 3rd = 2.7; 4th = 2.1), but again the trend test was not significant ($p = 0.10$). Similar estimates were obtained for half-life serum dioxin extrapolated to the time at which occupational exposure ended.

The results of this study indicated that estimated dioxin exposure levels were positively associated with LH and FSH levels and negatively associated with serum testosterone. As the authors correctly noted, the magnitude of the differences in hormone concentrations were small. A major issue in the interpretation of these findings, if real, is whether these changes in hormone concentration

ascribed to dioxin have any implications for reproductive failure. Clearly, the hormonal changes are rather subtle and are well below levels expected to result in gonadal failure.

Update of the Scientific Literature

An analysis of semen samples collected in 1991–1992 from 17 Vietnam veterans showed detectable levels of TCDD (an average of 3 parts per quadrillion), although the significance of this level of TCDD for adverse reproductive and developmental endpoints is not clear (Schecter et al., 1996).

A recent Ranch Hand publication addressed the relationship between serum dioxin and reproductive hormones (Henriksen et al., 1996). The investigators measured serum testosterone, FSH, LH, and testicular abnormality during clinic visits by Ranch Hand and comparison participants in 1982, 1985, 1987, and 1992. Serum dioxin was measured from the 1987 sample. Sperm count and percentage of abnormal sperm were assessed in 1982; testicular volume was measured in 1992. After exclusion of subjects for nondiagnostic (e.g., refusal, vasectomy, missing dioxin data) and diagnostic (scrotal varices, prostatitis, mumps, orchitis, and epididymitis) reasons, a total of 474 Ranch Hands and 532 comparisons were included in the analysis. In the baseline year of 1982 a total of 1,045 Ranch Hands and 1,224 comparisons participated in the study. Serum testosterone less than 400 ng/ml in 1982, or less than 260 ng/ml in 1987 or 1992, was defined as abnormally low; FSH levels greater than 25 IU/ml in 1982 or 17.2 IU/ml in 1987, or greater than 15 IU/ml in 1992, were defined as abnormally high; LH greater than 30 IU/ml in 1982, 25.1 IU/ml in 1987, or 9.8 IU/ml in 1992 was defined as abnormally high. Sperm counts less than or equal to 60 million per milliliter were defined as abnormally low, and a percentage of abnormal sperm greater than 30 percent was defined as abnormally high. Potential confounding factors adjusted for in the analysis included age, race, personality type, diabetes, current alcohol consumption, current cigarette smoking, and percentage of body fat. Sperm count and abnormality were only adjusted for age and exposure to industrial chemicals. Background dioxin levels were defined as less than or equal to 10 parts per trillion (ppt), the 98th percentile in the comparison group. For Ranch Hand participants with levels above background, current dioxin level was extrapolated to their initial level at the end of their tour of duty in Vietnam and at the time of semen collection in 1982. Ranch Hand participants with dioxin levels greater than 10 ppt were assigned to low- and high-exposure categories based on the median (130 ppt) initial dioxin level. The number of Ranch Hands in each category was 186 (background), 144 (low), and 144 (high). The age of the men in 1987 ranged from 37 to 74 years (mean age 49).

Elevated odds ratios were found for abnormally low testosterone levels among Ranch Hand men in the high-dioxin category in 1982 (OR = 1.1, CI 0.6–1.8; N = 16; 6%) and 1992 (OR = 1.6, CI 0.9–2.7; N = 18; 7.2%) and in the low category in

1987 (OR = 2.3, CI 1.1–4.9; *N* = 10; 3.8%). The other odds ratios for low testosterone were at or below 1.0. Some weakly elevated odds ratios were found for abnormally high FSH in the background and low-dioxin, but not high-dioxin, categories (e.g., 1992: background = 1.3, CI 0.7–2.4, low = 1.6, CI 0.8–3.0, high = 1.0, CI 0.5–2.1). There were no consistently elevated odds ratios found for abnormally high LH. A few odds ratios were weakly elevated for testicular abnormality (1987 high dioxin category OR = 1.2; 1992 low-dioxin category OR = 1.1). No association was found with percentage of abnormal sperm or low sperm count. No Ranch Hands in the low- or high-exposure categories had a high percentage of abnormal sperm. The odds ratio for low sperm count was 0.9 (CI 0.7–1.2) among men in the high dioxin category. There was also no relationship found between dioxin level and testicular volume. In a subsequent publication, Henriksen and Michalek (1996) analyzed the relationships between dioxin and hormone levels as continuous variables. The analysis used a linear model with adjustment for covariates (age, race, military occupation, personality type, and percentage of body fat) and transformation of testosterone (square root) and FSH and LH (logarithmic). The adjusted testosterone means decreased in relation to the dioxin category (comparison mean = 510.2; Ranch Hand background = 544.7; low = 542.1; high = 449.4). Adjusted FSH and LH means also decreased. Among the Ranch Hand subjects a regression model likewise showed a testosterone decrease (unadjusted slope = –0.4276; standard error = 0.0950). In the adjusted model an interaction with military occupation was found. The slope was the most strongly negative among Ranch Hand officers (–1.2381) and weakest among enlisted ground personnel (–0.3485). This relationship was viewed by the authors as inconsistent owing to the fact that Ranch Hand officers are the least exposed and ground personnel have the highest dioxin levels.

The study (Henriksen et al., 1996; Henriksen and Michalek, 1996) is noteworthy because of the unique nature of the Ranch Hand cohort and the fact that multiple reproductive end points were carefully evaluated. Nonetheless, some analyses were limited by relatively small numbers of highly exposed subjects. The study found a general pattern of no association between dioxin levels and several semen quality and hormone endpoints. The results for testosterone showed a weak pattern of decreasing hormone level with increasing dioxin level. This result is somewhat consistent with the industrial cohort study of Egeland et al. (1994); however, the dioxin body burdens are much lower in the Ranch Hand subjects than the workers. Further, the decrease in testosterone is not of a magnitude that is generally thought to be clinically meaningful.

Heacock et al. (1998) evaluated fertility in a sawmill worker cohort in British Columbia (referred to earlier in the discussion of birth defects). The worker cohort was linked with provincial marriage and birth files. The person-year contributions and live births for workers less than 55 years of age, who had worked for at least one year between 1950 and 1985 (*N* = 26,487) were included. The exposure of these workers to chlorophenates, possibly contaminated by dioxin, was estimated for each worker, along with an index of cumulative chlorophenate

exposure duration, and was analyzed in categories (<120 hours; 120–1,999; 2,000–3,999; 4,000–9,999; and ≥10,000 hours). For the external analysis, exposure was defined as sawmills in which chlorophenates were used; for the internal analysis, the cumulative exposure index was used. The standardized fertility ratio (SFR) was estimated from the number of live births divided by the number of expected births adjusted for age and calendar year based on person-years at risk for the worker cohort multiplied by male fertility rates in British Columbia. The internal analysis estimated the rate ratio to evaluate the effects of chlorophenate exposure and time since hire, with adjustment for age and calendar period.

The SFR was 0.7 (CI 0.7–0.8) for workers from mills where chlorophenates were used and 0.8 (CI 0.8–0.9) for workers from mills not using chlorophenates. The rate ratio for workers from chlorophenate mills was 0.9 (CI 0.8–0.9). Analysis of the cumulative chlorophenate exposure duration index did not show a pattern of decreasing SFRs with increasing cumulative hours of exposure (≥10,000 hours: SFR = 0.8, CI 0.7–0.8). Similar results for the analysis estimating rate ratios (RR for ≥10,000 hours relative to <120 hours: 1.1, CI 0.9–1.2; adjusted for age and calendar period). Increasing time since hire was associated with decreasing fertility for all sawmill workers (>20 years among workers from mills not using chlorophenates: SFR = 0.5, CI 0.2–0.9; >20 years among workers from mills using chlorophenates SFR = 0.4, CI 0.4–0.5). No association with age at hire was found. The results indicate reduced fertility among the sawmill workers; however, the decrease does not appear to be related to estimated chlorophenate exposure. The population study was based on a relatively large number of workers and included a detailed assessment of potential chlorophenate exposure. However, a number of potentially confounding factors such as smoking, alcohol use, contraceptive use, and history of illness were not included in the study.

A potentially related fertility end point is sex ratio. Sex ratio (proportion at birth, about 105–107 males per 100 females) has been used for a number of years as a potential marker of genetic damage. It has been hypothesized that the induction of lethal mutations prior to birth will alter the sex ratio at birth. In general it was thought that with paternal exposure there would be a reduction in the frequency of female offspring since sex-linked lethals on the paternal X chromosome would differentially affect female conceptuses. Investigators have evaluated the sex ratio among various species in relation to exposures such as radiation for a number of years. More recently, it has been suggested that the sex ratio is controlled by parental hormone levels at conception and that changes in gonadotropin and steroid levels may result in an altered sex ratio (James, 1997). The specific mechanisms involved (zygote formation, implantation, regulation of sex-determining factors, selective fetal loss) are uncertain, and direct experimental evidence for or against the hypothesis is lacking. James (1996) has suggested that a reduction in testosterone and high gonadotropin levels after dioxin exposure would result in an excess of female offspring. Potential confounding factors for altered sex ratio are uncertain, but parental age, social class, illness, race, smoking, and stress have been considered.

Mocarelli et al. (1996) evaluated the sex ratio (males to females) among offspring who were born in zone A of Seveso from 1977 to 1984. Among the 74 total births there was an excess of females (26 males/48 females; $p = <0.001$). Stored serum samples were used to determine TCDD levels in 13 families in which both parents were from zone A to further examine the relationship to sex ratio. From the data presented, it appeared that there were only female births among the nine families with "high" TCDD levels in 1976 (104–2340 ppt) and mostly male offspring of parents with "low" levels (26.6–65.4 ppt). In contrast, Garry et al. (1996) found that the sex ratio in county clusters with high chlorophenoxy herbicide/fungicide use was 2.8 among children of pesticide applicators with major anomalies and 1.5 among births in the general population in the same area. The sex ratios for children without anomalies among applicators and the general population in these areas were not presented. The British Columbia study of sawmill workers reported a sex ratio of 1.06:1 for offspring of chlorophenate-exposed workers, 1.08:1 for unexposed workers, and 1:05:1 for the province of British Columbia (Heacock et al., 1998). Results from the small Seveso study (Mocarelli et al., 1996) suggest an altered sex ratio with dioxin and/or herbicide exposure; however, other studies do not support this finding. Experimental animal evidence and further mechanistic data are also needed to evaluate the relationship between dioxin, or herbicides and sex ratio.

Synthesis

Three relatively well-designed studies have evaluated hormone, semen quality, and fertility endpoints in relation to potential dioxin exposure. The NIOSH study (Egeland et al., 1994) suggested a weak effect of dioxin on levels of LH, FSH, and testosterone. The recent Ranch Hand study (Henriksen et al., 1996) also reported a relatively small decrease in testosterone in relation to increased dioxin level. The study found no association with sperm count or percentage of abnormal sperm. The British Columbia sawmill worker study (Heacock et al., 1998) found reduced fertility rates among the worker cohort but could not consistently attribute this effect to chlorophenate exposure. Nonetheless, sufficient uncertainty remains because some studies had methodologic limitations including small sample sizes for higher exposure categories, failure to account for potential confounding factors, and exposure misclassification. Fertility studies are summarized in Table 9-2.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides considered in this report and altered hormone levels, semen quality parameters, or infertility.

TABLE 9-2 Selected Epidemiologic Studies—Fertility

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Heacock et al., 1998	Workers at sawmills using chlorophenates (RR)	18,016 births	0.9 (0.8-0.9)
	Workers at sawmills using chlorophenates (SFR)	18,016	0.7 (0.7–0.8)
	Cumulative exposure (hours) RR		
	120–1,999	7,139	0.8 (0.8–0.9)
	2,000–3,999	4,582	0.9 (0.8–0.9)
	4,000–9,999	4,145	1.0 (0.9–1.1)
	≥10,000	1,300	1.1 (0.9–1.2)
VIETNAM VETERANS			
New Studies			
Henriksen et al., 1996	Ranch Hands		
	Low testosterone		
	High dioxin (1992)	18	1.6 (0.9–2.7)
	High dioxin (1987)	3	0.7 (0.2–2.3)
	Low dioxin (1992)	10	0.9 (0.5–1.8)
	Low dioxin (1987)	10	2.3 (1.1–4.9)
	Background (1992)	9	0.5 (0.3–1.1)
	High FSH		
	High dioxin (1992)	8	1.0 (0.5–2.1)
	Low dioxin (1992)	12	1.6 (0.8–3.0)
	Background (1992)	16	1.3 (0.7–2.4)
	High LM		
	High dioxin (1992)	5	0.8 (0.3–1.9)
	Low dioxin (1992)	5	0.8 (0.5–3.3)
	Background (1992)	8	0.8 (0.4–1.8)
	Low Sperm Count		
	High dioxin	49	0.9 (0.7–1.2)
	Low dioxin	43	0.8 (0.6–1.0)
	Background	66	0.9 (0.7–1.2)
Reviewed in *VAO*			
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)
Stellman et al., 1988	American Legionnaires who served in Southeast Asia—difficulty having children	349	1.3 ($p < 0.01$)

Biologic Plausibility

Experimental animal evidence suggests that dioxin can alter testosterone synthesis, generally at relatively high doses, but does not provide direct clues as to the reproductive significance of hormone dysregulation of the magnitude found in available studies. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and reproductive and developmental disorders is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

STILLBIRTH, NEONATAL DEATH, AND INFANT DEATH

Background

The use of the terms "stillbirth" and "neonatal death" can be confusing and has differed in various epidemiologic studies. 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 grams at birth, regardless of gestational age at delivery (Kline et al., 1989). Neonatal death is usually defined as the death of a live-born infant within the first 28 days 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). Stillbirths occur in approximately 1 to 2 percent of all births (Kline et al., 1989). Among low-birthweight live- and stillborn infants (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).

Summary of *VAO* and *Update 1996*

A statistical association of herbicide exposure with stillbirth, neonatal, and infant death has been inconsistently 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, by uncertainty of correctly assigning herbicide exposure to study groups.

Update of the Scientific Literature

The study of sawmill workers in British Columbia also evaluated the association between chlorophenates and the risk of stillbirth and neonatal death (Dimich-Ward et al., 1996). The sawmill worker cohort was linked with stillbirth and infant death records maintained by the British Columbia Division of Vital Statistics. In this study, neonatal death was defined as the death of a live-born infant within the first year of life (usually known as infant death). Stillbirths included deaths of at least 28 weeks gestational age. No association was found for either stillbirths or infant deaths and any of the chlorophenate exposure measures. For the maximal index of exposure for any sawmill job held up to three months prior to conception the odds ratios were close to the null value (stillbirth OR = 1.0, CI 0.9–1.1; infant death OR = 1.0, CI 0.9–1.0).

A recent Ranch Hand publication reported results for the analysis of dioxin levels in relation to infant death (Michalek et al., 1998). Infant death was ascertained from medical records, vital statistics and autopsy records. Cause of death was coded based on record review. The analysis included a total of 5,151 children (2,082 conceived before the father's service in Southeast Asia [pre-SEA] and 3,069 post-SEA). Post-SEA children were stratified into four exposure categories including children of comparison veterans (current dioxin levels less than 10 ppt), "background" Ranch Hand children ($\leq$10 ppt), "low" Ranch Hand children (>10 ppt and $\leq$79 ppt), and "high" Ranch Hand children (>79 ppt). The cutpoint of 79 ppt is the median initial dioxin level based on extrapolated current levels (in 1987 or 1992). The stratified analyses adjusted for father's race, mother's smoking and alcohol consumption during pregnancy, parental age, and father's military occupation. The risk of infant death was increased in every Ranch Hand category. Post-SEA there were six deaths among the comparison children (0.5 percent); five deaths (1.6 percent) among the background infants (adjusted risk relative to the comparison group: 3.2, CI 1.0–10.3); two among the low-dioxin-category infants (RR = 1.5, CI 0.3–7.5); and six among the high-category infants (RR = 4.5, CI 1.5–14.0). The relative risk for the combined low and high categories was 3.0 (CI 1.1–8.7). Pre-SEA infant deaths were also associated with dioxin level (low and high RR = 1.8, CI 0.9–3.6; N = 15). The investigators also examined the distribution of post-SEA infant deaths in the low and high categories by quintile of initial dioxin. The number of infants at risk in each quintile was 107 in the first four quintiles and 108 in the last. Five of the eight deaths were in the 4th quintile (104.3 to 184 ppt), and one death was in the 5th quintile (184 to 1424.8 ppt). Another analysis was conducted excluding infants whose mothers had medical conditions such as hypertension during pregnancy, abruptio placentae, placenta previa, and Rh incompatibility. The results were similar to the analysis including these infants. Examination of the underlying cause of death showed that five of the six infant deaths in the post-SEA high category and all five deaths in the background group were caused by disorders related to short gestation and

unspecified low birthweight. An analysis of infant death by military occupation showed an increased risk for both officers (RR = 3.3) and enlisted ground personnel (RR = 2.6). The authors viewed the results as inconsistent because elevated risks were found for both background and high-dioxin-exposure categories, an excess of deaths was not found in the 5th dioxin quintile, and increased risks were also found for pre-SEA children. In addition, the findings by military occupation were also considered problematic because officers generally had the lowest exposure. Nevertheless, the findings remain intriguing and cannot be dismissed. The study is valuable because of the individual dioxin measurements, ascertainment of outcome from records, and adjustment for some potentially confounding factors. The analysis is limited by the relatively small number of exposed cases.

Synthesis

Previous occupational, environmental, and veteran studies did not consistently find an association between herbicide and dioxin exposure and an increased risk of fetal and infant death. The British Columbia study of chlorophenate-exposed sawmill workers did not find any association with stillbirth or infant death. The new Ranch Hand report suggested an association between dioxin exposure and an increased risk of infant death; however, the association was not consistently related to level of exposure and was also found for the period prior to service in southeast Asia. The evidence remains inadequate because some studies were limited by small sample sizes for higher exposure categories, failure to account for potential confounding factors, and exposure misclassification. The relevant studies are summarized in Tables 9-3, 9-4, and 9-5.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides considered in this report and stillbirth, neonatal death, and infant death.

Biologic Plausibility

Laboratory studies of the potential male-mediated developmental toxicity of TCDD and herbicides as a result of exposure of adult male animals are too limited to permit conclusions. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and reproductive and developmental disorders is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

TABLE 9-3 Selected Epidemiologic Studies—Stillbirth

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Dimich-Ward et al., 1996	British Columbia sawmill workers	159	1.0 (0.9–1.1)
	Maximal index of exposure for any job held up to three months prior to conception		
Studies Reviewed in *VAO*			
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	—
Townsend et al., 1982	Follow-up of Dow chemical plant employees	15	1.1 (0.5–2.1)
ENVIRONMENTAL			
Studies Reviewed in *VAO*			
Stockbauer et al., 1988	TCDD soil contamination in Missouri	4	1.6 (0.3–7.4)
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)
VIETNAM VETERANS			
Studies Reviewed in *VAO*			
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)
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)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	11	1.4 (0.5–3.5)

LOW BIRTHWEIGHT AND PRETERM BIRTH

Background

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

TABLE 9-4 Selected Epidemiologic Studies—Neonatal Death

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
Studies Reviewed in *VAO*			
Suskind and Hertzberg, 1990	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 death	1	
ENVIRONMENTAL			
Studies Reviewed in *VAO*			
Stockbauer et al., 1988	TCDD soil contamination in Missouri Perinatal deaths (includes stillbirths)	6	1.3 (0.4–4.2)
VIETNAM VETERANS			
Studies Reviewed in *VAO*			
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)
CDC, 1989	Vietnam Experience Study GBDS study—early neonatal death	16	2.0 (0.8–4.9)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	12	18.1 (2.4–134.4)

(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) or small for gestational age, 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). 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. 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 or ethnicity. Other potential risk factors for low birthweight include socioeconomic status (SES), maternal size, birth order, maternal complications during pregnancy (e.g., severe

TABLE 9-5 Selected Epidemiologic Studies—Infant Death

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Dimich-Ward et al., 1996	British Columbia sawmill workers		
	Maximal index of exposure for any job held up to three months prior to conception	300	1.0 (0.9–1.0)
Studies Reviewed in *VAO*			
Townsend et al., 1982	Follow-up of Dow chemical plant workers	9	0.6 (0.3–1.4)
ENVIRONMENTAL			
Studies Reviewed in *VAO*			
Stockbauer et al., 1988	TCDD soil contamination in Missouri	5	2.0 (0.5–8.7)
VIETNAM VETERANS			
New Studies			
Michalek et al., 1998	Ranch Hand—infant death post-service in southeast Asia		
	Background	5	3.2 (1.0–10.3)
	Low	2	1.5 (0.3–7.5)
	High	6	4.5 (1.5–14.0)
Studies Reviewed in *VAO*			
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)

preeclampsia) and obstetric history, job stress, and cocaine or caffeine use during pregnancy (Kallen, 1988). Established risk factors for preterm birth include race, marital status, socioeconomic status, previous low birthweight or preterm birth, multiple gestations, cigarette smoking, and cervical, uterine, or placental abnormalities (Berkowitz and Papiernik, 1993).

Summary of *VAO* and *Update 1996*

Given the lack of available occupational and environmental studies, the evidence on low infant birthweight was considered inadequate. Its inadequacy is due to the paucity of occupational studies and lack of consistent findings. One avail-

able epidemiologic study (Stockbauer et al., 1988) of dioxin exposure (soil contamination) reported a weak association (statistically nonsignificant) with low birthweight. Studies of veterans were inconsistent (AFHS, 1992; CDC, 1989; Field and Kerr, 1988); some indicated no increased risk, whereas others suggested an increased risk among certain subgroups.

Update of Scientific Literature

The study of sawmill workers in British Columbia also evaluated the association between chlorophenates and risk of low birthweight (Dimich-Ward et al., 1996). The sawmill worker cohort was linked with birth records maintained by the British Columbia Division of Vital Statistics. Low birthweight was defined as less than 2,500 grams, and small for gestational age as less than the 10th percentile based on the distribution of birthweight for gestational age in British Columbia. In addition, the investigators examined preterm birth (<37 weeks gestation). Adjustment was made for gender and, in the case of low birthweight, gestational age as well. No association was found for either low birthweight, small for gestational age, or PTD and any of the chlorophenate exposure measures. For the maximal index of exposure for any sawmill job held up to three months prior to conception, the odds ratios were close to 1.0 (low birthweight OR = 0.99, CI 0.97–1.0; small for gestational age OR = 1.0, CI 0.9–1.0; preterm birth OR = 0.9, CI 0.9–1.0). The recent Ranch Hand analysis described in the previous section also evaluated PTD and IUGR (Michalek et al., 1998). Preterm delivery (<37 weeks gestation) was determined using the mother's record of labor and delivery. IUGR was defined as birthweight less than the 10th percentile of birthweight for gestational week. The post-SEA analysis did not find a pattern of increased risk relative to dioxin exposure (background RR = 1.4, CI 0.9–2.3; low RR = 0.5, CI 0.2–1.5; high RR = 1.3, CI 0.8–2.3). With respect to IUGR, the relative risks were close to 1.0 for all exposure categories (background RR = 0.9, CI 0.6–1.4; low RR = 0.9, CI 0.6–1.3; high RR = 0.9, CI 0.6–1.3). As discussed previously, the Ranch Hand study is a valuable cohort because of exposure and outcome assessment, although again some of the analyses are limited by a small number of subjects.

Synthesis

Previous studies of Vietnam veterans (AFHS, 1992; CDC, 1989; Field and Kerr, 1988) did not consistently find an association between herbicide and dioxin exposure and an increased risk of low birthweight and preterm birth. The British Columbia study (Dimich-Ward et al., 1996) of chlorophenate-exposed sawmill workers did not find any association with low birthweight, small for gestational age, or preterm birth. The new Ranch Hand report (Michalek et al., 1998) did not find an association between dioxin exposure and an increased risk of preterm

birth or small for gestational age. The evidence remains inadequate because some studies were limited by small sample sizes for higher-exposure categories, failure to account for potential confounding factors, and exposure misclassification. Studies are summarized in Table 9-6.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides considered in the report and low birthweight and preterm birth.

Biologic Plausibility

Laboratory studies of the potential male-mediated developmental toxicity of TCDD and herbicides as a result of exposure of adult male animals are too limited to permit conclusions. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and reproductive and developmental disorders is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

CONCLUSIONS FOR REPRODUCTIVE EFFECTS

Strength of Evidence in Epidemiologic Studies

In *Update 1996* there was limited/suggestive evidence of an association between exposure to the herbicides considered in this report and spina bifida. The occupational studies of Dimich-Ward et al. (1996), Blatter et al. (1997), and Kristensen et al. (1997) provide some additional support for the association with this specific birth defect, although concerns remain including control of confounding, exposure determination, statistical imprecision, and isolation of exposure to specific herbicides and TCDD.

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides and fertility, stillbirth, neonatal and infant death, birth defects (other than spina bifida), and low birthweight and preterm birth.

Biologic Plausibility

Chapter 3 details the committee's evaluation of data from animals and studies with cells regarding the biological plausibility of a connection between exposure to dioxin or herbicides and various reproductive and developmental effects.

TABLE 9-6 Selected Epidemiologic Studies—Low Birthweight and Preterm Birth

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)
OCCUPATIONAL			
New Studies			
Dimich-Ward et al., 1996	Sawmill workers—Maximal index of exposure for any job held up to three months prior to conception		
	Low birthweight	848	0.9 (0.9–1.0)
	Preterm birth	867	0.9 (0.6–1.3)
Studies Reviewed in *VAO*			
Fitzgerald et al., 1989	Follow-up of an electrical transformer fire	3	81 (16.7–236.4)*
ENVIRONMENTAL			
Studies Reviewed in *VAO*			
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			
New Studies			
Michalek et al., 1998	Ranch Hand preterm birth		
	Background	20	1.4 (0.9–2.3)
	Low	6	0.5 (0.2–1.2)
	High	16	1.3 (0.8–2.3)
	Intrauterine growth retardation—post-service in southeast Asia		
	Background	29	0.9 (0.6–1.4)
	Low	22	0.9 (0.6–1.3)
	High	22	0.9 (0.6–1.3)
Studies Reviewed in *VAO*			
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)
CDC, 1989	Vietnam Experience Study (GBDS)	99	1.0 (0.8–1.4)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans	48	1.6 (1.0–2.5)
Stellman et al., 1988	Offspring of American Legion Vietnam veterans	—	—

*Standard incidence rate (SIR).

This section summarizes that evidence. Some of the preceeding discussions of reproductive and developmental outcomes include references to specific relevant papers.

TCDD is reported to cause a number of reproductive and developmental effects in laboratory animals. Administration of TCDD to male rats, mice, guinea pigs, marmosets, monkeys, and chickens elicits 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. The reproductive systems of adult male laboratory animals are considered to be relatively insensitive to TCDD, because high doses are required to elicit effects.

Limited research has been conducted on the offspring of male animals exposed to herbicides. A study of male mice fed varying concentrations of simulated Agent Orange mixtures concluded there were no adverse effects in offspring. A statistically significant excess of fused sternebra in the offspring of the two most highly exposed groups was attributed to an anomalously low rate of the defect in the controls. Another study reported an increase in the incidence of malformed offspring of male mice exposed to subacute levels of a mixture of 2,4-D and picloram in drinking water. However, the paternal toxicity observed in the high dosage levels used and inconsistent dose-response pattern are of concern.

Studies in male rats and hamsters show that decreased daily sperm production and cauda epididymal sperm number result from in utero and lactational TCDD exposure. Research suggests that in utero and lactational TCDD exposure selectively impairs rat prostate growth and development without inhibiting testicular androgen production or consistently decreasing prostate DHT concentrations.

Studies in female animals are limited but demonstrate in utero and lactational exposure reduced fertility, decreased ability to remain pregnant throughout gestation, decreased litter size, increased fetal death, impaired ovary function, and decreased levels of hormones such as estradiol and progesterone. 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.

Recent studies on female rats show that a single dose of TCDD administered on gestational day 15 results in malformations of the external genitalia in Long-Evans and Holtzman rats. There was complete to partial clefting of the phallus. Exposure on gestation day 8 was more effective in inducing functional reproductive alterations in female progeny (e.g., decreased fertility rate, reduced fecundity, cystic endometrial hyperplasia, and increased incidences of constant estrus). TCDD also induced changes in serum hormone levels in immature female rats administered TCDD by gastric intubation, increasing LH, FSH, and gonadotropin levels. This effect is partially due to the action of TCDD on the pituitary and is calcium dependent.

TCDD exposure did not increase egg mortality nor did it affect time-to-hatching of newly fertilized zebrafish eggs. However, pericardial edema and craniofacial malformations were observed in zebrafish larvae. In ovo TCDD exposure adversely affected the body and skeletal growth and hatchibility of the domestic pigeon but had no effect on the domestic chicken or great blue heron.

TCDD is teratogenic in mice, inducing cleft palate and hydronephrosis. Research indicates that co-exposure with either of two other chemicals, hydrocortisone or retinoic acid, synergistically enhances expression of cleft palate. This synergy suggests that the pathways controlled by these agents converge at one or more points in cells of the developing palate.

Several reports published during the reference period describe developmental deficits in the cardiovascular system of TCDD-treated animals. For example, the cardiotoxicity induced by TCDD was examined in the chick embryo. The spatial and temporal expression of AhR and Arnt suggests that the developing myocardium and cardiac septa are potential targets of TCDD induced teratogenicity, and such targets are also consistent with cardiac hypertrophy and septal defects observed following TCDD exposure. Evidence suggests that the endothelium lining of blood vessels is a primary target site of TCDD-induced cardiovascular toxicity. CYP1A1 induction in the endothelium may be linked to early lesions that result in TCDD-induced vascular derangements. CYP1A1 is induced in mammalian endothelial cells in culture in the vascular endothelium in lake trout of developing animals and in adult quail aortic smooth muscle cells. DNA damage and consequent cell death in the embryonic vasculature are key physiological mediators of TCDD-induced embryotoxicity in Medaka, a type of fish often used in laboratory studies. Treatment of TCDD-exposed Medaka embryos with an anti-oxidant provides significant protection against TCDD-induced embryotoxicity and suggests that reactive oxygen species may participate in the teratogenic effects of TCDD.

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. However, other, as-yet-unidentified factors also play a role, 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 vary among species. More generally, TCDD has a wide range of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. These effects may in turn influence reproductive and developmental outcomes.

Limited information is available on reproductive/developmental effects of the herbicides discussed in the report. Studies indicate that 2,4-D does not affect male or female fertility and does not produce fetal abnormalities, but when preg-

nant rats or mice are exposed it does reduce the rate of growth of offspring and increase their rate of mortality. Very high doses were required to elicit these effects. 2,4,5-T was toxic to fetuses when administered to pregnant rats, mice, and hamsters. The ability of 2,4,5-T to interfere with calcium homeostasis in vitro was documented and linked to the teratogenic effects of 2,4,5-T on the early development of sea urchin eggs. Cacodylic acid is toxic to rat, mouse, and hamster fetuses at high doses that are also toxic to the pregnant mother. Limited data suggested that picloram may produce fetal abnormalities in rabbits at doses that are also toxic to the pregnant animals.

The foregoing evidence suggests that a connection between TCDD or herbicide exposure and human reproductive and developmental disorders, is, in general, biologically plausible. However, differences in sensitivity and susceptibility across individual animals, strains and species, lack of strong evidence of organ-specific effects across species, and differences in route, dose, duration and timing of exposure complicate any more definitive conclusions about the presence or absence of a mechanism for induction of specific reproductive and developmental disorders by TCDD and herbicides.

Considerable uncertainty remains over how to apply this information to the evaluation of potential health effects of herbicides or dioxin exposure in Vietnam veterans. Scientists disagree over the extent to which information derived from animals and cellular studies predicts human health outcomes, and the extent to which the health effects resulting from high-dose exposure are comparable to those resulting from low-dose exposure. Research on biological mechanisms is burgeoning and subsequent Veterans and Agent Orange updates may have more and better information on which to base conclusions.

Increased Risk of Disease Among Vietnam Veterans

Under the Agent Orange Act of 1991, the committee is asked to determine (to the extent that available scientific data permit meaningful determinations) the increased risk of the diseases it studies among those exposed to herbicides during their service in Vietnam. Chapter 1 presents the committee's general findings regarding this charge. Where more specific information about particular health outcomes is available, this information is related in the preceding discussions of those health outcomes.

REFERENCES

Ahlborg G, Hogstedt C, Bodin L, Barany S. 1989. Pregnancy outcome among working women. Scandinavian Journal of Work, Environment, and Health 15:227–233.

Air Force Health Study (AHFS). 1992. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Reproductive Outcomes. Brooks AFB, TX: Armstrong Laboratory. AL-TR-1992-0090.

Air Force Health Study. 1985. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update: 1985. Brooks AFB, TX: USAF School of Aerospace Medicine.

Alberman E. 1984. Low birthweight. In: Bracken MB, ed. Perinatal Epidemiology. New York: Oxford University Press. 86–98.

Aschengrau A, Monson RR. 1990. Paternal military service in Vietnam and the risk of late adverse pregnancy outcomes. American Journal of Public Health 80:1218–1224.

Australia Department of Veterans Affairs. 1983. Case-Control Study of Congenital Abnormalities and Vietnam Service. Canberra, Australia: Dept. of Veterans Affairs.

Berkowitz GS, Papiernik E. 1993. Epidemiology of preterm delivery. Epidemiologic Reviews 15:414–443.

Blatter BM, Roeleveld N. 1996. Spina bifida and parental occupation in a Swedish register-based study. Scandinavian Journal of Work, Environment, and Health 22(6):433–437.

Blatter BM, Roeleveld N, Zielhuis GA, Gabreels FJM, Verbeek ALM. 1996. Maternal occupational exposure during pregnancy and the risk of spina bifida. Occupational and Environmental Medicine 53(2):80–86.

Blatter BM, Hermens R, Bakker M, Roeleveld N, Verbeek AL, Zielhuis GA. 1997. Paternal occupational exposure around conception and spina bifida in offspring. American Journal of Industrial Medicine 32(3):283–291.

Bloom AD, ed. 1981. Guidelines for Studies of Human Populations Exposed to Mutagenic and Reproductive Hazards. White Plains, NY: March of Dimes Foundation.

Bonde JP, Giwercman A. 1995. Occupational hazards to male fecundity. Reproductive Medicine Review 4:59–73.

Bryce R. 1991. The epidemiology of preterm birth. In: Kiely M, ed. Reproductive and Perinatal Epidemiology. Boca Raton, FL: CRC Press. 437–444.

Centers for Disease Control (CDC). 1988. Health status of Vietnam veterans. III. Reproductive outcomes and child health. Journal of the American Medical Association 259:2715–2717.

Centers for Disease Control. 1989. Health status of Vietnam veterans. Vietnam Experience Study, Vol. V, Reproductive Outcomes and Child Health. Atlanta: U.S. Department of Health and Human Services.

Correa-Villasenor A, Ferencz C, Boughman JA, Neill CA. 1991. Baltimore-Washington Infant Study Group: total anomalous pulmonary venous return: familial and environmental factors. Teratology 44:415–428.

Dimich-Ward H, Hertzman C, Teschke K, Hershler R, Marion SA, Ostry A, Kelly S. 1996. Reproductive effects of paternal exposure to chlorophenate wood preservatives in the sawmill industry. Scandinavian Journal of Work, Environment and Health 22(4):267–273.

Egeland GM, Sweeney MH, Fingerhut MA, Wille KK, Schnorr TM, Halperin WE. 1994. Total serum testosterone and gonadotropins in workers exposed to dioxin. American Journal of Epidemiology 139:272–281.

Erickson J, Mulinare J, Mcclain P, Fitch T, James L, McClearn A, Adams M. 1984a. Vietnam Veterans' Risks for Fathering Babies with Birth Defects. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control.

Erickson JD, Mulinare J, Mcclain PW. 1984b. Vietnam veterans' risks for fathering babies with birth defects. Journal of the American Medical Association 252:903–912.

Fenster L, Coye MJ. 1990. Birthweight of infants born to Hispanic women employed in agriculture. Archives of Environmental Health 45:46–52.

Field B, Kerr C. 1988. Reproductive behaviour and consistent patterns of abnormality in offspring of Vietnam veterans. Journal of Medical Genetics 25:819–826.

Fitzgerald EF, Weinstein AL, Youngblood LG, Standfast SJ, Melius JM. 1989. Health effects three years after potential exposure to the toxic contaminants of an electrical transformer fire. Archives of Environmental Health 44:214–221.

Garry VF, Schreinemachers D, Harkins ME, and Griffith J. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. Environmental Health Perspectives 104(4):394–399.

Goulet L, Theriault G. 1991. Stillbirth and chemical exposure of pregnant workers. Scandinavian Journal of Work, Environment, and Health 17:25–31.

Ha MC, Cordier S, Bard D, Thuy LTB, Hao HA, Quinh HT, Dai LC, Abenhaim L, Phuong NTN. 1996. Agent orange and the risk of gestational trophoblastic disease in Vietnam. Archives of Environmental Health 51(5):368–374.

Hanify JA, Metcalf P, Nobbs CL, Worsley KJ. 1981. Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science 212:349–351.

Heacock H, Hogg R, Marion SA, Hershler R, Teschke K, Dimich-Ward H, Demers P, Kelly S, Ostry A, Hertzman C. 1998. Fertility among a cohort of male sawmill workers exposed to chlorophenate fungicides. Epidemiology 9(1):56–60.

Hemminki K, Mutanen P, Luoma K. 1980. Congenital malformations by the parental occupation in Finland. International Archives of Occupational and Environmental Health 46:93–98.

Henriksen GL, Michalek JE. 1996. Serum dioxin, testosterone, and gonadotropins in veterans of Operation Ranch Hand. Epidemiology 7(4):454–455.

Henriksen GL, Michalek JE, Swaby JA, Rahe AJ. 1996. Serum dioxin, testosterone, and gonadotropins in veterans of Operation Ranch Hand. Epidemiology 7(4):352–357.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

James WH. 1996. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. Journal of Theoretical Biology 180(4):271–286.

James WH. 1997. The use of offspring sex ratios to detect reproductive effects of male exposure to dioxins. Environmental Health Perspectives 105(2):162–163.

Kallen B. 1988. Epidemiology of Human Reproduction. Boca Raton, FL: CRC Press.

Kalter H, Warkany J. 1983. Congenital malformations. Etiologic factors and their role in prevention (first of two parts). New England Journal of Medicine 308:424–491.

Kline J, Stein Z, Susser M. 1989. Conception to Birth: Epidemiology of Prenatal Development. New York: Oxford University Press.

Knobil E, Neill JD, Greenwald GS, Markert CL, Pfaff DW, eds. 1994. The Physiology of Reproduction. New York: Raven Press.

Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. 1996. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 97(5):700–706.

Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L. 1997. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 8(5):537–544.

Lerda D, Rizzi R. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). Mutation Research 262:47–50.

Lin S, Marshall EG, Davidson GK. 1994. Potential parental exposures to pesticides and limb reduction defects. Scandinavian Journal of Work, Environment, and Health 20:166–179.

May G. 1982. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. British Journal of Industrial Medicine 39:128–135.

McDonald AD, McDonald JC, Armstrong B. 1987. Occupation and pregnancy outcome. British Journal of Industrial Medicine 44:521–526.

Michalek JE, Rahe AJ, Boyle CA. 1998. Paternal dioxin, preterm birth, intrauterine growth retardation, and infant death. Epidemiology 9(2):161–167.

Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr, Needham LL. 1996. Change in sex ratio with exposure to dioxin. Lancet 348(9024):409.

Nurminen T, Rantala K, Kurppa K, Holmberg PC. 1995. Agricultural work during pregnancy and selected structural malformations in Finland. Epidemiology 6:23–30.

Olshan AF, Faustman EM. 1993. Male-mediated developmental toxicity. Annual Review of Public Health 14:159–181.

Restrepo M, Munoz N, Day NE, Parra JE, Hernandez C, Brettner M, Giraldo A. 1990. Prevalence of adverse reproductive outcomes in a population occupationally exposed to pesticides in Colombia. Scandinavian Journal of Work, Environment, and Health 16:232–238.

Savitz DA, Whelan EA, Kleckner RC. 1989. Effect of parents' occupational exposures on risk of stillbirth, preterm delivery, and small-for-gestational-age infants. American Journal of Epidemiology 129:1201–1218.

Schecter A, McGee H, Stanley JS, Boggess K, Brandt-Rauf P. 1996. Dioxins and dioxin-like chemicals in blood and semen of American Vietnam veterans from the State of Michigan. American Journal of Industrial Medicine 30(6):647–654.

Smith AH, Fisher DO, Pearce N, Chapman CJ. 1982. Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Archives of Environmental Health 37:197–200.

Stellman SD, Stellman JM, Sommer JF Jr. 1988. Health and reproductive outcomes among American Legionnaires in relation to combat and herbicide exposure in Vietnam. Environmental Research 47:150–174.

Stockbauer JW, Hoffman RE, Schramm WF, Edmonds LD. 1988. Reproductive outcomes of mothers with potential exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. American Journal of Epidemiology 128:410–419.

Suskind RR, Hertzberg VS. 1984. Human health effects of 2,4,5-T and its toxic contaminants. Journal of the American Medical Association 251:2372–2380.

Tas S, Lauwerys R, Lison D. 1996. Occupational hazards for the male reproductive system. Critical Reviews in Toxicology 26(3):261–307.

Teschke K, Hertzman C, Fenske RA, Jin A, Ostry A, van Netten C, Leiss W. 1994. A history of process and chemical changes for fungicide application in the western Canadian lumber industry: What can we learn? Applied Occupational and Environmental Hygiene 9:984–993.

Townsend JC, Bodner KM, Van Peenen PFD, Olson RD, Cook RR. 1982. Survey of reproductive events of wives of employees exposed to chlorinated dioxins. American Journal of Epidemiology 115:695–713.

White FMM, Cohen FG, Sherman G, McCurdy R. 1988. Chemicals, birth defects and stillbirths in New Brunswick: associations with agricultural activity. Canadian Medical Association Journal 138:117–124.

Wolfe WH, Michalek JE, Miner JC, Rahe AJ, Moore CA, Needham LL, Patterson DG Jr. 1995. Paternal serum dioxin and reproductive outcomes among veterans of Operation Ranch Hand. Epidemiology 6:17–22.

Yen SC, Jaffe RB. 1991. Reproductive Endocrinology. Philadelphia: WB Saunders Company.

10

Neurobehavioral Disorders

BACKGROUND

The nervous system is made up of a central portion—the central nervous system (CNS)—that consists of the brain and spinal cord, and the peripheral nervous system (PNS), which includes the nerve roots, the brachial and lumbar plexuses, and the peripheral nerves that pass to the extremities. The peripheral nerves are responsible for innervating the muscles and also are made up of afferent fibers that convey sensory information to the CNS. In addition, they contain autonomic fibers that regulate the activity of the heart, blood vessels, sweat glands, bladder, and bowels.

Disturbances of the CNS may lead to neurobehavioral abnormalities such as cognitive changes and neuropsychiatric disorders. Other disturbances related to CNS dysfunction include abnormalities of sensation, weakness, tremors, incoordination, and the development of abnormal movements.

Disturbances of the PNS lead to peripheral neuropathy. Peripheral neuropathy is an imprecise term that includes the symmetric involvement of numerous nerves in the extremities (polyneuropathy) or the selective involvement of one or several individual peripheral nerves (mononeuropathy simplex or multiplex). It is sometimes used to refer to pathology affecting the nerve roots, which emerge from the spinal cord and have branches that ultimately form the nerves to the extremities. Chronic peripheral neuropathy may occur for many different reasons; it varies in its pathology and severity in different cases. It may be a feature of a number of common general medical disorders, especially diabetes mellitus.

Numerous publications have addressed the neurotoxicity of herbicides and pesticides. Such reports have been based on studies related to occupational, environmental, or Vietnam veteran exposure. In *Veterans and Agent Orange: Health*

TABLE 10-1 Selected Neurobehavioral Studies of Herbicide Exposure

Reference	Study Group	Tests of Neurological Dysfunction	Exposure Measures	Comparison Group
OCCUPATIONAL				
Zober et al., 1994	158 German BASF employees	Medical record review	Chloracne and TCDD levels	161 reference comparisons
Berkley and Magee, 1963	1 farmer	Neurological examination	No	None
Todd, 1962	1 weed-sprayer	Neurological examination	No	None
Goldstein et al., 1959	2 farmers 1 book-keeper	Neurological examination EMG	No	None
Baader and Bauer, 1951	10 pentachlorophenol plant workers	Record review clinical evaluation	No	None
ENVIRONMENTAL				
Peper et al., 1993	19 German residents exposed to 2,3,7,8-TCDD	Neuropsychological battery and symptom questionnaires	Serum TCDD	None
VIETNAM VETERANS				
Visintainer et al., 1995	151,377 Michigan veterans who served in Vietnam	No: mortality data only	No	225,651 Non-Vietnam veterans
Decoufle et al., 1992	7,924 veterans	Self-report with neurological examinations in a subset	Self-report	7,364 Non-Vietnam veterans

Effects of Herbicides Used in Vietnam (henceforth called *VAO*) (IOM, 1994), attention was focused particularly on persistent neurobehavioral dysfunction. In *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996), attention was also directed at the occurrence of acute and subacute peripheral neuropathy, and earlier data relating to that aspect were reexamined. In the present report, at the specific request of the Department of Veteran Affairs, the possibility of chronic peripheral neuropathy developing in Vietnam veterans as a consequence of herbicide exposure has been reconsidered.

COGNITIVE AND NEUROPSYCHIATRIC EFFECTS

Summary of *VAO* and *Update 1996*

In *VAO*, the committee concluded that the literature was insufficient to determine whether an association existed between exposure to herbicides and related compounds and chronic cognitive or neuropsychiatric disorders. As suggested by Sharp et al. (1986), the delayed effects of such exposures 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 was no shortage of studies concerning this topic, methodologic problems made it difficult to reach definitive conclusions. Shortcomings in defining exposure included absent or poor exposure assessments; inconsistencies in identifying exposed individuals for study (i.e., some studies relied on the presence of chloracne for inclusion, whereas others assumed that all subjects had been exposed); and concomitant exposure to different chemicals, mixtures of chemicals, or concentrations of chemicals. 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.

The committee noted that in order to maximally define the direct effects of dioxin on cognitive and neuropsychiatric function, future studies should focus primarily on occupationally exposed groups for whom levels of exposure are better known and should include neurobehavioral testing in relative proximity to the time of exposure.

VAO also concluded that significantly exposed subjects should be followed for the development of neuropsychological dysfunction in middle and later life. It is possible that minor CNS changes acquired in early adulthood are too subtle to be detected by current neuropsychological testing methods, but they could manifest themselves later when compounded by "normal age-related changes" of the CNS. Theoretically, exposure to neurotoxins could produce "accelerated aging" of the brain due to premature neuronal loss, which could then result in neurobehavioral deficits.

In *Update 1996*, the committee reviewed the several publications that had appeared since the original report, concluding that there was still inadequate or insufficient evidence from occupational and other studies of any association between exposure to the herbicides under consideration and cognitive or neuropsychiatric disorders.

Update of the Scientific Literature

In a report from Australia, O'Toole et al. (1996) analyzed the self-reported psychiatric states of Vietnam veterans as determined 20–25 years after the war. It was found that the veterans had higher prevalences than the civilian population of alcohol abuse or dependence, posttraumatic stress disorder, and social and simple phobias. This related to combat rather than posting to a combat unit. No attempt was made in the report to relate these behavioral disorders to herbicide exposure. We are aware of no other new studies since the last report that have provided any further evidence of an association between herbicide exposure and cognitive or neuropsychiatric dysfunction.

MOTOR/COORDINATION DYSFUNCTION

Summary of *VAO* and *Update 1996*

In *VAO*, the committee concluded that there were no definitive studies to determine whether exposure to dioxin or related herbicides was associated with CNS motor/coordination problems. However, follow-up of veterans and, to a lesser extent, environmental observations suggested that motor and coordination difficulties should be assessed further in exposed subjects. It was determined that longitudinal assessments of motor and coordination problems were warranted in exposed subjects, especially those with high exposure, such as the National Institute for Occupational Safety and Health cohort studied by Fingerhut et al. (1991). Vietnam veterans represent the most systematically evaluated group with chronic TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) 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 accepted scales for movement disorders have been developed, and these scales should be used in future studies of such problems.

In addition to assessments that capture the disability related to any objective findings, *VAO* also stressed that in the past decade, an increasing concern—unrelated specifically to the question of TCDD and the CNS—has developed scientifically over the possible link between Parkinson's disease and chemicals used as herbicides and pesticides (Semchuk et al., 1992). It was suggested that as Vietnam veterans move into the decades when Parkinson's disease becomes more prevalent, attention to the frequency and character of new cases in exposed

versus nonexposed individuals may be highly useful in assessing whether dioxin exposure is a risk factor for eventual Parkinson's disease.

In *Update 1996* it was noted that no new data relating to this topic had been published. Nevertheless, it was pointed out that concern persisted about the role of herbicides and pesticides in the pathogenesis of parkinsonism. For example, Semchuk et al. (1993) had noted following multivariate statistical analysis that occupational herbicide use was the third highest predictor of eventual Parkinson's disease risk. Similarly, Butterfield et al. (1993) examined occupational and environmental factors associated with disease risk in patients with early-onset Parkinson's disease and found that the disease was positively associated with herbicide exposure, insecticide exposure, previous residence in a fumigated house, and residence in a rural area at the time of diagnosis. The committee emphasized the importance of cases of early-onset parkinsonism in testing the hypothesis that the disease relates to a toxic exposure, and pointed to the particular medical and scientific importance of a systematic and prospective study of Vietnam veterans for the development of early-onset parkinsonism.

Update of the Scientific Literature

As far as this committee is aware, no new studies relating directly to this aspect have been published. However, Schulte et al. (1996) examined the death certificates from 27 states in the National Occupational Mortality Surveillance System and calculated proportional mortality ratios by occupation for certain neurodegenerative disorders. They found an increase in proportionate mortality ratio for Parkinson's disease among male pesticide applicators, horticultural farmers, farm workers, and graders and sorters of agricultural products. Similarly, in Taiwan, an increased risk of Parkinson's disease has been found among those using paraquat and other herbicides and pesticides (Liou et al., 1997). This was a case-control study in which 120 patients with Parkinson's disease were age- and sex-matched with 240 controls, and data were then obtained on demographic and residential history and on potential exposure to occupational and environmental agents. In Germany significantly elevated odds ratios with pesticide exposure have been noted among patients with Parkinson's disease (Seidler et al., 1996). The implications of these studies for the health of Vietnam veterans are unclear. Such reports underscore the importance of a prospective study of Vietnam veterans for the development of parkinsonism.

CHRONIC PERSISTENT PERIPHERAL NEUROPATHY

Summary of *VAO* and *Update 1996*

Although some of the case reports reviewed in *VAO* suggested that an acute or subacute peripheral neuropathy can develop with exposure to TCDD and

related products, other reports with comparison groups did not offer clear evidence that TCDD exposure is associated with chronic peripheral neuropathy. The most rigorously conducted studies argued against a relationship between TCDD or herbicides and chronic persistent neuropathy.

As a group, the studies concerning peripheral neuropathy have been conducted with highly varying methodologies and have lacked uniform 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 based on assessments made only a short time after exposure. It was concluded that careful definition of neuropathy and standardization of protocols will be essential to future evaluations.

In *Update 1996* it was noted that several new articles had appeared on this topic related to occupational and other studies. Careful analysis, however, showed there to be inadequate or insufficient evidence of any association between exposure to the herbicides under consideration and the development of chronic persistent peripheral neuropathy.

Update of the Scientific Literature

No new information has appeared in the intervening two years that alters this conclusion. It is important, however, to review the context in which this conclusion was reached.

Synthesis

There have been only a limited number of epidemiologic studies of chronic peripheral neuropathy, except for the neuropathy associated with diabetes. A recent study from Europe estimated that a chronic symmetric polyneuropathy occurs in about 8 percent of people over the age of 55 years with a severity that is sufficient to lead to symptoms of the disorder (Italian General Practitioner Study Group, 1995). Prevalence studies of peripheral neuropathy performed both in Asia and in Europe also suggest that peripheral neuropathy is common, occurring in between 2 and 7 percent of the population. In the United States, the most common cause of chronic peripheral neuropathy is diabetes. Several types of peripheral neuropathy are associated with diabetes, including a predominantly sensory polyneuropathy, an asymmetric proximal neuropathy, involvement of individual cranial or limb nerves, and an autonomic neuropathy. In one study it was found that approximately 4 percent of diabetic patients develop a neuropathy within 5 years of diagnosis and 15 percent do so within 20 years (Palumbo et al., 1978). More recent population studies or studies of clinical case

series suggest that the prevalence of neuropathy is even higher among diabetic patients. Thus, in one study of insulin-dependent diabetic patients, the overall prevalence of distal polyneuropathy was 34 percent, and this increased to 58 percent in patients who were aged 30 years or more (Maser et al., 1989). In diabetics who are not dependent upon insulin, evidence of peripheral sensory loss has been found in 26 percent of instances (Franklin et al., 1990). A study from Finland in 1995 has indicated that among non-insulin-dependent diabetic subjects, 8 percent satisfied criteria for definite or probable neuropathy at the time of diagnosis; after a 10-year follow-up period, the prevalence of neuropathy had increased to 42 percent in the diabetic patients, compared to 6 percent in control subjects (Partanen et al., 1995). Among the factors that relate to the development of neuropathy in diabetics are the duration of the disease and the extent to which the blood glucose level is controlled (Orchard et al., 1990; DCCT Research Group, 1995). Further reference to diabetes can be found in Chapter 11.

The peripheral nerves are vulnerable to a number of toxic substances including some of the heavy metals such as lead, arsenic, and thallium; certain organic solvents such as *n*-hexane and methyl *n*-butyl ketone; and various organophosphates. A neuropathy is also a common complication of chronic alcohol abuse and, in this circumstance, may relate to either a direct toxic effect of alcohol, a concomitant nutritional deficiency, or both. Other causes of chronic peripheral neuropathy include infection (such as leprosy), diverse metabolic disorders, and a number of general medical diseases. Some neuropathies have a hereditary basis, even though they may not become symptomatic until adulthood.

It is often not possible to distinguish with confidence among various possible causes of neuropathy, except by the setting in which the neuropathy develops. Distinction depends on the results of a detailed family and general medical history, any associated clinical findings, the results of various investigations including electrophysiologic studies, and pathologic examination of nerve biopsy specimens. A neuropathy with acute onset suggests a metabolic or toxic cause or an infective or inflammatory disturbance. A toxic basis for a neuropathy is supported by the cessation of progression—and by subsequent clinical improvement—after discontinuation of exposure to the offending substance. A chronic peripheral neuropathy due to toxic exposure (such as might be attributed to herbicide exposure) would not be expected to develop years after exposure to that toxin ceases.

Despite intensive investigation, a specific cause of a chronic peripheral neuropathy may not be found in between 20 and 50 percent of cases (Dyck et al., 1981; McLeod et al., 1984; Notermans et al., 1993; McLeod, 1995). It is not possible to ascribe such neuropathies to exposure to a possible neurotoxin when that exposure occurred years earlier and is not continuing.

ACUTE AND SUBACUTE TRANSIENT PERIPHERAL NEUROPATHY

Summary of *VAO* and *Update 1996*

In *Update 1996* it was noted that the methodology used to establish associations between putative causal agents and persistent chronic neurological deficits relies heavily on epidemiologic studies with adequate control or comparison populations. Such methodology can rarely be set in motion with sufficient speed to assess relationships between unexpected chemical exposure and the development of acute or subacute transient neurological disturbance. Because of the very transient nature of the conditions, documenting signs and symptoms in association with documented exposures can be difficult to accomplish in a systematic manner. In such instances, greater reliance must be placed on isolated case histories and less well controlled studies. Based on an analysis of the data from studies reviewed in *VAO* and *Update 1996*, as well as those published more recently regarding occupational, environmental, and Vietnam veteran exposure to herbicides and herbicide components, this committee agrees with the conclusion of the last committee that there is limited/suggestive evidence of an association between exposure to certain herbicides used in Vietnam and the development of an acute or subacute transient peripheral neuropathy. Acute peripheral neuropathies have been reported following acute occupational exposure to 2, 4-dichlorophenoxyacetic acid (2,4-D) weedkiller by several authors (Goldstein et al., 1959; Todd, 1962; Berkley and Magee, 1963). Affected patients had not been examined prior to exposure, but the temporal relationship between clinical disturbance and herbicide exposure was well documented. It remains possible, however, that the neuropathy was unrelated to the herbicide exposure and related to other disorders, such as Guillain-Barré syndrome.

Update of the Scientific Literature

The committee is aware of no new publications that bear on this issue. If TCDD were associated with the development of transient acute and subacute peripheral neuropathy, the disorder would become evident shortly after exposure. The committee knows of no evidence that new cases developing long after service in Vietnam are associated with herbicide exposure.

CONCLUSIONS FOR NEUROBEHAVIORAL DISORDERS

Strength of Evidence in Epidemiologic Studies

As in the earlier reports, this committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides used in Vietnam and disorders involving cognitive and

neuropsychiatric dysfunction, motor/coordination deficits, and chronic persistent peripheral neuropathy. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components, as reviewed in previous reports. No new evidence has appeared since those reports.

In *Update 1996*, the committee indicated that there is limited/suggestive evidence of an association between exposure to the herbicides considered in this report and acute or subacute transient peripheral neuropathy. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components. This conclusion remains unchanged.

Biologic Plausibility

Chapter 3 details the committee's evaluation of data from animals and studies with cells regarding the biological plausibility of a connection between exposure to dioxin or herbicides and various neurobehavioral disorders. This section summarizes that evidence. Some of the preceding discussions of neurobehavioral outcomes include references to specific relevant papers.

Little information exists on the development of neurobehavioral disorders and TCDD exposure in laboratory animals. Acute doses of TCDD administered to rats affect the metabolism of serotonin, a neurotransmitter in the brain able to modulate food intake. This biochemical change is consistent with observations of progressive weight loss and anorexia in experimental animals exposed to TCDD. In primary cultures of rat hippocampal neuronal cells, there is evidence that TCDD may increase the uptake of intracellular calcium. This concentration-dependent increase in calcium is associated with a decrease in mitochondrial membrane potentiation and activation of α-protein kinase C. An experimental study in rats suggested that a single low dose of TCDD could cause a toxic polyneuropathy.

In general, TCDD has a wide range of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. These effects may in turn influence nerve cells. Studies in animals indicate that some TCDD effects are mediated through the Ah receptor (AhR), a protein in animal and human cells to which TCDD can bind. It is hypothesized that TCDD, together with the AhR, can interact with sites on DNA and alter the information obtained from DNA in a way that transforms normal cells into abnormal cells. Although structural differences in the AhR have been identified, this receptor operates in a similar manner in animals and humans. Evidence has also begun to accumulate for non-AhR-mediated effects. Animal studies and in vitro mechanistic studies continue to emphasize the importance of alterations in neurotransmitter systems as underlying mechanisms of TCDD induced behavioral dysfunction.

Limited information is available on health effects of exposure to the herbicides discussed in the report. Some studies have observed impairment of motor function in rats administered high single oral doses of 2,4-D. Another study showed that the toxic effects of 2,4-D in rats were observed within one-half hour after its oral administration and correlated with signs and symptoms of CNS depression. These data were interpreted to suggest that the toxic mechanism of 2,4-D is related to an action on the central nervous system. 2,4-D also renders the developing rat nervous system vulnerable by hindering the process of myelination in the brain. Evidence was recently presented suggesting that 2,4-D and 2,4,5-T access to the central nervous system is energy-dependent. Results from in vitro mechanistic studies suggest that 2,4,5-T may acutely affect neuronal and muscular function by altering cellular metabolism and cholinergic transmission.

The herbicide 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to produce parkinsonism in humans and experimentally in animals, including nonhuman primates. Paraquat, another herbicide, shares with MPTP the ability to increase free-radical load in target tissues and exposure to it has also been related to an increased incidence of parkinsonism. These results may be biologically relevant because it is suspected that TCDD and some of the herbicides used in Vietnam may indirectly generate free radicals or sensitize cells to free radical injury.

The foregoing evidence suggests that a connection between TCDD or herbicide exposure and human health effects is, in general, biologically plausible. However, differences in sensitivity and susceptibility across individual animals, strains and species, lack of strong evidence of organ-specific effects across species, and differences in route, dose, duration and timing of exposure complicate any more definitive conclusions about the presence or absence of a mechanism for the induction of neurobehavioral effects.

Considerable uncertainty remains over how to apply this information to the evaluation of potential health effects of herbicides or dioxin exposure in Vietnam veterans. Scientists disagree over the extent to which information derived from animals and cellular studies predicts human health outcomes, and the extent to which the health effects resulting from high-dose exposure are comparable to those resulting from low-dose exposure. Research on biological mechanisms is burgeoning and subsequent Veterans and Agent Orange updates may have more and better information on which to base conclusions.

Increased Risk of Disease Among Vietnam Veterans

Under the Agent Orange Act of 1991, the committee is asked to determine (to the extent that available scientific data permit meaningful determinations) the increased risk of the diseases it studies among those exposed to herbicides during their service in Vietnam. Chapter 1 presents the committee's general findings regarding this charge. Where more specific information about particular health

outcomes is available, this information is related in the preceding discussions of those diseases.

REFERENCES

Baader EW, Bauer H. 1951. Industrial intoxication due to pentachlorophenol. Industrial Medicine and Surgery 20:286–290.

Berkley MC, Magee KR. 1963. Neuropathy following exposure to a dimethylamine salt of 2,4-D. Archives of Internal Medicine 111:133–134.

Butterfield PG, Valanis BG, Spencer PS, Lindeman CA, Nutt JG. 1993. Environmental antecedents of young-onset Parkinson's disease. Neurology 43:1150–1158.

Decoufle P, Holmgreen P, Boyle CA, Stroup NE. 1992. Self-reported health status of Vietnam veterans in relation to perceived exposure to herbicides and combat. American Journal of Epidemiology 135:312–323.

Diabetes Control and Complications Trial (DCCT) Research Group. 1995. The effect of intensive diabetes therapy on the development and progression of neuropathy. Annals of Internal Medicine 122:561–568.

Dyck PJ, Oviatt KF, Lambert EH. 1981. Intensive evaluation of referred unclassified neuropathies yields improved diagnosis. Annals of Neurology 10:222–226.

Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. 1991. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New England Journal of Medicine 324:212–218.

Franklin GM, Kahn LB, Baxter J, Marshall JA, Hamman RF. 1990. Sensory neuropathy in non-insulin-dependent diabetes mellitus. The San Luis Valley Diabetes Study. American Journal of Epidemiology 131:633–643.

Goldstein NP, Jones PH, Brown JR. 1959. Peripheral neuropathy after exposure to an ester of dichlorophenoxyacetic acid. Journal of the American Medical Association 171:1306–1309.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Italian General Practitioner Study Group. 1995. Chronic symmetric symptomatic polyneuropathy in the elderly: a field screening investigation in two Italian regions. I. Prevalence and general characteristics of the sample. Neurology 45:1832–1836.

Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, Chen RC. 1997. Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. Neurology 48:1583–1588.

Maser RE, Steenkiste AR, Dorman JS, Nielsen VK, Bass EB, Manjoo Q, Drash AL, Becker DJ, Kuller LH, Greene DA, et al. 1989. Epidemiological correlates of diabetic neuropathy. Report from Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes 38:1456–1461.

McLeod JG, Tuck RR, Pollard JD, Cameron J, Walsh JC. 1984. Chronic polyneuropathy of undetermined cause. Journal of Neurology, Neurosurgery and Psychiatry 47:530–535.

McLeod JG. 1995. Investigation of peripheral neuropathy. Journal of Neurology, Neurosurgery and Psychiatry 58:274–283.

Notermans NC, Wokke JH, Franssen H, van der Graaf Y, Vermeulen M, van den Berg LH, Bar PR, Jennekens FG. 1993. Chronic idiopathic polyneuropathy presenting in middle or old age: a clinical and electrophysiological study of 75 patients. Journal of Neurology, Neurosurgery and Psychiatry 56:1066–1071.

O'Toole BI, Marshall RP, Grayson DA, Schureck RJ, Dobson M, French M, Pulvertaft B, Meldrum L, Bolton J, Vennard J. 1996. The Australian Vietnam Veterans Health Study: III. Psychological health of Australian Vietnam veterans and its relationship to combat. International Journal of Epidemiology 25:331–340.

Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, LaPorte RE, Kuller LH. 1990. Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II. Diabetes 39:1116–1124.

Palumbo PJ, Elvehack LR, Whisnant JP. 1978. Neurological complications of diabetes mellitus: transient ischaemic attack, stroke and periperal neuropathy. In: Schoenberg BS, ed. Neurological Epidemiology: Principles and Clinical Applications. New York: Raven Press. 593–601.

Partanen J, Niskanen L, Lehtinen J, Mervaala E, Siitonen O, Uusitupa M. 1995. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. New England Journal of Medicine 333:89–94.

Peper M, Klett M, Frentzel-Beyme R, Heller WD. 1993. Neuropsychological effects of chronic exposure to environmental dioxins and furans. Environmental Research 60:124–135.

Schulte PA, Burnett CA, Boeniger MF, Johnson J. 1996. Neurodegenerative diseases: occupational occurrence and potential risk factors, 1982 through 1991. American Journal of Public Health 86(9):1281–1288.

Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, Oertel WH, Ulm G, Schneider E. 1996. Possible environmental, occupational, and other etiologic factors for Parkinson's disease: a case-control study in Germany. Neurology 46(5):1275–1284.

Semchuk KM, Love EJ, Lee RG. 1992. Parkinson's disease and exposure to agricultural work and pesticide chemicals. Neurology 42:1328–1335.

Semchuk KM, Love EJ, Lee RG. 1993. Parkinson's disease: a test of the multifactorial etiologic hypothesis. Neurology 43:1173–1180.

Sharp DS, Eskenazi B, Harrison R, Callas P, Smith AH. 1986. Delayed health hazards of pesticide exposure. Annual Review of Public Health 7:441–471.

Todd RL. 1962. A case of 2,4-D intoxication. Journal of the Iowa Medical Society 52:663–664.

Visintainer PF, Barone M, McGee H, Peterson EL. 1995. Proportionate mortality study of Vietnam-era veterans of Michigan. Journal of Occupational and Environmental Medicine 37:423–428.

Zober A, Ott MG, Messerer P. 1994. Morbidity follow up study of BASF employees exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) after a 1953 chemical reactor incident. Occupational and Environmental Medicine 51:469–486.

11

Other Health Effects

INTRODUCTION

A variety of health outcomes are evaluated in this chapter, including chloracne, porphyria cutanea tarda, respiratory disorders, immune system disorders (immune suppression, autoimmunity), diabetes mellitus, lipid abnormalities, gastrointestinal and digestive disease (including liver toxicity), and circulatory disorders. Many of these outcomes have not been addressed as thoroughly in the epidemiologic literature as the health outcomes described in Chapters 7, 9, and 10.

For most of the health outcomes discussed in this chapter, a brief summary of the scientific evidence described in *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (henceforth called *VAO*) (IOM, 1994) and *Veterans and Agent Orange: Update 1996* (henceforth called *Update 1996*) (IOM, 1996) is presented, followed by an update of the recent scientific literature. Because of special interest expressed by the Department of Veterans Affairs, a complete discussion of the evidence, including the studies cited in *VAO* and *Update 1996*, is presented for diabetes and for lipid and lipoprotein disorders.

CHLORACNE

Background

Skin disorders are among the most common health problems encountered in combat, aside from traumatic injuries. Because of the tropical environment and

living conditions in Vietnam, veterans developed 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 and other herbicides, including the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, TCDD, or dioxin), is chloracne. Therefore, this discussion focuses on chloracne and its link to TCDD.

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 manifest the condition.

Chloracne has a variable natural history. 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). Like 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, a number of precautionary steps should be taken 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 and that there be careful comparison with an appropriate control group. Definitive diagnosis may require histologic confirmation from a biopsy specimen.

Chloracne can be viewed as both a toxic outcome of exposure to TCDD and a potential clinical marker of TCDD exposure. It is the latter that has generated the most controversy. In this section, the primary focus is on 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 5. A major unresolved issue is whether TCDD exposure below the level required to cause chloracne may have other adverse health consequences such as cancer.

Summary of *VAO* and *Update 1996*

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.

From the studies reviewed in *VAO* and *Update 1996*, it is apparent that higher levels of exposure to TCDD, as reflected by increased serum levels, are

associated in a general way with increased risk of developing chloracne. However, the great degree of variability in TCDD levels among subjects with a history of chloracne and among those with no evidence of the condition suggests a complex dose–response relationship, with highly variable individual susceptibility. In addition, in many subjects the serum TCDD levels were measured many years after first exposure or onset of chloracne. Based on current data, it is not possible to assign a "threshold" TCDD level associated with chloracne.

Update of the Scientific Literature

No new informative publications were identified that related chloracne to exposure to herbicides or dioxin in humans. Because TCDD-associated chloracne becomes evident shortly after exposure, there is no risk that new cases will occur in veterans due to Vietnam service-related exposures.

Conclusions

Strength of Evidence in Epidemiologic Studies

Evidence is sufficient to conclude that there is a positive association between exposure to the herbicides considered in this report and chloracne. The evidence regarding association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

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. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and chloracne is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

PORPHYRIA CUTANEA TARDA

Background

Porphyria cutanea tarda (PCT) is an uncommon disorder of porphyrin metabolism manifest in patients by thinning and blistering of the skin in sun-exposed areas, as well as by 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 of uroporphyrinogen decarboxylase (UROD), a cytoplasmic enzyme in the pathway of hemoglobin synthesis (Sweeney, 1986). In the hereditary form, no precipitat-

ing exposure is necessary for the appearance of excess uroporphyrin and coproporphyrin in the urine and the development of clinical symptoms.

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).

Summary of *VAO* and *Update 1996*

The occurrence of clinical PCT is rare and may be influenced by genetic predisposition of individuals with low enzyme levels of UROD. The cases reported have occurred relatively shortly after exposure to specific chemicals, including TCDD, and have improved 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 and may represent a preclinical stage of PCT.

There is no suggestion of an increase in PCT in studies of Vietnam veterans or Ranch Hands, possibly because of comparatively low dioxin exposure even for Ranch Hand veterans or a fortuitous absence of genetically predisposed individuals who could develop PCT after exposure to TCDD. Further studies of PCT incidence in Vietnam veterans would not be called for, since the biologic and clinical evidence indicates 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 exposure and PCT that do not simultaneously assess alcohol consumption.

Jung et al. (1994) presented porphyrin data on former workers in a German pesticide plant that had manufactured 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The study found no difference in porphyrin levels or abnormal liver function tests between subjects with and without chloracne. There was also no relationship between porphyrin levels in urine, red blood cells, or plasma and TCDD levels in adipose tissue. Three cases of chronic hepatic porphyria (none with overt PCT and none with chloracne) were identified, which did not exceed the expected prevalence in this population.

Calvert et al. (1994) analyzed porphyrin levels and TCDD serum levels in a cross-sectional medical study of the National Institute for Occupational Safety and Health (NIOSH) cohort of workers who had been previously exposed to TCDD through manufacture of sodium trichlorophenol, 2,4,5-T, or hexachlorophene. There were no cases of overt PCT, and three exposed and three unexposed subjects had subclinical PCT. Porphyrin levels did not differ between exposed and unexposed workers, and there was no significant relationship between urinary porphyrin levels and serum TCDD levels.

Taken together, the studies reviewed did not support the hypothesis that TCDD caused disturbances in heme metabolism in humans, even at the relatively

high exposure levels experienced by these cohorts. Reports that some persons employed in herbicide production have evidence of increased porphyrins in urine warrant further investigation.

Update of the Scientific Literature

No informative publications were identified relating PCT to exposure to herbicides or dioxin in humans. Because TCDD-associated PCT becomes evident shortly after exposure, there is no risk that new cases will occur in veterans due to Vietnam service-related exposures.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is limited/suggestive evidence of an association between exposure to the herbicides considered in this report and porphyria cutanea tarda. Evidence regarding the association is drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

There is some evidence that TCDD 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. A more thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and PCT is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

RESPIRATORY DISORDERS

Background

The studies reviewed in this section cover a wide range of respiratory conditions encompassed by *International Classification of Diseases, Ninth Edition* (ICD·9) codes 460–519, including acute respiratory infections, other diseases of the upper respiratory tract, pneumonia, influenza, chronic obstructive pulmonary disease, chronic bronchitis, emphysema, asthma, pleurisy, and pneumoconiosis. In the morbidity studies, a variety of methods were used to assess the respiratory system, including assessment of symptoms, physical examination of the chest, lung function tests, and chest radiographs. Lung function (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 volume (which can occur because of lung scarring or inflammation). Tests that measure airflow obstruction include FEV_1 (forced expirating volume, the amount of air that can be exhaled forcefully in one second); the ratio FEV_1/FVC (forced vital capacity, the total amount of air that can be forcefully exhaled); $FEF_{25–75}$ (forced expiratory flow, the rate of airflow in the middle range of total volume); and FEF_{max} (rate of airflow at highest lung volume). The test that measures restriction is FVC, which determines the total amount of air that can be exhaled with sustained effort. Chest radiographs, which were used in several studies, can assess whether inhaled agents have damaged the lungs; damage is usually apparent as opacities such as scarring, inflammation, or both.

Summary of *VAO* and *Update 1996*

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 involved in herbicide spraying, the respiratory tract could be viewed as a target organ for aerosol or other particulate deposition. In general, the lack of working hypotheses about respiratory disease outcomes associated with herbicides, the nonuniformity in methods and reported results, and the variable ability to adjust for the effects of cigarette smoking make it difficult to interpret much of the morbidity data, especially reports of symptoms and radiographic data.

Interpretation of many of the mortality studies was generally limited by the small number of deaths observed. These studies also tended to use the ICD·9 codes for all respiratory diseases. The wide range of diverse conditions and small number of total deaths make it difficult to assess any particular respiratory outcome using mortality studies. In some studies, the specific ICD·9 codes used were not stated, thus making comparisons with other studies difficult. Overall, there was little evidence of any associations with herbicide or dioxin exposure.

Update of the Scientific Literature

Occupational Studies

Becher et al. (1996) examined mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population included workers who had a least one month of employment, and resulted in a cohort consisting of 2,479 male workers. Standardized mortality ratios (SMRs) and 95 percent confidence intervals (95% CIs) were calculated using West German mortality rates by five-year age and calendar intervals. SMRs for respiratory system disease among the four plants ranged from 0.5 to 0.8.

Svensson et al. (1995) studied mortality and disease incidence in two cohorts

of Swedish fishermen. One group (2,896 men) resided on the east coast of Sweden and consumed fish from the Baltic Sea. These fatty fish (particularly salmon and herring) are reported to contain elevated levels of polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). The other group of fishermen (8,477 men) resided on the west coast of Sweden and were presumed to have a higher intake of lean (and less contaminated) fish, including cod and flat fish. Bronchitis and emphysema rates (ICD·9 codes 490–493), were reported, and no significant increase was found among east coast fishermen (SMR 0.5, 95% CI 0.1–1.2).

Ott and Zober (1996) updated the experience of workers exposed to TCDD during the cleanup of a trichlorophenol (TCP) reactor that exploded in 1953 at a BASF plant in Ludwigshafen, Germany. They studied disease incidence and mortality up to 1992 for a group of 243 men and developed TCDD dose estimates based on work activity information, blood TCDD determinations on a subset of the population, and estimates of TCDD elimination rates. Expected numbers of incident cases and cause-specific deaths were obtained from German sources by five-year age and calendar intervals. The overall SMR (95% CI) for respiratory system diseases was 0.1 (0.0–0.8); in the highest TCDD dose group, the SMR (95% CI) was 0.4 (0.0–2.0).

Ramlow et al. (1996) examined mortality in a cohort of workers exposed to pentachlorophenol (PCP), as part of a larger study of Dow chemical manufacturing workers exposed to the higher chlorinated dioxins. The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential PCDD exposure between 1937 and 1980. From this cohort, 770 workers were identified who were considered to have potential PCP exposure based on work history records. Cumulative exposure indices for PCP and dioxin were calculated using scores described by Ott et al. (1987). In the study analysis, the U.S. white male death rates (five-year age and calendar time specific) and the death rates of non-PCP and non-PCDD male Dow Michigan employees for 1940 to 1989 were both used as reference values to calculate expected deaths. The overall SMR (95% CI) for respiratory system disease was 0.9 (0.5–1.5), with no significant effect of latency or estimated exposure level.

In an update and expansion of the International Agency for Research on Cancer (IARC) cohort study, Kogevinas et al. (1997) examined mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. Exposure information varied between cohorts, but in general exposures were reconstructed from job records. Based on job categories and information on production processes and the composition of the materials used, exposed workers were classified into three categories: exposed to TCDD or higher chlorinated dioxins, unexposed to the same, and unknown exposure to the same. Analysis was performed by calculating SMRs and 95% CI, using the World Health Organization (WHO) mortality data bank to calculate national

mortality rates by sex, age (five-year intervals), and calendar period (five years). Overall, a decrease in respiratory system disease was observed in exposed workers compared to unexposed workers for males (SMR 0.8, 95% CI 0.7–0.9), with no significant differences observed for women (SMR 1.1, 95% CI 0.4–2.2).

Studies of Vietnam Veterans

In a study of postservice suicide among Vietnam veterans, Bullman and Kang (1996) reported subsequent cause-specific mortality for 34,534 veterans who had been hospitalized for wounds suffered in Vietnam, compared to U.S. men overall. They observed no significant difference in respiratory system disease deaths (SMR 0.9, 95% CI 0.7–1.2) compared to U.S. men.

O'Toole et al. (1996) described the results of a simple random sample of Australian Army Vietnam veterans on self-reported health status. Data were obtained on 641 veterans from the Australian Bureau of Statistics Health Interview Survey 1989–90, and illness rates were compared to the age- and sex-matched Australian population. They observed no significant increase in overall respiratory system disease among veterans; the relative risk (RR) was 2.0 (99% CI 0.0–7.1). Hay fever (RR 1.6, [CI, 1.3–1.9]), bronchitis or emphysema (4.1 [CI 2.8–5.5]), and other respiratory disease (4.0 [CI 2.2–5.9]) were significantly elevated compared to the general population, although none of these conditions were related to an index of combat exposure. The veterans were significantly less likely to have never smoked than the general population (0.7 [CI 0.5–0.8]), a finding similar to data reported by McKinney et al. (1997) on U.S. Vietnam veterans. No information was available on other potential confounding factors such as occupational exposures.

Watanabe and Kang (1996) reported on the mortality of 33,833 U.S. Army and Marine Corps Vietnam veterans who died during 1965–1988, compared to mortality among 36,797 deceased non-Vietnam veterans, using proportionate mortality ratios (PMRs). They observed no increase in respiratory system disease mortality among Army Vietnam veterans (PMR 0.9) or among Marine Vietnam veterans (PMR 1.1).

Dalager and Kang (1997) reported a study comparing 2,872 Vietnam veterans with 2,737 non-Vietnam veterans (all of whom served in Chemical Corps specialties). All study subjects served at least 18 months' active duty between 1965 and 1973, and vital status ascertainment was complete for both groups. They reported no significant increase in respiratory system disease mortality among Vietnam veterans, with an RR (95% CI) of 2.6 (0.6–12.2).

A report on Australian Vietnam veterans (Crane et al., 1997a) compared cause-specific mortality rates of 59,036 male Vietnam veterans with those of other Australian males. They found a significant decrease in respiratory system disease (SMR [95% CI] for deaths in 1964–1979 (0.1 [0.0–0.3]) and no significance from expected rates for 1980–1994: 0.9 (0.7–1.1).

A second cohort study of Australian veterans compared the mortality for 1982–1994 for 18,949 national servicemen who had served in Vietnam (veterans) with 24,646 national servicemen who had not served in Vietnam (nonveterans) (Crane et al., 1997b). They observed an RR (95% CI) for veterans compared to nonveterans of 0.9 (0.3–2.7) for all respiratory diseases and 0.3 (0.0–3.2) for chronic obstructive airways disease.

The ongoing study of Ranch Hand veterans (AFHS, 1996) reported cause-specific mortality among 1,261 Ranch Hand personnel compared to 19,080 Air Force veterans from the same era who did not handle herbicides. No increase was observed in respiratory system disease deaths (SMR 0.5).

Synthesis

Although there were sporadic reports of increased respiratory disease potentially related to exposure to herbicides or TCDD, the results were inconsistent across studies. In addition, interpretation of individual studies was generally limited by a lack of information on cigarette smoking. In the one study that showed the strongest association between potential exposure and respiratory disease (O'Toole et al., 1996), veterans were much more likely to have smoked than nonveterans. Additional research, with adequate information on cigarette smoking and other risk factors for respiratory disease, is required to adequately assess the potential association between respiratory disease and herbicide or TCDD exposure.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to herbicides and 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. The evidence regarding association is drawn from occupational and veteran studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

A thorough discussion of biologic plausibility with respect to exposure to TCDD or herbicides and respiratory disorders is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

IMMUNE SYSTEM DISORDERS

Background

Immunotoxicology is the study of the effects of xenobiotics (chemical compounds that are foreign to the human body) on the immune system. The compounds may produce an impaired immune response (immunosuppression) or an enhanced immune response (immune-mediated disease). Although alterations in the immune system can be related to increases in the incidence of infection and neoplasm (immune suppression) and immune-mediated diseases (allergy and autoimmunity), there has been no observed increase in infectious or immune-mediated disease in the populations examined after exposure to herbicides. However, alterations have been observed in measures of immune function or populations of immune cells. The question of possible increases in neoplastic diseases is dealt with in Chapter 7.

Immune Suppression

The immune system helps defend the host against foreign invaders. It confers resistance to infection by bacteria, viruses, and parasites; it is involved in the rejection of allografts (tissue transplants); and it may eliminate spontaneously occurring tumors (Paul, 1993). Proper function of the immune system is exquisitely sensitive to disruptions in physiologic homeostasis. The immune response is highly redundant, and several different mechanisms may be employed to eliminate an antigen. 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.

Suppression of the immune system leads to increased susceptibility to infection and neoplasia. However, the degree of immune suppression necessary to cause disease is unknown 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-1] can lead to AIDS), and exposure to immunosuppressive agents (Paul, 1993). Immune suppression can also occur in patients with autoimmune disease (discussed 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, the immune system may prevent or limit tumor growth, and a high incidence of tumors may follow immune suppression (Paul, 1993).

Allergy and Autoimmunity

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 agents, including plant pollens and epithelial products of domestic 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 on subsequent exposure to antigen. The mediators of allergic reactions, such as histamine, are then released. The alterations discussed below reflect only in vitro immune parameters, not disease incidence. In fact, no increase in allergic disease related to herbicide exposure has been reported in any of the studies reviewed.

In general, the immune response is directed against foreign antigens. However, in some instances antibodies can be demonstrated that react with endogenous antigens (i.e., autoantibodies). Autoimmune disease is the pathological consequence of an immune response to autologous antigen. Some autoimmune diseases result when autoantibodies activate the complement cascade or interact with "killer" mononuclear cells to induce antibody-dependent cell-mediated cytotoxicity. Others 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 an autoimmune response from autoimmune disease. Autoimmunity, as indicated by the presence of autoantibodies, is relatively common, whereas autoimmune disease is relatively rare. Detecting 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.

Summary of *VAO* and *Update 1996*

The effects of herbicide exposure on the level of several immune parameters were presented in studies reviewed in *VAO* and *Update 1996*. The data are divided into two categories: immune suppression and immune enhancement. No studies investigating the association of autoimmune disease or allergy with exposure to herbicides were identified.

Several studies were carried out on individuals occupationally exposed to TCDD, examining immunological markers, including immunoglobulin levels, complement components, and lymphocyte subpopulations. The changes described were marginal and varied from study to study, some showing increases and some decreases in these parameters. No changes in the incidence of infectious disease were noted. 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 these alterations in humans. Currently, the level of alteration in immune parameters necessary to affect the incidence of disease is unknown. Furthermore, since so many immune parameters were assessed in these studies, there is a high probability that at least some positive results would be noted based on chance alone, which would undermine interpretation of the few

positive results. The earlier committees reached the conclusion that no clinically significant changes in the human immune response could be attributed at that time to TCDD or other halogenated aromatic hydrocarbons.

Update of the Scientific Literature

Two studies have appeared relating to the incidence of infectious diseases in American Vietnam veterans. Visintainer et al. (1995) studied a cohort of 377,028 veterans who are on the Michigan Department of Management and Budget's Vietnam-Era Bonus List. This database differentiates between two groups of Vietnam era veterans: those who served in Vietnam (151,377) and those who served elsewhere (225,651). Vietnam veterans compared with non-Vietnam veterans had a slightly elevated proportionate mortality ratio from infectious and parasitic diseases (PMR = 1.6, CI 1.2–2.1, $N = 56$). The study, however, did not distinguish Vietnam veterans exposed to Agent Orange from those with no known exposure. A retrospective cohort mortality study was reported by Watanabe and Kang (1995). They studied all marines on active duty during 1967 through 1969. A total of 281,196 records were obtained, from which a sample of 26,158 was drawn. A search of their records divided this group into 11,167 who served in Vietnam and 9,412 who never served in Vietnam. The remaining 5,579 were not traced. Based on cause-specific mortality, comparing Vietnam Marines with non-Vietnam Marines, an estimated risk for infectious diseases of 2.8 (CI 0.8–10.3) was obtained. This difference was not determined to be significant. Two studies of Australian Vietnam veterans failed to show any increase in mortality due to infectious or parasitic diseases (Crane et al., 1997a,b).

Several studies were undertaken to relate exposure to halogenated aromatic hydrocarbons to shifts in lymphocyte subpopulations. Lovik et al. (1996) studied 24 fishermen with possible occupational and environmental exposure to a number of halogenated aromatic hydrocarbons, such as PCDDs and polychlorinated furans (PCFs) through fish and crab consumption. In a preliminary report, they found a tendency toward negative correlation between B lymphocytes and blood levels of PCDD or PCF. On the other hand, there was a positive correlation between the mitogenic responses of T lymphocytes and the blood levels of halogenated aromatic hydrocarbons. No relationship between NK (natural killer) cell number or function and blood level of these compounds was found. Of the serum immunoglobulins, there was a negative correlation with serum immunoglobulin M (IgM) levels and a tendency toward positive correlation for IgE. A negative relationship with autoantibodies, such as antinuclear antibodies, was observed. These preliminary results do not allow any firm conclusions to be drawn.

A study of 10 farmers who mixed and applied chlorophenoxy herbicides was carried out by Faustini et al. (1996). After one to twelve days of exposure, the proportions of circulating CD4+ T cells, CD8+ T cells, activated T cells, and NK

cells were significantly reduced compared with values before exposure. There was also a reduction in lymphocyte responses to mitogenic stimulation by phytohemagglutinin and concanavalin A. No correlation was found between the amount of herbicide applied and the decrease in percentage of lymphocyte subsets. These studies suggested that short-term immunological changes may occur following exposure to phenoxy herbicides in an agricultural setting. The changes, however, were short-lived, because most of the values returned to normal or near-normal ranges 50–70 days after exposure.

A study in Germany of the effects of inhalative exposure to TCDD and related compounds in wood preservatives on cell-mediated immunity in teachers at day care centers was reported by Wolf and Karmaus (1995). The study population consisted of 221 exposed persons and an unexposed control group of 189 persons. Exposed and unexposed populations did not differ with regard to the number of peripheral CD4+ T cells, CD8+ T cells, and the CD4:CD8 ratio. In a multitest format for delayed hypersensitivity, exposed subjects had a slightly higher overall score than unexposed controls. When exposure to TCDD was estimated, subjects with a TCDD burden of >0.6 pg/mg had a statistically significant higher risk of hypoergy than subjects who did not experience any exposure at their day care center workplace. There were, however, some methodological limitations that require caution in attributing the observed decrease in skin test responsiveness to inhalative TCDD exposure. The TCDD burden could not be assessed with precision, and the relatively low prevalence of hypoergy or anergy limited the estimation of an effect. Recruitment of the study population was also a problem because of the possibility of selection bias toward people with ill health or presumed harmful exposures.

The immunological effects of pre- and postnatal exposure to PCBs or TCDD in 207 Dutch infants from birth to 18 months of age were explored by Weisglas-Kuperus et al. (1995). Prenatal exposure was estimated from the total toxic equivalent level of each compound in human milk multiplied by the weeks of breastfeeding. No evidence was found of increased upper or lower respiratory tract symptoms or altered antibody production to mumps, measles, or rubella in relation to PCB or TCDD exposure. Although there were small differences in the T-cell, B-cell, and NK-cell populations between high- and low-exposed infants, all values were within the normal range. The investigators did find, however, that prenatal PCB or TCDD exposure was associated with changes in T-cell subpopulations in the blood. These changes were seen mainly at 18 months of age. At this age, higher prenatal exposure was associated with an increase in the number of CD4+ T cells, resulting in a relative change in the CD4:CD8 ratio. Higher prenatal as well as postnatal PCB or TCDD exposure was associated with lower monocyte and granulocyte counts only at 3 months of age. These results suggest that perinatal exposure to chlorinated aromatic hydrocarbons may influence the human fetal and neonatal immune system, but the changes are not reflected in increased susceptibility to infection.

Tonn et al. (1996) examined the long-term effects of TCDD in 11 industrial workers who 20 years earlier had been exposed to high levels of TCDD for several years. Their current TCDD body burdens were still at least 10 times higher than the general population. No significant differences could be detected between individuals tested and controls with respect to lymphocyte subpopulations or mitogen-induced lymphocyte proliferation. The investigators noted a decrease in interleuken-2 (IL-2)- induced proliferation, suggesting a decrease in preactivated T cells in the blood. TCDD-exposed subjects showed a reduced response to allogeneic stimulation. This effect was attributed in part to an increase in a lymphocyte population that displayed a suppressive activity on allogeneic responses. There is no known relationship between this decreased alloresponse and any disease state.

One study was reported of autoimmunity following TCDD exposure. Chinh et al. (1996) examined 25 Vietnamese veterans and 36 control males. They found no increase in autoantibodies to sperm or in antinuclear antibodies.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides considered in this report and immune suppression or autoimmunity.

Biological Plausibility

Evidence for the effects of TCDD and other halogenated aromatic hydrocarbons on the immune system of animals is presented in Chapter 3; a summary is presented in the conclusion of this chapter. Similar observations have not been reported in humans.

Increased Risk of Disease Among Vietnam Veterans

No evidence is available to associate defects in the immune response with Agent Orange exposure. A more thorough discussion of the issue of increased risk of disease among Vietnam veterans is included in Chapter 1.

DIABETES

Background

Primary diabetes (i.e., not secondary to another known disease or condition, such as pancreatitis or pancreatic surgery) is a heterogeneous metabolic disorder

characterized by hyperglycemia and quantitative and/or qualitative deficiency of insulin action (Orchard et al., 1992). Two main types have been recognized based on the 1979 National Diabetes Data Group (NDDG) criteria and those of the World Health Organization (WHO, 1980, 1985): insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). In June 1997, the American Diabetes Association (ADA, 1997) suggested a revised classification, with IDDM being termed Type I and NIDDM, Type II. This new terminology is used in the remainder of this review, although the older diagnostic criteria are utilized as appropriate.

Type I diabetes is generally accepted to result from β-cell dysfunction, caused by a genetically based autoimmune destruction. It comprises approximately 10 percent of all cases of diabetes and characteristically has an abrupt onset in youth or young adulthood, although it may appear at any age. The usual autoimmune form results in complete β-cell destruction and complete insulinopenia, hence the "insulin dependency" of earlier classifications. The genetic basis of the autoimmune form is linked to the human lymphocyte antigen (HLA) system (class II antigens). A number of environmental triggers of the autoimmune process and/or of symptomatic disease in genetically susceptible subjects have been proposed including viral infections.

Type II diabetes accounts for the majority (approximately 90 percent) of cases of primary diabetes. It is rare before age 30, but increases steadily with age thereafter. The age, sex, and ethnic prevalences are given in Table 11-1. The etiology of Type II is unclear, but three cardinal components have been proposed: (1) peripheral insulin resistance (thought by many to be primary) in target tissues (e.g., muscle, adipose and liver); (2) β-cell insulin secretory defect; and (3) hepatic glucose overproduction. Although the relative contributions of these features are controversial, it is generally accepted that the main factors for increased risk of Type II diabetes include age (with older individuals at higher risk), obesity, central fat deposition, a history of gestational diabetes (if female), physical inactivity, ethnicity (prevalence is greater in African Americans and Hispanic Americans, for example), and perhaps most important, a positive family

TABLE 11-1 Three-year Mean Prevalence of Diagnosed Diabetes (per 1,000 population) by Gender, Age, and Race: 1990–1992

Age	Total	Male	Female	White (men and women)	Black (men and women)
25–44	13.9	12.2	15.5	13.9	19.5
45–54	35.6	31.2	39.8	32.9	63.0
55–64	77.5	79.5	75.6	72.2	128.1
>65	101.1	101.4	100.8	93.5	178.6

SOURCE: Kenny et al., 1995, Appendix 4.5, Chapter 4 (1990–1992 National Health Interview Surveys).

history of Type II (for example, more than 90 percent of monozygotic twins are concordant for diabetes compared to less than 50 percent for dizygotic twins). Defects at many intracellular sites could account for the impaired insulin action and secretion seen in Type II diabetes (Kruszynska and Olefsky, 1996). The insulin receptor itself, insulin receptor tyrosine kinase activity, insulin receptor substrate proteins, insulin-regulated glucose transporters, enhanced protein kinase C (PKC) activity, tumor necrosis factor-α, rad (ras associated with diabetes), and PC1 have all been proposed as potential mediators of insulin resistance; impaired insulin secretion has been linked to hyperglycemia itself, to abnormalities of glucokinase and hexokinase activity, and to abnormal fatty acid metabolism.

Finally, an increasing number of "other" types of diabetes have been described that are linked to specific genetic mutations, for example, maturity-onset diabetes of youth, which results from a variety of mutations of the β-cell glucokinase gene.

The diagnosis of diabetes is problematic and a major concern for clinicians and investigators. Whereas Type I is often clearly diagnosed at onset (a blood sugar >200 mg/dl plus symptoms), up to half of the Type II population goes undiagnosed. This occurs because the degree of metabolic disturbance needed to meet both the old and the recently revised criteria does not necessarily produce symptoms, but nonetheless is likely to lead to the late complications of diabetes (cardiovascular disease, nephropathy, retinopathy, and neuropathy). It is partly because of this large population of undiagnosed cases and the impracticability of the standard diagnostic test (oral glucose tolerance test) in busy clinical practice that a more simplified diagnostic approach has been recommended by the ADA based on the fasting plasma glucose. Table 11-2 shows the earlier NDDG (WHO, 1979) and the current ADA (ADA, 1997) criteria. It should be noted that the vast majority of undiagnosed cases of diabetes under the 1979 criteria were diagnosable only by the 2-hour postglucose criterion (>200 mg/dl) and had fasting plasma glucose levels below the diagnostic level (140 mg/dl). This was one of the main reasons that current ADA recommendations have lowered the fasting criterion to 126 mg/dl (i.e., to capture those cases with the simpler [and more reproducible] fasting glucose test, as 126 mg/dl fasting approximates the 2-hour post challenge diagnostic level).

TABLE 11-2 Diagnostic Criteria for Diabetes (mg/dl plasma glucose)

	1979 NDDG/1980 WHO	1997 ADA
Fasting	≥140	≥126
2 hr[a]	≥200	≥200[b]
Random glucose[c]	≥200	≥200

[a]Post 75-gm glucose load; midtest value also has to be >200 mg/dl for NDDG.
[b]Not recommended for routine use.
[c]In the presence of diabetes symptoms.
SOURCE: WHO, 1980; ADA, 1997.

Epidemiologic Concerns in the Study of Diabetes

As can be surmised from the above brief description, the epidemiologic study of diabetes is filled with problems. Pathogenetic diversity and diagnostic uncertainty are two of the more significant problems.

Pathogenetic Diversity

Given the multiple likely pathogenetic mechanisms leading to diabetes, which include diverse genetic susceptibilities (ranging from autoimmunity to obesity) and a variety of potential environmental and health behavior factors (e.g., viruses, nutrition, activity), it is probable that many agents or behaviors contribute to diabetes risk, especially in genetically susceptible individuals. These multiple mechanisms may also lead to heterogeneous responses to various exposures.

Diagnostic Uncertainty

Because up to half the affected diabetic population is currently undiagnosed, the potential for ascertainment bias is high (i.e., more intensively followed groups or those with more frequent health care contact are more likely to be diagnosed), and the need for formal standardized testing (to detect undiagnosed cases) is great. Furthermore, the division of cases developing during young to middle age (i.e., 20–44 years) into Type I or Type II (which indicates the more likely pathogenetic mechanism) is very difficult. Indeed, it is now thought that as many as 10 percent of clinical "Type II" subjects may well have an incomplete form of "Type I" diabetes (Tuomi et al., 1993).

Epidemiologic Studies

Pazderova-Vejlupkova et al. (1981) reported on the 10-year follow-up of 55 workers who had become acutely ill while producing TCP and 2,4,5-T: 95 percent (52) developed chloracne and 8 percent (4) had diabetes at the onset of intoxication. Ten years later, one-fifth ($N = 11$) were reported to have a diabetic glucose tolerance test (diagnostic criteria are not stated, and the role of confounders is not addressed). In a survey of subjects up to 10 years after another industrial incident, May (1982) reported only two clinically recognized cases of diabetes in a total study group of 126 subjects including controls, with a mean age in the low forties. Reported diabetes did not increase in another study (after age adjustment) of 117 2,4,5-T production workers with chloracne compared to 109 without, 10–20+ years after mixed accidental and chronic TCDD exposure (Moses et al., 1984). Two mortality studies provide further negative data. Cook et al. (1987) examined mortality among 2,187 chemical workers and found a decreased SMR

(0.7) for diabetes; Bertazzi et al. (1989) reported the 10-year mortality of those living in the area of Seveso, Italy, at the time of the incident in 1976. The relative risk of diabetes mortality was 1.3 (95% CI 0.7–2.3) for men and 1.5 (0.9–2.5) for women. It should be noted that vital statistics data are known to be unreliable in terms of complete ascertainment of diabetes-related mortality.

More recently, Ott et al. (1994), reporting on 138 BASF workers exposed to TCDD in a 1953 industrial incident, found borderline ($p = 0.06$) increased fasting glucose levels approximately 37 years later. Further analysis suggested that this association was limited to subjects without chloracne who happened to be more obese. The authors raise the possibility that the TCDD–glucose association may be secondary to the link between obesity and diabetes. In a morbidity follow-up of 158 TCDD-exposed BASF workers, significantly fewer (6.3 percent versus 14.3 percent) exposed subjects had medical insurance diagnoses of diabetes (Zober et al., 1994). Interestingly, thyroid disease was increased ($p < 0.05$) in the exposed population. There is a considerable overlap between the subjects in this study and those in Ott et al. (1994). Reporting on a mortality study of 883 pulp and paper workers, Henneberger et al. (1989) did not find a statistically significant increase in diabetes (SMR 1.4, 95% CI 0.7–2.7). A German Cancer Research Center report (Von Benner et al., 1994) also found no TCDD effect on blood sugar levels in 153 TCDD-exposed workers from six chemical plants. Two recent mortality follow-up studies also found no increased diabetes (Ramlow et al., 1996) or endocrine mortality (Kogevinas et al., 1997) in chemical workers exposed to dioxins.

Early reports from the Air Force Health Study (the "Ranch Hand" study) of Vietnam veterans exposed to herbicide spraying and an unexposed comparison cohort suggested little relationship. At the first baseline exam in 1982, 10–20 years after exposure, no difference in the prevalence of an abnormal blood sugar (>120 mg/dl 2 hours after a standard carbohydrate load) was seen between the two groups (15 percent versus 17 percent) (AFHS, 1984). Reporting data using lipid-adjusted serum TCDD levels as a measure of exposure from the same cohort study, the Ranch Hand Study (AFHS, 1991) found a significant association between diabetic status on a 3-point scale—normal, impaired (2-hour post prandial glucose 140–200 mg/dl), and diabetic (verified past history or $\geq$ 200 mg/dl 2-hour post prandial glucose)—and TCDD level in both the Ranch Hands ($p = 0.001$) and the comparison group ($p = 0.028$). However, this correlation may be influenced by the strong correlation between obesity (percentage of body fat) and TCDD level in the same analysis ($r = 0.3$, $p < 0.001$ in Ranch Hands; $r = .15$, $p < 0.01$ in comparison). It should also be noted that the prevalence of an abnormal 2-hour blood glucose, either impaired or diabetic together (25 percent versus 22 percent Ranch Hand versus comparisons, respectively), or diabetic alone (10 percent versus 8 percent), is not markedly increased despite a nearly fourfold difference in mean dioxin levels between the Ranch Hand and comparison groups. The major impact of obesity in deter-

TABLE 11-3 Selected Epidemiologic Studies—Diabetes

Reference	Study Population	Exposed Cases	Estimated Risk (95% CI)	*p* Value
OCCUPATIONAL				
New Studies				
Ramlow et al., 1996	Pentachlorophenol production workers	4	1.2 (0.3–3.0)[a]	
Studies reviewed in *Update 1996*				
Ott et al., 1994	Trichlorophenol production workers	134 [g]		0.06[c]
Von Benner et al., 1994	West German chemical production workers	N/A	N/A	
Zober et al., 1994	BASF production workers	10	0.5 (0.2–1.0)	
Studies reviewed in *VAO*				
Sweeney et al., 1992	NIOSH production workers	26	1.6 (0.9–3.0)	
Henneberger et al., 1989	Paper and pulp workers	9	1.4 (0.7–2.7)	
Cook et al., 1987	Production workers	4	0.7 (0.2–1.9)[a]	
Moses et al., 1984	2,4,5-T and TCP production workers	22 (chloracne)	2.3 (1.1–4.8)	
May, 1982	TCP production workers	2	Not available	
Pazderova-Vejlupkova et al., 1981	2,4,5-T and TCP production workers	11	No referent group	

ENVIRONMENTAL				
Studies reviewed in *VAO*				
Bertazzi et al., 1989[b]	Seveso residents			
	Males	15	1.3 (0.7–2.3)	
	Females	19	1.5 (0.9–2.5)	
VIETNAM VETERANS				
New Studies				
Henriksen et al., 1997[c]	Ranch Hands			
	High-exposure category	57	1.5 (1.2–2.0)	
	All Ranch Hands	146	1.1 (0.9–1.4)	
O'Toole et al., 1996	Australian Vietnam veterans	12	1.6 (0.4–2.7) [c,d]	
Studies reviewed in *VAO*				
AFHS, 1991[b]	Ranch Hands	85		0.001,[e] 0.028[f]
AFHS, 1984[a]	Ranch Hands	158		0.234

[a]Standardized mortality ratio compared to U.S. population.
[b]Mortality compared to referent population.
[c]Comparison of fasting glucose values to referents.
[d]Compared to Australian population.
[e]Differences for mean dioxin level across three groups—normal, impaired, and diabetic glucose tolerance—of Ranch Hands.
[f]Differences for mean dioxin level across three groups—normal, impaired, and diabetic glucose tolerance—of comparisons.
[g]Total sample size listed.

mining both diabetes risk and serum dioxin level has to be fully controlled for before firm conclusions can be drawn.

In view of the potential importance of the most recent report from this ongoing Ranch Hand study, it is reviewed here in more detail. Henriksen et al. (1997) compared 989 dioxin-exposed Operation Ranch Hand veterans (1962–1971) to 1,276 nonexposed veterans serving at the same time. Exposure was classified on the basis of original exposure calculated from serum (lipid-adjusted) dioxin levels determined in 1987 or 1992. At follow-up (1992), the mean age of the comparison group was 53.5 years (±7.6) and that of the exposed group was 54.6 ± 7.2, 54.9 ± 7.6, and 50.9 ± 7.4 years, according to increasing exposure category. The prevalence of diabetes mellitus by 1995 was 13.2 percent in the comparison group and increased from 9.5 percent to 17.2 percent to 20.1 percent across the three Ranch Hand exposure categories. There was a statistically significant increase of the prevalence of the highest-exposure category relative to the comparison group (RR 1.5, 95% CI 1.2–2.0). Of the diabetic veterans, 41 percent were not following any treatment regimen, 27 percent were treated with diet alone, 21 percent with oral medications, and 10 percent by insulin.

Two concerns about this potentially important and well-conducted study are case definition and the focus on subgroup analysis with only limited control of confounders. Two somewhat conflicting definitions are given of a case of diabetes: one implies that all cases were clinically verified in medical records; the other is a combination of history and glucose testing after a standard meal or 100-g oral glucose tolerance test (OGTT).

Generally, half of the cases of diabetes go undiagnosed, and, in most cases, those that are diagnosed are found only after formal OGTT testing; the total prevalence of diabetes is the sum of previously diagnosed and currently discovered cases. (Technically an abnormal OGTT has to be repeated before a clinical diagnosis is made, but in epidemiologic studies this is not often done.) Although OGTTs were performed in the current study (at least in the 1992 examination; earlier reports refer to postprandial values), a 100-g glucose load was used, which inflates the positive rate a little compared to the recommended 75-g load. Since the OGTT was given only to those without a diagnosis of diabetes, the prevalence of undiagnosed diabetes is approximated in Henriksen et al. (1997, Table 8) by the 2-hour "postprandial" glucose values that are labeled abnormal (>200 mg/dl). Compared to the rates for 50–59-year-old, non-Hispanic whites from a recent national study (National Health and Nutrition Evaluation Survey III [NHANES III] 1988–1994), only the high-exposure group has a marked increase in prevalence of known diabetes, whereas all exposure groups have lower rates of discovered diabetes than reported in NHANES. Total prevalences (known and discovered) are therefore similar or lower for the background (9.5 percent) or low exposure group (17.2 percent) than in NHANES III (16.7 percent), with only the high-exposure group having an increased prevalence (20 percent). These results are consistent with the hypothesis that, generally, Ranch Hands have somewhat

lower rates of diabetes (which might be expected for a healthy military population) and that relatively more of the diabetic veterans have been diagnosed (reflecting their more intensive medical follow-up).

A high proportion (41 percent) of all cases are not being treated (even with diet), particularly if the cases were verified in medical records and thus carried a clinical diagnosis. Although comparable data are difficult to find, the 1989 National Health Interview Survey (NHIS) suggests that 43.6 percent of NIDDM subjects age 55–64 years use insulin and 51.7 percent use oral agents. Even given some overlap of these groups (i.e., those who use both insulin and oral agents), it would seem that the proportion of Ranch Hands with diabetes, but not on treatment (diet, insulin, or oral agents), is two to four times higher than expected.

The analyses are problematic since they partially ignore the matched design employed in the study. In the report, each exposure group is compared to the entire comparison group (which was chosen by an original matched design to be comparable to Ranch Hands as a whole). The three exposure groups should ideally be compared to appropriate subgroups of comparison subjects matched to the specific exposed group. Although the availability of serum dioxin levels enables a better measure of exposure and a focus on the risks of the low- and high-exposed groups is understandable, Ranch Hands as a group do not have an increased risk of diabetes:

Comparison group	13.2% (169/1,276)
Ranch Hands (all groups)	14.8% (146/989)
RR (95% CI)	1.1 (0.9–1.4)

The other major analytic concern involves the limited analyses concerning confounding. The authors note (Henriksen et al., 1997, Table 3) that the high-risk Ranch Hand group has both increased (body mass index [BMI]) and decreased (age) diabetes risk factors. Tables 4 and 5 in the paper list relative risks of diabetes based on the actual (or "raw") numbers of cases in each dioxin exposure category (Michalek and Ketchum, 1997). One analysis presented controls for obesity (Henriksen et al., 1997, Table 7) and appears to eliminate the significance of the negative coefficient of "time to onset of diabetes." A further matched analysis is described, including matching within 3 percent body fat, but relative risks (without confidence intervals) are given only for glucose and insulin values and not for diabetes risk or diabetes severity. In addition, the authors also reanalyzed the data using revised initial doses to take into account 1982 baseline body fat. The results are similar although the relative risk for the high exposure category is now lower than the low-exposure group. No confidence intervals are given so it is difficult to more fully assess these data. A fully adjusted multivariate model is strongly recommended (e.g., Cox Proportional Hazard with time to diabetes as the outcome), fully controlling for baseline age and obesity (BMI) and, if possible, for family history of diabetes, central fat

distribution, diabetogenic drug exposure, and a measure of obesity at the time of Vietnam service.

O'Toole et al. (1996), reporting on 641 Australian Vietnam veterans compared to the Australian population, found a response-adjusted RR of 1.6 (99% CI 0.4–2.7). There are a number of methodologic problems inherent in this study, including a lack of health outcome validation and the use of a control group that is not adequately representative of the cohort.

In a report of a NIOSH medical study of 281 dioxin-exposed workers from two chemical plants in New Jersey and Missouri, Sweeney et al. (1996, 1997) note a slight, statistically significant increase in the risk of diabetes (OR 1.1, $p < 0.003$) and high (≥140 mg/dl) fasting serum glucose level ($p < 0.001$) with increasing serum concentrations of 2,3,7,8-TCDD. The authors suggest, without further documentation, that known diabetes risk factors (age, weight, family history of diabetes) appear more influential than TCDD exposure in explaining this result. An earlier report on this same cohort, published as a conference abstract (Sweeney et al., 1992), finds increased diabetes prevalence (9.2 percent) in the exposed workers compared to 258 nonexposed workers (5.8 percent).

Although not reported, this difference is not significant. However, in a multiple logistic regression analysis, significant associations between serum TCDD level and diabetic status or (in those without diabetes) fasting blood sugar, were apparently noted that were independent of major confounders (age, body mass index, race, and family history of diabetes). Since an OGTT was not performed, many cases (in both groups) may not have been detected. It is recommended that this study be documented more completely and published in the peer-reviewed literature, so that these potentially important findings can be evaluated fully.

Synthesis

The evidence suggesting that a connection between herbicide exposure and diabetes risk is equivocal. Consistency across studies is lacking in terms of early reports; however, the two recent studies using serum TCDD levels appear to have some consistency (Henriksen et al., 1997; Sweeney et al., 1996, 1997). In many studies, no association is detected and even in NIOSH and Ranch Hand studies it is not significant in univariate analyses for exposed subjects overall. Thus, only a small fraction of cases to date could be linked to herbicide exposure. However, the increased risk reported for the highest exposure groups suggests dose–responsiveness in both the Ranch Hand and the NIOSH studies. On the other hand, the much higher serum TCDD levels in the exposed groups in the NIOSH (Sweeney et al., 1996, 1997) and Ranch Hand study (Henriksen et al., 1997) compared to each study's control group do not lead to proportionately higher rates. Indeed in the 1991 Ranch Hand report (AFHS, 1991), the association with TCDD level was also seen in the comparison group. These observations raise the possibility of residual confounding by obesity. As obesity is a powerful determinant of both

TCDD level and diabetes, it is very difficult to determine whether TCDD has an independent pathogenetic role. More rigorous statistical analyses are, as suggested, needed to address the issue of residual confounding. A different possibility, namely that obesity is a mediator of TCDD-enhanced diabetes risk, has not been formally addressed in the analyses to date. This possibility remains open but difficult to explore as obesity or percent body fat measures at the time of initial Vietnam service would be needed along with equally precise TCDD exposure measures. Animal data suggest that rather than being associated with obesity, TCDD exposure, if anything, leads to a wasting syndrome. Other possibilities, for example, that there is some interaction between TCDD and obesity, could be more fully explored with statistical analyses of existing data, and researchers with relevant data are encouraged to critically examine these possibilities.

Potential pathogenetic mechanisms add to the biologic plausibility of herbicide exposure increasing diabetes risk. Empirically, the TCDD association with triglyceride and high-density lipoprotein (HDL) concentrations suggests a general consistency because these are the hallmarks of altered lipid metabolism in diabetes, since fatty acid metabolism, insulin resistance, and glucose metabolism are closely linked. The nature of the cases (i.e., few treated with insulin) does not suggest a Type I diabetes process with autoimmune β-cell destruction or chemical toxicity as seen with the rat poison Vacor (Drash et al., 1989). Nonetheless, measurement of glutamic acid decarboxylase (GAD) and insulin antibodies may be worthwhile given the uncertain nature of young adult-onset diabetes (Tuomi et al., 1993).

The well-established effect of TCDD on glucose transport in a variety of cells including human granulosa cells (by a cAMP [cyclic adenosine 5′-monophosphate] dependent protein kinase [Enan et al., 1996]), guinea pig adipose tissue (Enan and Matsumura, 1993), and mice and rats (by an Ah receptor-mediated mechanism [Enan and Matsumura, 1994]) provides some basis for biological plausibility. Furthermore, the association between TCDD and decreased PKC activity is of particular interest (Matsumura, 1995). TCDD may exert an influence on PKC activity which, in turn, may relate to insulin receptor kinase activity. Kruszynska and Olefsky report that increased PKC appears to inhibit insulin receptor kinase activity in humans (1996). Information about TCDD modulation of PKC is growing; for example, in vascular smooth muscle cells it appears to exhibit cell cycle dependence and isoform specificity (Weber et al., 1996) and is biphasic (Weber et al., 1994), while Bagchi et al. (1997) have shown that TCDD is a particularly strong stimulant of hepatic PKC in Sprague-Dawley rats. TCDD also has been reported to decrease glucose transporter 4 in adipose tissue and glucose transporter 1 in mice brains by the Ah receptor-dependent process operating at different levels (mRNA and protein, respectively) (Liu and Matsumura, 1995). Finally, since TCDD has been shown to affect hormone (including insulin) signaling, the likelihood that TCDD may be diabetogenic is further increased (Liu and Safe, 1996).

Thus, in summary, many animal studies provide potential biological mechanisms for an association between herbicide exposure and diabetes risk, and although the majority of earlier reports on humans suggest little association, the potentially more definitive 1997 report from the Ranch Hand study (Henriksen et al., 1997) raises the possibility that veterans in the highest herbicide exposure category may be at increased risk. Such a conclusion may be supported by a currently unpublished NIOSH study of workers exposed to TCDD. It is important to note that these studies used serum TCDD levels as the measure of exposure. At this time, questions concerning case definition and full control for obesity and other confounders (in the Ranch Hand study) preclude determining whether or not an association exists between herbicide exposure and diabetes in these studies.

The committee strongly urges that the NIOSH study be documented more completely and published in the peer-reviewed literature, so that its potentially important findings can be evaluated fully. It strongly recommends that the Ranch Hand study develop a fully adjusted multivariate model (e.g., Cox Proportional Hazard with time to diabetes as the outcome), fully controlling for baseline age and obesity (BMI) and, if possible, for family history of diabetes, central fat distribution, diabetogenic drug exposure, and a measure of obesity at the time of Vietnam service. The committee recommends that consideration be given to a combined analysis of the Ranch Hand and NIOSH studies to further examine the possibility that herbicide or dioxin exposure leads to an increased risk of diabetes. Using the new ADA definition of diabetes (i.e., fasting plasma glucose $\geq$126 mg/dl), outcome data from both studies could be made comparable.

Conclusions

Strength of Evidence in Epidemiologic Studies

When viewed in the context of the total literature, the committee concludes that, at this time, there is inadequate/insufficient evidence to determine whether an association exists between herbicide or dioxin exposure and increased risk of diabetes. Further analyses and full publication of existing studies may justify a reevaluation of this conclusion.

Biologic Plausibility

It is plausible that TCDD exposure could affect glucose metabolism and insulin action and thereby increase diabetes risk, although much of the evidence comes from animal studies. Whether these often short-term effects in animals and, occasionally in human cells can explain the possible small increase in diabetes prevalence 20–30+ years after exposure requires further human study, as does the apparent lack of a dose–response relationship across cohorts with very differ-

ent exposures. A discussion of the research related to biologic plausibility with respect to exposure to TCDD or herbicides and diabetes is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

Increased Risk of Disease Among Vietnam Veterans

Currently, it is uncertain whether any increased risk of diabetes has been experienced by Vietnam veterans exposed to herbicides. Although the most recent Ranch Hand study report suggests that those with the highest exposure may experience an increased risk (up to 50 percent), it is not clear whether this is independent of other risk factors for diabetes, especially obesity. A more thorough discussion of the issue of increased risk of disease among Vietnam veterans is contained in Chapter 1.

LIPID AND LIPOPROTEIN DISORDERS

Background

Plasma lipid concentrations (notably cholesterol) have been shown to predict cardiovascular disease and are considered fundamental to the underlying atherosclerotic process (Kuller and Orchard, 1988). The two major lipids, cholesterol and triglycerides, are carried in the blood attached to proteins to form lipoproteins, which are classed according to their density: very low density lipoprotein (VLDL—the major "triglyceride" particle) produced in the liver and progressively catabolized (hydrolyzed) mainly by an insulin-mediated enzyme (lipoprotein lipase) to form intermediate-density lipoprotein (IDL) or VLDL remnants, most of which are rapidly cleared by the liver B/E receptors, with the rest going to form low-density lipoprotein (LDL), the major "bad" cholesterol particle. This is cleared by LDL receptors in the liver and other tissues. The "good cholesterol" particle, high-density lipoprotein (HDL), is produced in the small intestine and the liver, and also results from of the catabolism of VLDL. Although LDL is thought to be involved in delivery of cholesterol to the tissues, HDL in contrast is involved in "reverse" transport and facilitates the return of cholesterol to the liver for biliary excretion (LaRosa, 1990).

Disorders of lipoprotein metabolism usually result from overproduction or decreased clearance of lipoproteins, or both. Common examples are hypercholesterolemia, which may be familial (due to an LDL receptor genetic defect) or polygenic (due to multiple minor genetic susceptibilities); familial hypertriglyceridemia (sometimes linked to diabetes susceptibility); and mixed hyperlipidemias in which both cholesterol and triglycerides are elevated. This group includes familial combined hyperlipidemia, thought by many to result from hepatic overproduction of VLDL and apoprotein B, and type III dyslipidemia (defective clearance of IDL–VLDL remnants, leading to a buildup of these athero-

genic particles). Although the bulk of blood lipid concentrations are genetically determined, diet, activity, and other factors (concurrent illness, drugs, age, gender, hormones, etc.) do have major effects. In particular, the saturated fat content of the diet may raise LDL cholesterol concentrations via decreased LDL receptor activity, whereas obesity and a high-carbohydrate diet may increase VLDL triglycerides, possibly linked to insulin resistance and reduced lipoprotein lipase activity. Intercurrent illness may increase the triglyceride (and lower the cholesterol) concentration. Diabetes is also associated with increased triglycerides and decreased HDL cholesterol, whereas other diseases (e.g., thyroid, renal) often result in hypercholesterolemia. It is thus evident that multiple host and environmental factors influence lipid and lipoprotein concentrations and that these influences must be accounted for before the effect of a new factor can be assessed (LaRosa, 1990). In the current context, obesity as a primary determinant of both triglyceride and TCDD concentrations has to be fully controlled for in any analysis. Furthermore, the ability of acute or chronic illness to raise triglycerides (and glucose) concentrations and lower HDL (and LDL) cholesterol must be recognized.

Epidemiologic Studies

Pazderova-Vejlupkova et al. (1981) reported that although increased lipid levels were seen in 50 percent of the sample of TCP and 2,4,5-T production workers at the time of an industrial accident in 1968, lipids were normal 10 years later. However, increased VLDL and decreased HDL lipoprotein fractions were noted compared to a control group. Potential confounders were not described. May (1982) reported on the results of a study of 126 British TCP production workers, laboratory, and administrative staff, which included 41 subjects who had been exposed to 2,3,7,8-TCDD in an industrial accident and subsequently developed chloracne. A nonsignificant increase in triglycerides and decrease in cholesterol were noted in the exposed groups. In a similar analytic approach, Martin (1984) studied the same group but used a different control group matched for age, social class, height–weight ratio, and smoking and alcohol intake habits. In this analysis, triglyceride and cholesterol concentrations were significantly increased in the exposed group compared to the controls, and HDL cholesterol concentrations were nonsignificantly lower. Moses et al. (1984), reporting on a mixed group of accidentally and chronically exposed TCP and 2,4,5-T production workers, found no difference 10–40 years after exposure in cholesterol concentrations between those with and without chloracne and only borderline higher triglycerides ($p = 0.057$, adjusted for age but not obesity) in those with chloracne.

In a detailed analysis of lipid values from a mixed accidentally and chronically exposed cohort of 2,4,5-T and TCP production workers from a plant in Nitro, West Virginia, no differences were noted 10–30 years after exposure in mean total HDL and LDL cholesterol and triglyceride levels for those exposed

($N = 204$) compared to those not exposed ($N = 163$), although out-of-range HDL and LDL cholesterol levels were associated with chloracne status in the exposed group (Suskind and Hertzberg, 1984). Other negative studies include one of 1,500 children aged 6–10 years at the time of the 1976 Seveso accident, of whom 597 were exposed. No differences in cholesterol or triglycerides were noted compared to 874 nonexposed children, up to six years later (Mocarelli et al., 1986). A further follow-up (Assennato et al., 1989) of 193 subjects exposed at Seveso, again mainly children, who developed chloracne also failed to show triglyceride or cholesterol elevations up to nine years later in those with chloracne compared to a matched control group. However, in the exposed group, the 1983 values were significantly lower than at the time of accident which supports the hypothesis of an early elevation of both cholesterol and triglycerides. Confounding factors (e.g., obesity), were not considered beyond age and sex.

A recent report (Calvert et al., 1996) of 281 TCP production workers and 260 referents examined 15 years after exposure, revealed only modest associations between exposure category and lipid or lipoprotein concentrations. This detailed study adjusted for multiple confounders. No association with serum TCDD levels was observed for total cholesterol concentration ($p = 0.44$) or for the prevalence of abnormally high cholesterol concentrations ($p = 0.71$). In examining the association of serum TCDD levels and HDL cholesterol, weak trends were seen for concentration ($p = 0.15$) and the prevalence of low HDL concentration ($p = 0.09$); a borderline significant trend ($p = 0.05$) was seen for triglyceride concentration, but no difference ($p = 0.21$) was noted for prevalence of abnormally high levels ($p = 0.21$).

Four reports from the Air Force Health Study, which has followed a cohort of Vietnam veterans exposed to herbicide spraying, have included lipid measures. The first (AFHS, 1984; Wolfe et al., 1990), which reported data 10–20 years after exposure, showed minimally lower cholesterol and triglyceride concentrations in the exposed Ranch Hands compared to the comparison group, with no evidence of a trend for increased values with increased herbicide exposure.

Two reports include data from the 1987 examination (up to 25 years after exposure). The Air Force Health Study (AFHS, 1990) reported identical mean total and HDL cholesterol and triglyceride concentrations in Ranch Hands and comparison subjects. The second report of the same examination data (AFHS, 1991) using serum (lipid weight-adjusted) dioxin analyses as a measure of exposure, revealed low-order but significant correlations between HDL cholesterol and dioxin level ($r = –0.14$ for Ranch Hands and $r = –0.10$ for comparisons). Similar correlations for total cholesterol were nonsignificant, whereas for triglycerides in a different analytical format, significant positive associations with serum TCDD were seen ($p < 0.001$). It is possible, however, that these correlations at least partially reflect the correlations in the two groups between dioxin and percentage of body fat ($r = 0.3$ for the Ranch Hands and .15 for the comparison group). The more recent 1992 examination data (AFHS, 1995) did not show

any of the dioxin-determined categories of Ranch Hands to differ from the comparison group for prevalence of high triglyceride levels or low HDL concentrations, or for a high ratio of total cholesterol to HDL cholesterol in a longitudinal analysis.

Four German reports have also reported on lipid values after industrial exposure to dioxin. Ott et al. (1994) studied 138 BASF subjects exposed to TCDD following a plant accident in 1953 and found nonsignificantly lower total and HDL cholesterol and triglycerides in the exposed subjects compared to controls. A medical insurance follow-up (Zober et al., 1994) of 158 BASF workers—including some of the same workers studied by Ott et al. (1994)—showed a nonsignificant increase in the recorded medical diagnosis of lipid disorders in exposed subjects compared to the referent population. Ott and Zober (1996) in another study of 34 male production workers potentially exposed to polybrominated dibenzo-*p*-dioxins found no significant elevations of total and LDL cholesterol or triglycerides and a borderline ($p = 0.05$) higher HDL cholesterol compared to nonexposed controls. The German Cancer Research Center, reporting on employees from six West German chemical plants found no apparent effect of TCDD on lipid metabolism (Von Benner et al., 1994).

A study of self-reported health problems of 641 Australian Vietnam veterans (O'Toole et al., 1996) found an increased frequency of elevated cholesterol compared to that expected from national Australian data (RR = 3.0, 95% CI 1.3–4.7).

Synthesis

The majority of studies reported do not suggest any major disturbance of cholesterol or triglyceride concentration in herbicide-exposed chemical workers or veterans. Five studies report increased triglycerides (two are of borderline significance), and six studies report lowered HDL cholesterol (four are of borderline significance). Given the liability of triglyceride concentration, the marginal nature of the few positive studies, and the equal or greater number of negative studies for each lipid or lipoprotein (except HDL cholesterol, which was not examined in more than half of the studies), the effect of herbicide exposure on lipid and lipoproteins is likely to be small. Furthermore, few studies have fully controlled for obesity and the many other potential confounders including diet.

The difficulties of interpreting relationships between serum dioxin levels (even lipid weight-adjusted levels) and lipid orlipoprotein concentrations, which may be a determinant as well as a consequence of dioxin level, have been fully discussed (Flanders et al., 1992). Lipoproteins are blood and possibly intracellular carriers of TCDD (Soues et al., 1989). This problem is further complicated by the effect of obesity, which independently plays a role in determining lipid or lipoprotein concentration, and the extent of available fat stores, thereby further confounding any attempt to determine the relationship between dioxin level and lipoprotein.

The relatively weak and conflicting human data stand in contrast to the extensive animal data, which offer considerable biologic plausibility for the concept that herbicide and TCDD exposure may cause lipid and lipoprotein disturbances. The multiple effects of dioxin-type chemicals have been reviewed by Matsumura (1995) and include reduction in adipose tissue lipoprotein lipase in guinea pigs, hypertriglyceridemia in rabbits, and down-regulation of LDL receptors on the plasma membrane in guinea pig hepatocytes. These effects appear to be mediated by the Ah receptor. However the relevance of these observations to humans may be limited. These effects, even in animals, show considerable differences according to species, age, strain, and gender. For example, male and female guinea pigs differ in the effect of TCDD on lipoprotein lipase activity, lipid peroxidation, and glucose uptake by adipose tissue (Enan et al., 1996). Furthermore, TCDD exposure in guinea pigs causes a wasting syndrome with major loss of fat tissue. Such a syndrome does not seem to be present in the humans studied who have marginal lipid disturbances.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate/insufficient evidence to conclude that an association exists between herbicide exposure and lipid or lipoprotein levels. Further research is needed to better elucidate the small effects on HDL cholesterol and triglyceride concentrations observed in some studies.

Biologic Plausibility

Although animal studies suggest potential mechanisms whereby TCDD may cause lipid disturbances, human data (e.g., lipoprotein kinetic studies) are needed to determine whether, and how, TCDD-exposed subjects have altered lipoprotein metabolism. A discussion of biologic plausibility with respect to exposure to TCDD or herbicides and lipid and lipoprotein disorders is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

Increased Risk of Disease Among Vietnam Veterans

Ranch Hand study data (AFHS, 1991) suggest that lipid and lipoprotein concentrations of Ranch Hands and comparison subjects do not differ overall, although significant correlation between HDL cholesterol (negative) or triglycerides (positive) and TCDD level are seen. Whether these correlations are fully independent of obesity is unresolved. It is concluded that Vietnam veterans have not experienced major lipid or lipoprotein disease as a result of herbicide expo-

sure. A more thorough discussion of the issue of increased risk of disease among Vietnam veterans is contained in Chapter 1.

GASTROINTESTINAL AND DIGESTIVE DISEASE, INCLUDING LIVER TOXICITY

Background

The discussion in this section of gastrointestinal and digestive disease, including liver toxicity, encompasses a variety of conditions included under ICD·9 codes 520–579. Conditions in this category include diseases of the esophagus, stomach, intestines, rectum, liver, and pancreas. As in *VAO* and *Update 1996*, the focus of this section is primarily ulcer disease and liver toxicity, since these were the conditions most frequently discussed in the literature reviewed. The symptoms and signs of gastrointestinal disease and liver toxicity are highly varied and often vague, depending on the specific condition involved.

The essential function of the gastrointestinal tract is to absorb nutrients and eliminate waste products. This complex task involves numerous chemical and molecular interactions at the mucosal surface, as well as complex local and distant neural and endocrine factors. One of the most common conditions affecting the gastrointestinal tract is motility disorder, which may be present in as many as 15 percent of adults. The range of diseases affecting the gastrointestinal system can most conveniently be categorized by the anatomic segment involved. These conditions include esophageal disorders that predominantly affect swallowing, gastric disorders related to acid secretion, and conditions affecting the small and large intestine and reflected by alterations in nutrition, mucosal integrity, and motility. Systemic disorders may also affect the gastrointestinal system; these include inflammatory, vascular, infectious, and neoplastic conditions.

Peptic Ulcer Disease

Peptic ulcer disease refers to ulcerative disorders of the gastrointestinal tract that are caused by the action of acid and pepsin on the stomach duodenal mucosa. Peptic ulcer disease is characterized as gastric ulcer or duodenal ulcer, depending on the anatomic site of origin. Peptic ulcer disease occurs when the corrosive action of gastric acid and pepsin exceeds the normal mucosal defense mechanisms protecting against ulceration. Approximately 10 percent of the population has clinical evidence of duodenal ulcer during their lifetime, with a similar percentage affected by gastric ulcer. The peak incidence for duodenal ulcer occurs in the fifth decade of life, whereas the peak for gastric ulcer occurs approximately ten years later. The natural history of duodenal ulcer is one of spontaneous remission (healing) and recurrences. It is estimated that 60 percent of healed

duodenal ulcers may recur in the first year and that 80–90 percent will recur within two years.

Increasing evidence indicates that the bacterium *Helicobacter pylori* (*H. pylori*) may be closely linked to both duodenal and gastric ulcer disease. This bacterium colonizes the gastric mucosa in 95–100 percent of patients with duodenal ulcer and 75–80 percent of patients with gastric ulcer. Healthy subjects in the United States under 30 years of age have gastric colonization rates of approximately 10 percent. Over age 60, colonization rates exceed 60 percent. Colonization alone, however, is not sufficient for the development of ulcer disease; only 15–20 percent of subjects with *H. pylori* colonization will develop ulcer disease in their lifetimes.

There are other risk factors for peptic ulcer disease. Genetic predisposition appears to be important; first-degree relatives of duodenal ulcer patients have approximately three times the risk of developing duodenal ulcer as the general population. Certain blood groups are associated with increased risk for duodenal ulcer, and HLA-B5 antigen appears to be increased among white males with duodenal ulcer. Cigarette smoking has also been linked to duodenal ulcer prevalence and mortality. Finally, psychological factors, particularly chronic anxiety and psychological stress, may act to exacerbate duodenal ulcer disease.

Liver Disease

Blood tests reflecting liver function are the mainstay of diagnosis for liver disease. Increases in serum bilirubin levels and in the serum activity of certain hepatic enzymes—including aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), alkaline phosphatase, and gamma-glutamyltransferase (GGT)—are commonly noted in many liver disorders. The relative sensitivity and specificity of these enzymes for liver disease vary, and several different tests may be required for diagnosis. The only regularly reported abnormality in liver function associated with TCDD exposure in humans is an elevation in GGT. Estimates of the serum activity of this enzyme provide a sensitive indicator of a variety of conditions, including alcohol and drug hepatotoxicity, infiltrative lesions of the liver, parenchymal liver disease, and biliary tract obstruction. 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., 1992). Moreover, elevation in GGT may be considered a normal biologic adaptation to chemical, drug, or hormone exposure.

Cirrhosis of the liver is the most commonly reported liver disease outcome in epidemiologic studies of herbicide and/or TCDD exposure. Pathologically, cirrhosis reflects irreversible chronic injury of the liver, with extensive scarring and resultant loss of liver function. Clinical symptoms and signs include jaundice, edema, abnormalities in blood clotting, and metabolic disturbances. Ultimately,

cirrhosis may lead to portal hypertension with associated gastroesophageal varices, enlarged spleen, abdominal swelling due to ascites, and ultimately hepatic encephalopathy, which may progress to coma. It is generally not possible to distinguish the various causes of cirrhosis by the clinical signs and symptoms or pathological characteristics. The most common cause of cirrhosis in North America and many parts of Western Europe and South America is excessive alcohol consumption. Other causes include chronic viral infections (hepatitis B or hepatitis C), a poorly understood condition called primary biliary cirrhosis, chronic right-sided heart failure, and a variety of less common metabolic and drug-related causes.

Summary of *VAO* and *Update 1996*

In *VAO*, the risk of ulcers in populations exposed to TCDD or herbicides had not been sufficiently studied to determine an association. However, the detection of a specific association was felt to be unlikely, given the frequency of ulcer disease and the many factors 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 was considered unlikely that new cases of ulcer disease directly related to herbicide exposure would occur. Subsequent occupational studies by Zober et al. (1994) and Ott et al. (1994) revealed no increases in the frequency of ulcers in the exposed group versus the controls (even in the highest TCDD subgroup), and no increases with increasing severity of chloracne.

Changes in liver function in humans exposed to TCDD have been limited to an increase in GGT and alkaline phosphatase; 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. Results from studies relating liver enzyme measurements or the diagnosis of chronic liver disease to serum TCDD levels or clinical indices of dioxin exposure (e.g., chloracne) have been inconsistent. 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 toxic chemical exposures. Given the long observation period since TCDD exposure in most studies and the 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.

Update of the Scientific Literature

Occupational Studies

Becher et al. (1996) examined mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population

included workers who had a least one month of employment and resulted in a cohort consisting of 2,479 male workers. SMRs and 95% CI were calculated using West German mortality rates by five-year age and calendar intervals. SMRs for digestive disease among the four plants ranged from 0.7 to 0.8; none were statistically significant.

Ott and Zober (1996) updated the experience of workers exposed to TCDD during the cleanup of a TCP reactor that exploded in 1953 at a BASF plant in Ludwigshafen, Germany. They studied disease incidence and mortality up to 1992 for a group of 243 men and developed TCDD dose estimates based on work activity information, blood TCDD determinations on a subset of the population, and estimates of TCDD elimination rates. Expected numbers of incident cases and cause-specific deaths were obtained from German sources by five-year age and calendar intervals. The overall SMR (CI) for digestive diseases was 0.7 (0.2–1.7); in the highest TCDD dose group, the SMR was 1.6 (0.4–4.2).

Ramlow et al. (1996) examined mortality in a cohort of workers exposed to pentachlorophenol, as part of a larger study of Dow chemical manufacturing workers exposed to the higher chlorinated dioxins. The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential PCDD exposure between 1937 and 1980. From this cohort, 770 workers were identified who were considered to have potential PCP exposure based on work history records. Cumulative exposure indices for PCP and dioxin were calculated using scores described by Ott et al. (1987). In the study analysis, the U.S. white male death rates (five-year age and calendar time specific) and the non-PCP and non-PCDD male Dow Michigan employees for 1940 to 1989 were both used as reference values to calculate expected deaths. The overall SMR (CI) for digestive system disease was 1.3 (0.7–2.2). For gastric and duodenal ulcer (ICD·9 531–533) the SMR was 3.6 (1.2–8.3) when considering no latency, and 5.6 (1.8–13.0) when allowing for 15 years of latency. The SMRs for liver cirrhosis were 1.1 (0.4–2.3), and 1.5 (0.6–34) with allowance for a 15-year latency. Overall digestive disease was higher in subjects with higher estimated PCP exposure (RR 2.3, CI 1.3–4.2) in more highly exposed workers versus 0.9 (CI 0.3–2.7) in workers with lower exposures ($p = 0.01$). However, the RR for ulcer disease decreased significantly with exposure ($p < 0.01$), whereas liver cirrhosis significantly increased with estimated exposure level ($p = 0.04$). Four of the six cirrhosis deaths were clearly identified as related to alcoholism by death certificate review.

In the updated and expanded IARC cohort study, Kogevinas et al. (1997) examined mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. Exposure information varied between cohorts, but in general, exposures were reconstructed from job records. Based on job categories and information on production processes and the composition of the materials used, exposed workers were classified into three categories: exposed to TCDD or higher chlorinated dioxins, unexposed to the same, and

unknown exposure to the same. Analysis was performed by calculating SMRs and 95% CI, using the WHO mortality data bank to calculate national mortality rates by sex, age (five-year intervals), and calendar period (five years). Overall, a decrease in gastrointestinal and digestive disease was observed in exposed workers compared to unexposed workers for males (SMR 0.8, CI 0.7–1.0), with no significant differences observed for women (SMR 1.3, CI 0.6–2.5).

Veterans Studies

In a study of postservice suicide among Vietnam veterans, Bullman and Kang (1996) reported subsequent cause-specific mortality for 34,534 veterans who had been hospitalized for wounds suffered in Vietnam, compared to U.S. men. They observed a significant decrease in deaths due to digestive disease (SMR 0.7, CI 0.6–0.9).

O'Toole et al. (1996) described the results of a simple random sample of Australian Army Vietnam veterans on self-reported health status. Data were obtained on 641 veterans from the Australian Bureau of Statistics Health Interview Survey 1989–90, and illness rates were compared to the age- and sex-matched Australian population. Ulcer disease (RR 2.7, 99% CI 1.7–3.8) and other digestive diseases (RR 4.0, 99%, CI 2.2–5.9) were reported significantly more frequently by veterans. Ulcer disease was significantly associated with an index of combat exposure. The authors conjectured that this relationship may be related to long-term psychological factors associated with battle (e.g., post-traumatic stress disorder). They also observed that veterans were significantly more likely to report high alcohol consumption than the age- and sex-matched Australian general population (RR 2.0, 99% CI 1.5–2.5).

Watanabe and Kang (1996) reported on the mortality of 33,833 U.S. Army and Marine Corps Vietnam veterans who died during 1965–1988, compared to mortality among 36,797 deceased non-Vietnam veterans, using PMRs. They observed no increase in digestive disease mortality among Army Vietnam veterans (PMR 1.0) and a slight decrease among Marine Vietnam veterans (PMR 0.9, $p < 0.05$).

Dalager and Kang (1997) reported a study comparing 2,872 Vietnam veterans with 2,737 non-Vietnam veterans (all of whom served in Chemical Corps specialties). All study subjects served at least 18 months' active duty between 1965 and 1973, and vital status ascertainment was complete for both groups. They reported a RR (CI) of 3.9 (1.1–13.5) for digestive system disease overall and 4.4 (1.0–20.2) for liver cirrhosis. No data were available on alcohol use, but the Centers for Disease Control and Prevention (CDC) Vietnam Experience Study had reported that 13 percent of Vietnam veterans and 11 percent of non-Vietnam veterans described heavy alcohol use (CDC, 1988).

A report on Australian Vietnam veterans (Crane et al., 1997a) compared cause-specific mortality rates of 59,036 male Vietnam veterans with those of

other Australian males. They found no significant increase in digestive system disease (SMR [CI]) for deaths 1964–1979: 0.7 [0.5–1.1] and for 1980–1994: 1.0 [0.8–1.2]. There was specifically no increase in cirrhosis of the liver.

A second cohort study of Australian veterans (Crane et al., 1997b) compared mortality for 1982–1994 for 18,949 national servicemen who had served in Vietnam (veterans) with 24,646 national servicemen who had not served in Vietnam (nonveterans). An RR (95% CI) for veterans compared to nonveterans of 1.0 (0.7–1.3) for all circulatory diseases and 1.0 (0.7–1.4) for ischemic heart disease (IHD) was observed.

The ongoing study of Ranch Hand veterans (AFHS, 1996), reported cause-specific mortality among 1,261 Ranch Hand personnel compared to 19,080 Air Force veterans from the same era who did not handle herbicides. An increase was observed in deaths due to digestive disease (SMR 1.8, 0.9–3.2), mostly from chronic liver disease and cirrhosis (seven of nine deaths).

Synthesis

Although there have been sporadic reports of increased gastrointestinal disease potentially related to exposure to herbicides or TCDD, the results are inconsistent across studies. In addition, interpretation of individual studies was generally limited by a lack of information on alcohol consumption and other risk factors. In the studies that showed the strongest association between potential exposure and gastrointestinal disease (cirrhosis of the liver), there was strong evidence that excess alcohol consumption was the etiology for the cirrhosis. Additional research, with adequate information on alcohol consumption and other risk factors for gastrointestinal disease, is required to adequately assess the potential association between gastrointestinal disease and herbicide or TCDD exposure.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides considered in this study and gastrointestinal and digestive disease including liver toxicity. The evidence regarding association is drawn from occupational and veterans studies in which subjects were exposed to a variety of herbicides and herbicide components.

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. A more thorough discussion of biologic plausibility with respect to exposure to

TCDD or herbicides and gastrointestinal disease, digestive disorders, and liver toxicity is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

CIRCULATORY DISORDERS

The circulatory diseases reviewed in this section cover a variety of conditions encompassed by ICD·9 codes 390–459, including hypertension, heart failure, arteriosclerotic heart disease, peripheral vascular disease, and cerebrovascular disease. In morbidity studies, a variety of methods were used to assess the circulatory system, including analysis of symptoms or history, physical examination of the heart and peripheral arteries. Doppler measurement of peripheral pulses, electrocardiograms, and chest radiographs. Doppler measurements and physical examinations of the pulses in the arms and legs are used to detect decreased strength of the pulses, which can be caused by thickening and hardening of the arteries. Electrocardiograms 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. Mortality studies attribute cause of death to one or more of the circulatory disorders, with varying degrees of diagnostic confirmation.

Summary of *VAO* and *Update 1996*

In general, the usefulness of mortality studies has been limited, because in most studies, no a priori hypotheses were provided regarding herbicide exposure and particular circulatory outcomes. Their usefulness is also limited by a failure to adjust for independent risk factors for circulatory disease. Among the morbidity studies, strong rationales for examining circulatory outcomes were not given. However, the Air Force Health Study (AFHS, 1991) reported associations between serum TCDD and both diabetes and blood lipids, suggesting a reason to examine coronary artery disease in subjects exposed to dioxins because of the possible association between risk factors for coronary artery disease and serum TCDD level.

A follow-up of the Ranch Hand cohort (AFHS, 1992) found significant associations between dioxin levels and several lipid-related variables and some cardiovascular effects. The authors suggested that these effects may be related to diabetes mellitus, since no consistent evidence of an adverse effect of dioxin was seen in nondiabetic individuals. An additional complicating factor in these analyses was that many of the effects correlated with dioxin level were related to body fat content. Causal relationships could not be established, because of the relationship between body fat itself and dioxin level. A more complete discussion of this study is included in Chapter 3.

Update of the Scientific Literature

Occupational Studies

Becher et al. (1996) examined mortality among workers in four German facilities that produced phenoxy herbicides and chlorophenols. The population included workers who had a least one month of employment, resulting in a cohort consisting of 2,479 male workers. SMRs (95% CI) were calculated using West German mortality rates by five-year age and calendar year intervals. SMRs for circulatory system disease among the four plants ranged from 0.3 to 1.1; none were significantly elevated.

Flesch-Janys et al. (1995) described cancer and circulatory system mortality among 1,189 male workers in a chemical plant in Hamburg, Germany. Workers had been exposed in varying degrees to herbicides contaminated with PCDD/F. The authors developed a quantitative estimate of PCDD/F exposure for the entire cohort derived from blood and adipose tissue levels measured in a subgroup of 190 workers. An unexposed cohort of gas workers served as an external reference group. Overall circulatory system disease mortality was elevated among exposed workers in a dose-dependent fashion (p for trend = 0.01), with an RR of 2.0 (95% CI 1.2–3.3) among men in the highest decile of estimated TCDD exposure. The increased risk appeared restricted to IHD (ICD·9 codes 410–414) where the RR was 2.5 (1.3–4.7) in the highest estimated exposure group. Information was not available for confounding factors related to IHD; the authors reasoned that the use of an unexposed referent population combined with the strong dose–response relationship argued against attributing the results to confounding factors. They also noted that the smoking rates and socioeconomic status of both cohorts appeared to be similar.

Svensson et al. (1995) studied mortality and disease incidence in two cohorts of Swedish fishermen. One group (2,896 men) resided on the east coast of Sweden and consumed fish from the Baltic Sea. These fatty fish (particularly salmon and herring) are reported to contain elevated levels of PCB, PCDD, and PCDF. The other group of fishermen (8,477) resided on the west coast of Sweden and were presumed to have a higher intake of lean (and less contaminated) fish, including cod and flat fish. A slight but significant decrease in cardiovascular disease was observed among east coast fishermen, attributed to the protective effect of some components of fish oil.

Ott and Zober (1996) updated the experience of workers exposed to TCDD during cleanup of a TCP reactor, Germany. They studied disease incidence and mortality up to 1992 for a group of 243 men and developed TCDD dose estimates based on work activity information, blood TCDD determinations on a subset of the population, and estimates of TCDD elimination rates. Expected numbers of incident cases and cause-specific deaths were obtained from German sources by five-year age and calendar intervals. The overall SMR (95% CI) for circulatory

system diseases was 0.8 (0.6–1.2); in the highest TCDD dose group, the SMR was 1.0 (0.5–1.7). There was also no significant elevation in deaths from ischemic heart disease when evaluated separately.

Ramlow et al. (1996) examined mortality in a cohort of workers exposed to PCP, as part of a larger study of Dow chemical manufacturing workers exposed to the higher chlorinated dioxins. The study cohort was assembled from company records, starting with a cohort of 2,192 workers ever employed in a department with potential PCDD exposure between 1937 and 1980. From this, 770 workers were identified who were considered to have potential PCP exposure based on work history. Cumulative exposure indices for PCP and dioxin were calculated using the method described by Ott et al. (1987). The U.S. white male death rates (five-year age and calendar time specific) and the non-PCP and non-PCDD male Dow Michigan employees for 1940 to 1989 were used as reference values to calculate expected deaths. The overall SMR (95% CI) for circulatory system disease was 1.0 (0.8–1.1), with no significant increase in specific disease subsets and no influence of latency or estimated PCP exposure.

In the update and expansion of the IARC cohort study, Kogevinas et al. (1997) examined mortality in a cohort of 26,615 male and female workers engaged in the production or application of phenoxy herbicides. Exposure information varied between cohorts, but in general exposures were reconstructed from job records. Based upon job categories and information on production processes and the composition of the materials used, the exposed workers were classified into three categories: exposed to TCDD or higher chlorinated dioxins, unexposed to the same, and unknown exposure to the same. Analysis was performed by calculating SMRs and 95% CI, using the WHO mortality data bank to calculate national mortality rates by sex, age (five-year intervals), and calendar period (five years). Overall, a decrease in circulatory system disease was observed in exposed workers compared to unexposed workers for males (SMR 0.9, CI 0.9–1.0), with no significant differences observed for women (SMR 1.0, CI 0.7–1.3).

Mortality among a cohort of rice growers in northern Italy was investigated by Gambini et al. (1997). Using a set of registered farm owners consisting of 1,493 males who worked on farms from 1957 to 1992, they examined the cause of death for 958 subjects and compared this with expected numbers calculated from national rates. No direct exposure information was available, so employment on the farm served as a surrogate for exposure to the range of phenoxy herbicides used during the study period. They observed a decrease in deaths from myocardial infarction (SMR 0.7, 95% CI 0.6–0.9) and other ischemic heart disease (SMR 0.4, CI 0.3–0.5), but not stroke (SMR 1.0, CI 0.8–1.1) among rice growers.

Studies of Vietnam Veterans

In a study of postservice suicide among Vietnam veterans, Bullman and Kang (1996) reported subsequent cause-specific mortality for 34,534 veterans

who had been hospitalized for wounds suffered in Vietnam, compared to U.S. men. They observed a significant decrease in circulatory system disease deaths (SMR 0.7, 95% CI 0.6–0.8).

O'Toole et al. (1996) described the results of a simple random sample of Australian Army Vietnam veterans on self-reported health status. Data were obtained on 641 veterans from the Australian Bureau of Statistics Health Interview Survey 1989–1990, and illness rates were compared to the age- and sex-matched Australian population. Hypertension (RR 2.2, 99% CI 1.7–2.6) and other circulatory system disease (RR 2.4, CI 1.6–3.2) were reported significantly more frequently by veterans. None were significantly associated with an index of combat exposure. Veterans were significantly more likely to be current or former smokers than the general population (RR for never smoked 0.7, CI 0.5–0.8). O'Toole et al. (1996) also observed that veterans were significantly more likely to report high alcohol consumption than the age- and sex-matched Australian general population (RR 2.0, CI 1.5–2.5).

Watanabe and Kang (1996) reported on the mortality experience of 33,833 U.S. Army and Marine Corps Vietnam veterans who died during 1965–1988, compared to mortality among 36,797 deceased non-Vietnam veterans, using PMRs. They observed no increase in circulatory system disease mortality among Army Vietnam veterans (PMR 1.0) and a slight decrease among Marine Vietnam veterans (PMR 0.9, $p < .05$).

Dalager and Kang (1997) compared 2,872 Vietnam veterans with 2,737 non-Vietnam veterans (all of whom served in Chemical Corps specialties). All study subjects served at least 18 months active duty between 1965 and 1973, and vital status ascertainment was complete for both groups. They reported an RR (95% CI) of 1.1 (0.6–1.8) for circulatory system disease overall. No data was available on alcohol use, but a previous CDC study had reported that 13 percent of Vietnam veterans and 11 percent of non-Vietnam veterans described heavy alcohol use (CDC, 1988).

A report on Australian Vietnam veterans (Crane et al., 1997a) compared cause-specific mortality rates of 59,036 male Vietnam veterans with those of other Australian males. They found no significant increase in circulatory system disease (SMR [95% CI]) for deaths in 1964–1979 (0.7 [0.6–0.9]) and 1980–1994 (1.0 [1.0–1.1]). There was also no increase in any subset of circulatory system disease.

A second cohort study of Australian veterans compared mortality for 1982–1994 for 18,949 national servicemen who had served in Vietnam (veterans) with 24,646 national servicemen who had not served in Vietnam (nonveterans) (Crane et al., 1997b). They observed a RR (95% CI) for veterans compared to nonveterans of 1.0 (0.7–1.3) for diseases of the circulatory system.

The ongoing study of Ranch Hand veterans (AFHS, 1996), reported cause-specific mortality among 1,261 Ranch Hand personnel compared to 19,080 Air Force veterans from the same era who did not handle herbicides. They observed

a significant increase among ground troops (SMR 1.5, CI 1.0–2.2), with nearly half of the increase due to atherosclerotic heart disease (SMR 1.4, CI 0.8–2.1). Data on smoking and alcohol use were not available.

Synthesis

Although there were sporadic reports of increased circulatory disease potentially related to exposure to herbicides or TCDD, the results were inconsistent across studies. In addition, interpretation of individual studies was generally limited by a lack of information on cigarette smoking, obesity, serum lipid levels, presence of diabetes, and other risk factors. In studies that showed the strongest association between potential exposure and gastrointestinal disease, there was evidence that cigarette smoking was greater among veterans than nonveterans. Additional research, with adequate information on the numerous risk factors for circulatory disease, is required to adequately assess the potential association between circulatory disease and herbicide or TCDD exposure. Further research on the potential relationships between TCDD exposure and diabetes or lipid abnormalities may also shed further light on any potential relationships to circulatory diseases.

Conclusions

Strength of Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the herbicides considered in this report and the following circulatory outcomes: circulatory disease mortality, various subgroups of cardiovascular disease, and symptoms or history of circulatory illnesses (e.g., heart disease, hypertension, coronary artery disease, angina, or myocardial infarction). The evidence regarding association is drawn from occupational and veteran studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biological Plausibility

A discussion of biologic plausibility with respect to exposure to TCDD or herbicides and circulatory disease is contained in Chapter 3; a summary is presented in the conclusion to this chapter.

SUMMARY

Based on the occupational, environmental, and veterans studies reviewed, the committee reached one of four conclusions about the strength of the evi-

dence regarding association between exposure to herbicides and/or TCDD and each of the other health effects under study. As explained in Chapter 4, these distinctions 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 Committee by the Secretary of Veterans Affairs in Public Law 102-4 and with accepted standards for scientific reviews, the distinctions between these standard conclusions are based on statistical association, not on causality. The committee used the same criteria to categorize diseases by the strength of the evidence as were used in *VAO* and *Update 1996*.

Health Outcomes with Sufficient Evidence of an Association

In *VAO* and *Update 1996*, the committee found sufficient evidence of an association between exposure to herbicides and/or TCDD and chloracne. The scientific literature continues to support the classification of chloracne in the category of sufficient evidence. Based on the literature, there are no additional health effects discussed in this chapter that satisfy the criteria necessary for this category.

For diseases 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 also regarded evidence from several small studies that are free from bias and confounding, and that show an association that is consistent in magnitude and direction, as sufficient evidence for an association.

Health Outcomes with Limited/Suggestive Evidence of Association

In *Update 1996*, the committee found limited/suggestive evidence of an association between herbicide/dioxin exposure and porphyria cutanea tarda. The scientific literature continues to support the classification of this disorder in the category of limited/suggestive evidence. Based on the literature, there are no additional health effects discussed in this chapter that satisfy the criteria necessary for this category.

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.

TABLE 11-4 Selected Epidemiologic Studies—Lipids and Lipoprotein Disorders

Reference	Study Population	Exposed Cases	Cholesterol	Triglycerides	HDL Cholesterol
OCCUPATIONAL					
New Studies					
Calvert et al., 1996[c]	Production workers	18, 16, 7	1.0 (0.5–1.7)	1.7 (0.6–4.6)	2.2 (1.1–4.7)
Ott and Zober, 1996[a]	Production workers	42 NS	NS	↑ $p = 0.05$	
Studies reviewed in *VAO*					
Martin, 1984[a]	Production workers	53 (some exposure)	↑ $p < 0.005$	↑ $p < 0.05$	NS
		39 (chloracne)	↑ $p < 0.005$	↑ $p < 0.01$	NS
Moses et al., 1984[b]	TCP and 2,4,5-T production workers	118 (chloracne)	NS	NS	No data
Suskind and Hertzberg, 1984[a]	TCP production workers	204	NS	NS	NS
May, 1982[a]	TCP production workers	94 NS	NS	No data	
Pazderova-Vejlupkova et al., 1981[a]	TCP and 2,4,5-T production workers	55 NS	↑ VLDL $p = 0.01$	No data	
ENVIRONMENTAL					
Studies reviewed in *VAO*					
Assennato et al., 1989[a]	Adults exposed near Seveso	193 (chloracne)	NS	NS	No data
Mocarelli et al., 1986[a]	Children exposed near Seveso	63 NS	NS	No data	

VIETNAM VETERANS					
New Studies					
AFHS, 1996[i]	Longitudinal analysis (1992 exam data)	884 (cholesterol: HDL ratio)	NS	NS (cholesterol: HDL ratio)	NS
O'Toole et al., 1996[d]	Australian Vietnam veterans	20 3.0 (1.3–4.7)	No data	No data	
Studies reviewed in *VAO*					
AFHS, 1991[g]	Serum dioxin analysis (1987 exam data)	283–304[f]	$p = 0.175$	$p < 0.001$[h]	$p < 0.001$
AFHS, 1990[f]	Original exposure group analysis (1987 exam data)	8–142[f]	1.2 (0.9–1.5)	1.3 (0.9–1.8)	1.0 (0.4–2.4)
AFHS, 1984[e] Wolfe et al., 1990	Vietnam Veterans exposed to herbicide spraying (1982 data)	1,027 total exposed	NS	NS	NS

Estimated risk and 95% confidence interval reported unless P-values are specified
NS = Not significant
[a]P-values comparing means to controls. Univariate analysis.
[b]P-values comparing means in production workers with subsequent chloracne to those without.
[c]OR for abnormal lipid in highest exposure category.
[d]Compared to Australian population.
[e]Comparing means.
[f]Number exposed RH with "high" lipid values.
[g]Comparing mean dioxin across lipid groups.
[h]Continuous analysis.
[i]Comparing change over time between exposed and comparison groups.

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

The scientific data for many of the health effects reviewed by the committee were inadequate or insufficient to determine whether an association exists. For the health effects discussed in this chapter, 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 category includes respiratory disorders, immune system disorders (immune suppression and autoimmunity), diabetes, lipid and lipoprotein disorders, gastrointestinal diseases, digestive diseases, liver toxicity, and circulatory disorders.

Health Outcomes with Limited/Suggestive Evidence of *No* Association

In *VAO* and *Update 1996*, the committee did not find any evidence to conclude that there is limited/suggestive evidence of *no* association between the health effects discussed in this chapter and exposure to TCDD or herbicides. The most recent scientific evidence continues to support this conclusion.

In order to classify outcomes in this category, several adequate studies covering the full range of levels of exposure that human beings are known to encounter must be mutually consistent in not showing a positive association between exposure to herbicides and the outcome at any level of exposure. These studies must also 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.

Biologic Plausibility

Chapter 3 details the committee's evaluation of evidence from animal and cellular-level studies regarding the biological plausibility of a connection between exposure to dioxin or herbicides and various noncancer health effects. This section summarizes that evidence. Some of the preceding discussions of other health outcomes include references to specific relevant papers.

TCDD has been shown to elicit a diverse spectrum of sex-, strain-, age-, and species-specific effects, including immunotoxicity, hepatotoxicity, chloracne, loss of body weight, and numerous biological responses, including the induction of phase I and phase II drug-metabolizing enzymes, the modulation of hormone systems and factors associated with the regulation of cellular differentiation and proliferation.

Effects of TCDD on the liver include modulation of the rate at which liver cells multiply, increasing the rate of cell death for other cell types, 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 in itself 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, but the extent to which TCDD effects mediate noncancer end points is not clear.

The mechanism by which TCDD induces hepatotoxicity is still under investigation. Some studies provide evidence that hepatotoxicity of TCDD involves AhR-dependent mechanisms. Specifically, there is evidence that the Ah-receptor plays a role in the co-mitogenic action of TCDD with epidermal growth factor and in the induction of liver enzymes involved in the metabolism of xenobiotics. Acute exposures to TCDD have been correlated with effects on intermediary metabolism and hepatomegaly.

TCDD has been shown to inhibit hepatocyte DNA synthesis, decrease hepatic plasma membrane epidermal growth factor receptor; inhibit hepatic pyruvate carboxylase activity, and induce porphyrin accumulation in fish and chick embryo hepatocyte cultures. Studies have been conducted to examine the short- and long-term effects of TCDD on rat EROD activity and liver enzymes. Oral dosing induced EROD and glutamyltranspeptidase (GT) activity and inhibited hepatic phosphoenolpyruvate carboxykinase (PEPCK) activities. EROD and PEPCK activity reverted to normal levels after ninety days while GT activity remained elevated. Hepatomegaly has been shown to occur following high subchronic doses. The myocardium has also been shown to be a target of TCDD toxicity; impairment of a cAMP-modulated contraction has been implicated.

Recent evidence suggests that the inhibition of glucose transport in adipose tissue, pancreas, and brain may be one of the major contributing factors to the wasting syndrome. In vitro studies have identified glomerular mesangial cells as sensitive cellular targets. These findings are consistent with epidemiologic reports that aromatic hydrocarbons result in glomerulonephritis.

TCDD has also been shown to have a number of effects on the immune systems of laboratory animals. Studies in mice, rats, guinea pigs, and monkeys indicated 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 suppressed cell-mediated immunity, primarily by affecting the T-cell arm of the immune response. It is not known whether TCDD directly affects T-cells. TCDD may indirectly affect T-cells and cell-mediated immunity by altering thymus gland function or cytokine production. The generation of antibodies by B cells, an indication of humoral immunity, may also be affected by TCDD.

Increased susceptibility to infectious disease has been reported following TCDD administration. In addition, TCDD increased the number of tumors that formed in mice following injection of tumor cells. It should be emphasized,

however, that very little change to the overall immune competence of the intact animal has been reported.

Despite considerable laboratory research, the mechanisms underlying the immunotoxic effects of TCDD are still unclear. TCDD immunotoxicity appears to be primarily mediated through Ah-receptor-dependent processes, but some components of immunosuppression have been shown to act independently of the Ah receptor. Some studies indicate that an animal's hormonal status may contribute to its sensitivity to immunotoxicity. 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.

Generally, TCDD has a wide range of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells. These effects may influence the formation of noncancer health disorders. Studies in animals indicate that some TCDD effects are mediated through the Ah-receptor, a protein in animal and human cells to which TCDD can bind. It is hypothesized that TCDD, together with the AhR, can interact with sites on DNA and alter the information obtained from DNA in a way that transforms normal cells into abnormal cells. Although structural differences in the AhR have been identified, this receptor operates in a similar manner in animals and humans. Evidence has also begun to accumulate for non-Ah receptor mediated effects.

TCDD has been shown to induce differentiation in human keratinocytes, which may be initiated by TCDD binding to the AhR. This effect is antagonized by retinoids and may involve interactions between TCDD and retinoids in the regulation of epithelial differentiation. TCDD has been reported to decrease an acidic type I Keratin involved in epidermal development, leading to keratinocyte hyperproliferation and skin irritations such as chloracne.

Limited information is available on health effects of the herbicides discussed in the report. These herbicides, however, have 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 was reported in animals exposed to 2,4-D and cacodylic acid. Exposure to 2,4-D has also been associated with effects on blood, such as reduced levels of heme and red blood cells. Cacodylic acid was reported to induce renal lesions in rats. Other studies provide evidence that 2,4-D binds covalently to hepatic proteins and lipids; the molecular basis of this interaction and its biologic consequences are unknown.

The potential immunotoxicity of the herbicides used in Vietnam has been studied to only a very limited extent. Effects on the immune system of mice were 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 (i.e., to produce an allergic response on the skin) was tested, but other aspects of immunotoxicology were not examined.

The foregoing evidence suggests that a connection between TCDD or herbicide exposure and human health effects is, in general, biologically plausible. However, differences in sensitivity and susceptibility across individual animals, strains, and species; lack of strong evidence of organ-specific effects across species; and differences in route, dose, duration, and timing of exposure complicate any more definitive conclusions about the presence or absence of a mechanism for the induction of specific noncancer health disorders.

Considerable uncertainty remains over how to apply this information to the evaluation of potential health effects of herbicides or dioxin exposure in Vietnam veterans. Scientists disagree over the extent to which information derived from animals and cellular studies predicts human health outcomes, and the extent to which the health effects resulting from high-dose exposure are comparable to those resulting from low-dose exposure. Research on biological mechanisms is burgeoning, and subsequent Veterans and Agent Orange updates may have more and better information on which to base conclusions.

Increased Risk of Disease Among Vietnam Veterans

Under the Agent Orange Act of 1991, the committee is asked to determine (to the extent that available scientific data permit meaningful determinations) the increased risk of the diseases it studies among those exposed to herbicides during their service in Vietnam. Chapter 1 presents the committee's general findings regarding this charge. Where more specific information about particular health outcomes is available, this information is related in the preceding discussions of those diseases.

REFERENCES

Air Force Health Study (AFHS). 1984. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Baseline Morbidity Study Results. Brooks AFB, TX: USAF School of Aerospace Medicine. NTIS AD-A138 340.

Air Force Health Study. 1990. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. 2 vols. Brooks AFB, TX: USAF School of Aerospace Medicine. USAFSAM-TR-90-2.

Air Force Health Study. 1991. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Serum Dioxin Analysis of 1987 Examination Results. 9 vols. Brooks AFB, TX: USAF School of Aerospace Medicine.

Air Force Health Study. 1992. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Reproductive Outcomes. Brooks AFB, TX: Armstrong Laboratory. AL-TR-1992-0090.

Air Force Health Study. 1995. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. 1992 Followup Examination Results. 10 vols. Brooks AFB, TX: Epidemiologic Research Division. Armstrong Laboratory.

Air Force Health Study. 1996. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Mortality Update 1996. Brooks AFB, TX: Epidemiologic Research Division. Armstrong Laboratory. AL/AO-TR-1996-0068.

American Diabetes Association (ADA). 1997. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 20(7):1183.

Assennato G, Cervino D, Emmett E, Longo G, Merlo F. 1989. Follow-up of subjects who developed chloracne following TCDD exposure at Seveso. American Journal of Industrial Medicine 16:119–125.

Bagchi D, Bagchi M, Tang L, Stohs SJ. 1997. Comparative in vitro and in vivo protein kinase C activation by selected pesticides and transition metal salts. Toxicology Letters 91(1):31–37.

Becher H, Flesch-Janys D, Kauppinen T, Kogevinas M, Steindorf K, Manz A, Wahrendorf J. 1996. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. Cancer Causes and Control 7(3):312–321.

Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. 1989. Mortality in an area contaminated by TCDD following an industrial incident. Medicina Del Lavoro 80:316–329.

Bullman TA, Kang HK. 1996. The risk of suicide among wounded Vietnam veterans. American Journal of Public Health 86(5):662–667.

Calvert GM, Hornung RV, Sweeney MH, Fingerhut MA, Halperin WE. 1992. Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. Journal of the American Medical Association 267:2209–2214.

Calvert GM, Sweeney MH, Fingerhut MA, Hornung RW, Halperin WE. 1994. Evaluation of porphyria cutanea tarda in U.S. workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. American Journal of Industrial Medicine 25:559–571.

Calvert GM, Willie KK, Sweeney MH, Fingerhut MA, Halperin WE. 1996. Evaluation of serum lipid concentrations among U.S. workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Archives of Environmental Health 51(2):100–107.

Cantoni L, Dal Fiume D, Ruggieri R. 1984. Decarboxylation of uroporphyrinogen I and III in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced porphyria in mice. International Journal of Biochemistry 16:561–565.

Centers for Disease Control (CDC). 1988. Health status of Vietnam veterans. II. Physical health. Journal of the American Medical Association 259:2708–2714.

Chinh TT, Phi PT, Thuy NT. 1996. Sperm auto-antibodies and anti-nuclear antigen antibodies in chronic dioxin-exposed veterans. Chemosphere 32(3):525–530.

Cook RR, Bond GG, Olson RA, Ott MG. 1987. Update of the mortality experience of workers exposed to chlorinated dioxins. Chemosphere 16:2111–2116.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997a. Mortality of Vietnam Veterans: The Veteran Cohort Study: A Report of the 1996 Retrospective Cohort Study of Australian Vietnam Veterans. Canberra: Department of Veterans' Affairs.

Crane PJ, Barnard DL, Horsley KW, Adena MA. 1997b. Mortality of National Service Vietnam Veterans: A Report of the 1996 Retrospective Cohort Study of Australian Vietnam Veterans. Canberra: Department of Veterans' Affairs.

Dalager NA, Kang HK. 1997. Mortality among Army Chemical Corps Vietnam veterans. American Journal of Industrial Medicine 31(6):719–726.

De Verneuil H, Sassa S, Kappas A. 1983. Effects of polychlorinated biphenyl compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, phenobarbital and iron on hepatic uroporphyrinogen decarboxylase. Implications for the pathogenesis of porphyria. Biochemical Journal 214:145–151.

Drash A, Cho N, Tajima N, Rewers M, LaPorte R. 1989. The epidemiology of diabetes in childhood with special reference to the Orient: implications for mechanism of beta cell damage. Indian Journal of Pediatrics 56(Suppl 1):S15–S32.

Enan E, Matsumura F. 1993. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin induced alterations in protein phosphorylation in guinea pig adipose tissue. Journal of Biochemical Toxicology 8(2):89–99.

Enan E, Matsumura F. 1994. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)-induced changes in glucose transporting activity in guinea pigs, mice, and rats in vivo and in vitro. Journal of Biochemical Toxicology 9(2):97–106.

Enan E, Lasley B, Stewart D, Overstreet J, Vandevoort CA. 1996. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) modulates function of human luteinizing granulosa cells via cAMP signaling and early reduction of glucose transporting activity. Reproductive Toxicology 10(3):191–198.

Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F. 1996. Immunological changes among farmers exposed to phenoxy herbicides: Preliminary observations. Occupational and Environmental Medicine 53(9):583–585.

Flanders WD, Lin L, Pirkle JL, Caudill SP. 1992. Assessing the direction of causality in cross-sectional studies. American Journal of Epidemiology 135:926–935.

Flesch-Janys D, Berger J, Gurn P, Manz A, Nagel S, Waltsgott H, Dwyer. 1995. Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. American Journal of Epidemiology 142(11):1165–1175.

Gambini GF, Mantovani C, Pira E, Piolatto PG, Negri E. 1997. Cancer mortality among rice growers in Novara Province, Northern Italy. American Journal of Industrial Medicine 31(4):435–441.

Grossman ME, Poh-Fitzpatrick MB. 1986. Porphyria cutanea tarda: diagnosis, management, and differentiation from other hepatic porphyrias. Dermatologic Clinics 4:297–309.

Henneberger PK, Ferris BG Jr, Monson RR. 1989. Mortality among pulp and paper workers in Berlin, New Hampshire. British Journal of Industrial Medicine 46:658–664.

Henriksen GL, Ketchum NS, Michalek JE, Swaby JA. 1997. Serum dioxin and diabetes mellitus in veterans of operation ranchhand. Epidemiology 8(3):252–258.

Institute of Medicine (IOM). 1994. Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam. Washington, DC: National Academy Press.

Institute of Medicine. 1996. Veterans and Agent Orange: Update 1996. Washington, DC: National Academy Press.

Jung D, Konietzko J, Reill-Konietzko G, Muttray A, Zimmermann-Holz HJ, Doss M, Beck H, Edler L, Kopp-Schneider A. 1994. Porphyrin studies in TCDD-exposed workers. Archives of Toxicology 68:595–598.

Kenny SJ, Aubert RE, and Geiss LS. 1995. Appendix 4.5, Chapter 4. Diabetes in America, 2nd Edition. Harris MI, ed. Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases. NIH Publication No. 95-1468.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M, Green L, Kauppinen T, Littorin M, Lynge E, Mathews JD, Neuberger M, Pearce N, Saracci R. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. American Journal of Epidemiology 145(12):1061–1075.

Kruszynska YT, Olefsky JM. 1996. Cellular and molecular mechanisms of non-insulin dependent diabetes mellitus. Journal of Investigative Medicine 44(8):413–428

Kuller LH, Orchard TJ. 1988. The epidemiology of atherosclerosis in 1987: Unraveling a common-source epidemic. Clinical Chemistry 34(8B):B40–B48

LaRosa JC. 1990. Lipid Disorders. Endiocrinology and Metabolism Clinics of North America. Philadelphia: WB Saunders Company.

Liu PC, Matsumura F. 1995. Differential effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the "adipose-type" and "brain-type" glucose transporters in mice. Molecular Pharmacology 47(1):65–73.

Liu H, Safe S. 1996. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on insulin-induced responses in MCF-7 human breast cancer cells. Toxicology and Applied Pharmacology 138(2): 242–250.

Lovik M, Johansen HR, Gaarder PI, Becher G, Aaberge IS, Gdynia W, Alexander J. 1996. Halogenated organic compounds and the human immune system: preliminary report on a study in hobby fishermen. Archives of Toxicology Supplement 18:15–20.

Martin JV. 1984. Lipid abnormalities in workers exposed to dioxin. British Journal of Industrial Medicine 41:254–256.

Matsumura F. 1995. Mechanism of action of dioxin-type chemicals, pesticides, and other xenobiotics affecting nutritional indexes. American Journal of Clinical Nutrition 61(3 Suppl):695S–701S.

May G. 1982. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. British Journal of Industrial Medicine 39:128–135.

McKinney WP, McIntire DD, Carmody TJ, Joseph A. 1997. Comparing the smoking behavior of veterans and nonveterans. Public Health Reports 112(3):212–217.

Michalek JE, Ketchum NS. 1997. Personal Communication. Subject: Answers to Questions Regarding Dioxin and Diabetes Mellitus posed by the Committee to Review the Health Effects of Exposure to Herbicides in Vietnam Veterans. Population Research Branch, Epidemiologic Research Division, Armstrong Laboratory, Brooks Air Force Base, Texas. October 15.

Mocarelli P, Marocchi A, Brambilla P, Gerthoux P, Young DS, Mantel N. 1986. Clinical laboratory manifestations of exposure to dioxin in children. A six-year study of the effects of an environmental disaster near Seveso, Italy. Journal of the American Medical Association 256:2687–2695.

Moses M, Lilis R, Crow KD, Thornton J, Fischbein A, Anderson HA, Selikoff IJ. 1984. Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. American Journal of Industrial Medicine 5:161–182.

Muhlbauer JE, Pathak MA. 1979. Porphyria cutanea tarda. International Journal of Dermatology 18:767–780.

National Diabetes Data Group. 1979. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057.

O'Toole BI, Marshall RP, Grayson DA, Schureck RJ, Dobson M, Ffrench M, Pulvertaft B, Meldrum L, Bolton J, Vennard J. 1996. The Australian Vietnam Veterans Health Study: II. Self-reported health of veterans compared with the Australian population. International Journal of Epidemiology 25(2):319–330.

Odom R. 1993. Dermatological Disorders in Vietnam Veterans. Presentation to the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. February 8, 1993.

Orchard TJ, LaPorte RE, Dorman JS. 1992. Diabetes. In: Last JM, Wallace RB, eds., Public Health and Preventive Medicine, 13th Ed. Stamford, Conn.: Appleton and Lange. Chapter 51:873–883.

Ott MG, Zober A. 1996. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occuptional and Environmental Medicine 53(9): 606–612.

Ott MG, Olson RA, Cook RR, Bond GG. 1987. Cohort mortality study of chemical workers with potential exposure to the higher chlorinated dioxins. Journal of Occupational Medicine 29:422–429.

Ott MG, Zober A, Germann C. 1994. Laboratory results for selected target organs in 138 individuals occupationally exposed to TCDD. Chemosphere 29:2423–2437.

Paul W, ed. 1993. Fundamental Immunology, 3rd ed. New York: Raven Press.

Pazderova-Vejlupkova J, Lukas E, Nemcova M, Pickova J, Jirasek L. 1981. The development and prognosis of chronic intoxication by tetrachlorodibenzo-*p*-dioxin in men. Archives of Environmental Health 36:5–11.

Ramlow JM, Spadacene NW, Hoag SR, Stafford BA, Cartmill JB, Lerner PJ. 1996. Mortality in a cohort of pentachlorophenol manufacturing workers, 1940–1989. American Journal of Industrial Medicine 30(2):180–194.

Smith AG, De Matteis F. 1990. Oxidative injury mediated by the hepatic cytochrome P-450 system in conjunction with cellular iron. Effects on the pathway of haem biosynthesis. Xenobiotica 20:865–877.

Soues S, Fernandez N, Souverain P, Lesca P. 1989. Intracellular lipoproteins as carriers for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzo(a)pyrene in rat and mouse liver. Biochemical Pharmacology 38(17):2841–2847.

Suskind RR, Hertzberg VS. 1984. Human health effects of 2,4,5-T and its toxic contaminants. Journal of the American Medical Association 251:2372–2380.

Svensson BG, Mikoczy Z, Stromberg U, Hagmar L. 1995. Mortality and cancer incidence among swedish fishermen with a high dietary intake of persistent organochlorine compounds. Scandinavian Journal of Work, Environment and Health 21(2):106–115.

Sweeney MH, Hornung RW, Wall DK, Fingerhut MA, Halperin WE. 1992. Diabetes and serum glucose levels in TCDD-exposed workers. Abstract of a paper presented at the 12th International Symposium on Chlorinated Dioxins (Dioxin '92), Tampere, Finland, August 24–28.

Sweeney MH, Calvert G, Egeland GA, Fingerhut MA, Halperin WE, Piacitelli. 1996. Review and update of the results of the NIOSH medical study of workers exposed to chemicals contaminated with 2,3,7,8-tetrachlorodibenzodioxin. Presented at the symposium, Dioxin Exposure and Human Health—An Update, June 17, Berlin, Germany.

Sweeney MH, Calvert GM, Egeland GA, Fingerhut MA, Halperin WE, Piacitelli LA. 1997. Review and update of the results of the NIOSH medical study of workers exposed to chemicals contaminated with 2,3,7,8-tetrachlorodibenzodioxin. Teratogenesis, Carcinogenesis, and Mutagenesis 17(4–5):241–247.

Sweeney GD. 1986. Porphyria cutanea tarda, or the uroporphyrinogen decarboxylase deficiency diseases. Clinical Biochemistry 19:3–15.

Tonn T, Esser C, Schneider EM, Steinmann-Steiner-Haldenstatt W, Gleichmann E. 1996. Persistence of decreased T-helper cell function in industrial workers 20 years after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environmental Health Perspectives 104(4):422–426.

Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. 1993. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 42(2):359–362.

Visintainer PF, Barone M, McGee H, Peterson EL. 1995. Proportionate mortality study of Vietnam-era veterans of Michigan. Journal of Occupational and Environmental Medicine 37(4):423–428.

Von Benner A, Edler L, Mayer K, Zober A. 1994. 'Dioxin' investigation program of the chemical industry professional association. Arbeitsmedizin Sozialmedizin Praventivmedizin 29:11–16.

Watanabe KK, Kang HK. 1995. Military service in Vietnam and the risk of death from trauma and selected cancers. Annals of Epidemiology 5(5):407–412.

Watanabe KK, Kang HK. 1996. Mortality patterns among Vietnam veterans: a 24-year retrospective analysis. Journal of Occupational and Environmental Medicine 38(3):272–278.

Weber TJ, Ou X, Merchant M, Wang X, Safe SH, Ramos KS. 1994. Biphasic modulation of protein kinase C (PKC) activity by polychlorinated dibenzo-*p*-dioxins (PCDDs) in serum-deprived rat aortic smooth muscle cells. Journal of Biochemical Toxicology 9(3):113–120.

Weber TJ, Chapkin RS, Davidson LA, Ramos KS. 1996. Modulation of protein kinase C-related signal transduction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exhibits cell cycle dependence. Archives of Biochemistry and Biophysics 328(2):227–232.

Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A, Hooijkaas H, and Sauer PJ. 1995. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatric Research 38(3):404–410.

Wolf N, Karmaus W. 1995. Effects of inhalative exposure to dioxins in wood preservatives on cell-mediated immunity in day-care center teachers. Environmental Research 68(2):96–105.

Wolfe WH, Michalek JE, Miner JC, Rahe A, Silva J, Thomas WF, Grubbs WD, Lustik MB, Karrison TG, Roegner RH, Williams DE. 1990. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. I. Physical health. Journal of the American Medical Association 264:1824–1831.

World Health Organization. 1980. WHO Expert Committee on Diabetes Mellitus (Tech. Rep. Ser., No. 646). Second Report. Geneva: World Health Organization.

World Health Organization. 1985. Diabetes Mellitus: Report of a WHO Study Group (Tech. Rep. Ser., No. 727). Geneva: World Health Organization.

Zober A, Ott MG, Messerer P. 1994. Morbidity follow up study of BASF employees exposed to 2,3,7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) after a 1953 chemical reactor incident. Occupational and Environmental Medicine 51:479–486.

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. Appendix A of the 1994 report contains a complete description of the methods used to perform literature searches, including a discussion of databases and keywords. Because this report focuses on reviewing literature published since the completion of *Veterans and Agent Orange: Update 1996*, databases were searched for articles published between 1995 and 1997. Indexing and critiquing of all other epidemiologic studies was performed by the committee with the support of Institute of Medicine staff.

ORAL AND WRITTEN TESTIMONY PRESENTED TO THE COMMITTEE

Public meetings were held on June 19, 1997, in Washington, D.C., and on October 20, 1997, in Irvine, California, to solicit scientific information on the health effects of exposure to dioxin and other chemical compounds in the herbicides used in Vietnam during the Vietnam era. In order to reach a broad range of interested individuals, notices of the meeting were sent to members of veterans' organizations, Congress, government agencies, academic institutions, environmental groups, chemical companies, the pulp and paper industry, medical research associations, and other groups, as well as to those who attended the previous committees' public meetings in September 1992 and April 1995. At the 1997

public meetings, which were attended by approximately 50 people, 17 individuals presented oral testimony. Written testimony was also received and was accorded the same weight as the oral testimony.

Oral Testimony Presented at the Public Meetings

Gerald Bender, Minnesota Department of Veterans Affairs, St. Paul
Turner Camp, Veterans of Foreign Wars, Washington, DC
George Clark, Xenobiotic Detection Systems, Durham, NC
Le Cao Dai, Hanoi University Medical School, Hanoi, Vietnam
Michael Eckstein, Vietnam Veterans of America, Stanhope, NJ; also representing the New Jersey Agent Orange Commission
Julio Gonzales, Vietnam Veterans of America, Chicago
Thomas Joyce, Vietnam Veterans of America, Friedens, PA
Jenny LeFevre, Shady Side, MD
Betty Mekdeci, Association of Birth Defect Children, Orlando, FL
Charles Outlaw, Bloomfield, CT
Arnold Schecter, State University of New York at Binghamton
Linda Schwartz, Pawcatuck, CT
Garold Schwartzenberger, Silverado, CA
John Patrick Sherlock, Levittown, PA
Michael Sovick, The Oklahoma Agent Orange Foundation, Norman; also presented on behalf of the Desert Storm Justice Foundation, Oklahoma City
Charles Stone, Stone and Associates, Silver Spring, MD
Kelli Willard West, Vietnam Veterans of America, Washington, DC

Written Testimony Presented to the Committee

Turner Camp, Veterans of Foreign Wars Medical Consultant, Washington, DC
Topic: Strengths and weaknesses of Ranch Hands studies, particularly as they relate to diabetes and spina bifida.

Richard Clapp, Boston University, Boston, MA
Topic: Provided the committee with a document for its review.

William Dean, Vietnam Veteran, Apple Valley, MN
Topic: Personal account of health problems, including multiple bilateral fibromatosis tumors (MBF), which he believes to be related to his exposure to Agent Orange. Mr. Dean also provided the results of his research on exposures that may be related to MBF and requested that the Department of Veterans Affairs (DVA) compensate Vietnam veterans for benign soft-tissue tumors, including Dupuytrens contractures.

Patrick Dockery, Baltimore, MD

Topic: Research regarding the health effects of exposure to trace contaminants of the herbicides used in Vietnam and the synergistic impact of multiple exposures.

Albert Donnay, Baltimore, MD

Topic: Evidence of relationship between Agent Orange or dioxin exposure and porphyrin disorders, specifically porphyria cutanea tarda (PCT).

Pedro Gamboa, Puerto Real, Puerto Rico

Topic: Provided the committee with a document for its review.

Julio Gonzales, Vietnam Veterans of America, Chicago, IL

Topic: Provided the committee with documents for its review.

Rockne Harmon, Vietnam Veteran, Alameda, CA

Topic: Personal account of the health problems of his child, which he believes to be related to exposure to Agent Orange. Mr. Harmon is also requesting that the committee more closely examine the issue of health effects in the children of Vietnam veterans.

Harold Jackson, Vietnam Veteran, Houston, TX

Topic: Personal account of health problems, including peripheral neuropathy and autoimmune disease, that he believes to be related to Agent Orange exposure.

Ross Jones, Texas Instruments, Dallas, TX

Topic: Provided the committee with a document for its review.

George Losoncy, Vietnam Veteran, Temple, PA

Topic: Personal account of health problems, including alopecia, which he feels are related to exposure to Agent Orange. Mr. Losoncy is also requesting that the committee look closely at the relationship between exposure to Agent Orange and autoimmune disease and hair loss.

John Martignetti, Vietnam Veteran, Bethlehem, NH

Topic: Personal account of health problems, including chloracne and multiple sclerosis, which he believes to be related to Agent Orange exposure.

Betty Mekdeci, Association of Birth Defect Children, Orlando, FL

Topic: Results of research into incidence rates of conditions in children of Vietnam veterans compared to children of non-veterans.

Gerald Pierce, Vietnam Veteran, Sierra Vista, AZ
Topic: Mr. Pierce wrote to express his frustration with the federal government's handling of the Agent Orange issue.

Garold Schwartzenberger, Silverado, CA
Topic: Results of personal research on genetic mutations and Agent Orange.

Claire Simon, Wife of Vietnam Veteran, Newport News, VA
Topic: Personal account of Vietnam veteran Robert Simon's health problems and eventual death from cardiac failure, which she believes are related to exposure to Agent Orange.

Carol Solberg, Wife of Vietnam Veteran, Grand Forks, MN
Topic: Personal account of her husband's chondrosarcoma and their son's primary immune deficiency, both of which she believes are related to her husband's exposure to Agent Orange. She also provided information on soft-tissue sarcomas.

Hope Tinoco, Wife of Vietnam Veteran, Olathe, KS
Topic: Personal account of Vietnam veteran John Tinoco's health problems, including autoimmune disease and peripheral neuropathy, as well as the health problems of their son, both of which she believes to be related to exposure to Agent Orange.

Kelli Willard West, Vietnam Veterans of America, Washington, DC
Topic: Provided the committee with documents for its review and praised the credibility the committee's work has provided to the DVA's benefits programs.

Shelia Winsett, Friend of Vietnam Veteran, Jasper, AL
Topic: Personal account of Vietnam veteran Gary Jacks' health problems and eventual death from cardiac failure, which she believes to be related to exposure to Agent Orange.

E. R. Zumwalt, Admiral, U.S. Navy (Ret.), Arlington, VA
Topic: Provided the committee with several published reports for its review, and called the committee's attention to the Environmental Protection Agency's draft reassessment of dioxin. Admiral Zumwalt also expressed his belief in the importance of beginning research in Vietnam on the effects of Agent Orange.

Appendix B

ICD·9 Codes for Cancer Outcomes

The International Classification of Diseases, Ninth Edition (ICD·9) is a system used by physicians and researchers around the world to group related disease entities and procedures for the reporting of statistical information. It is used for the purposes of classifying morbidity and mortality information for statistical purposes, indexing hospital records by disease and operations, reporting diagnosis by physicians, data storage and retrieval, reporting national morbidity and mortality data, and reporting and compiling of health care data. Many of the studies reviewed by the committee use ICD·9 classifications. The following table lists the codes for the various forms of cancer.

Surveillance, Epidemiology, and End Results (SEER) Program Site Groupings for ICD·9 National Center for Health Statistics (NCHS) Data

Site	ICD·9 Codes
Buccal cavity and pharynx	
Lip	140.0–140.9
Tongue	141.0–141.9
Salivary glands	142.0–142.9
Floor of mouth	144.0–144.9
Gum and other mouth	143.0–143.9, 145.0–145.6, 145.8–145.9
Nasopharynx	147.0–147.9
Tonsil	146.0–146.2
Oropharynx	146.3–146.9
Hypopharynx	148.0–148.9
Other buccal cavity and pharynx	149.0–149.9

continued

Continued

Site	ICD·9 Codes
Digestive system	
Esophagus	150.0–150.9
Stomach	151.0–151.9
Small intestine	152.0–152.9
Colon excluding rectum	153.0–153.9, 159.0
Rectum and rectosigmoid	154.0–154.1
Anus, anal canal and anorectum	154.2–154.3, 154.8
Liver and intrahepatic bile duct	
Liver	155.0, 155.2
Intrahepatic bile duct	155.1
Gallbladder	156.0
Other biliary	156.1–156.9
Pancreas	157.0–157.9
Retroperitoneum	158.0
Peritoneum, omentum and mesentery	158.8–158.9
Other digestive organs	159.8–159.9
Respiratory system	
Nasal cavity, middle ear and accessory sinuses	160.0–160.9
Larynx	161.0–161.9
Lung and bronchus	162.2–162.9
Pleura	163.0–163.9
Trachea, mediastinum and other respiratory organs	162.0, 164.2–165.9
Bones and joints	170.0–170.9
Soft tissue (including heart)	171.0–171.9, 164.1
Skin	
Melanomas—skin	172.0–172.9
Other non-epithelial skin	173.0–173.9
Breast	174.0–174.9, 175._
Female genital system	
Cervix	180.0–180.9
Corpus	182.0–182.1, 182.8
Uterus, NOS	179._
Ovary	183.0
Vagina	184.0
Vulva	184.1–184.4
Other female genital organs	181._, 183.2–183.9, 184.8, 184.9
Male genital system	
Prostate	185._
Testis	186.0–186.9
Penis	187.1–187.4
Other male genital organs	187.5–187.9

Continued

Site	ICD·9 Codes
Urinary system	
Urinary bladder	188.0–188.9
Kidney and renal pelvis	189.0, 189.1
Ureter	189.2
Other urinary organs	189.3–189.4, 189.8–189.9
Eye and orbit	190.0–190.9
Brain and other nervous system	
Brain	191.0–191.9
Other nervous system	192.0–192.3, 192.8–192.9
Endocrine system	
Thyroid	193._
Other endocrine (including thymus)	164.0, 194.0–194.9
Lymphomas	
Hodgkin's disease	201.0–201.9
Non-Hodgkin's lymphomas	200.0–200.8, 202.0–202.2, 202.8–202.9
Multiple myeloma	203.0, 203.2–203.8
Leukemias	
Lymphocytic	
Acute lymphocytic	204.0
Chronic lymphocytic	204.1
Other lymphocytic	204.2–204.9
Granulocytic (myeloid)	
Acute myeloid	205.0
Chronic myeloid	205.1
Other myeloid	205.2–205.9
Monocytic	
Acute monocytic	206.0
Chronic monocytic	206.1
Other monocytic	206.2–206.9
Other	
Other acute	207.0, 208.0
Other chronic	207.1, 208.1
Aleukemic, subleukemic and NOS	202.4, 203.1, 207.2, 207.8, 208.2–208.9
Ill-defined and unspecified sites	159.1, 195.0–195.8, 196.0–196.9, 199.0–199.1, 202.3, 202.5–202.6

NOTE: NOS = not otherwise specified.
SOURCE: Table A-4 in Ries LAG, Kosary CL, Hankey BF, Miller BA, Harras A, Edwards BK (eds). SEER Cancer Statistics Review, 1973–1994, National Cancer Institute. NIH Pub. No. 97-2789. Bethesda, MD, 1997.

Appendix C

Committee and Staff Biographies

COMMITTEE BIOGRAPHIES

David Tollerud, M.D., M.P.H. (*Chairman*), is Professor of Public Health, Medicine, and Community and Preventative Medicine, and Director of the Center for Environmental and Occupational Health at MCP Hahnemann University. 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, the American Public Health Association, and the American Association for the Advancement of Science, and a member of the editorial review board of the *American Industrial Hygiene Association Journal*.

Michael Aminoff, M.D., is Professor of Neurology, Director of the Clinical Neurophysiology Laboratories, and Director of the Movement Disorders Clinic and the Epilepsy Program at the University of California Medical Center, San Francisco. He has published extensively on topics related to clinical neurology

and neurophysiology, has authored or edited 15 textbooks, is on the editorial board of several medical and scientific journals, and is Editor of the journal *Muscle & Nerve*.

Steven Goodman, M.D., M.H.S., Ph.D., is an Associate Professor of Oncology, Pediatrics, Epidemiology and Biostatistics at the Johns Hopkins School of Medicine. He trained in Pediatrics at Washington University, and received degrees in Biostatistics and Epidemiology in 1989 from Johns Hopkins University, where he is currently in the Oncology Center's Division of Biostatistics. As statistician for the Hopkins Oncology Center, General Clinical Research Center and Pediatric Clinical Research Unit, he has participated in the design and analysis of a wide range of clinical and epidemiologic studies. He has served as Statistical Editor at the *Annals of Internal Medicine* since 1987, and been on a variety of NIH committees. His research interests include meta-analysis, statistical inference, the ethics of clinical trials, and the use of likelihood and Bayesian methodology in clinical research.

Robert F. Herrick, Sc.D., is a Lecturer on Industrial Hygiene at the Harvard School of Public Health. His educational background includes a BA degree in Chemistry from the College of Wooster, a MS in Environmental Health Science from the University of Michigan, and a Doctor of Science in Industrial Hygiene from the Harvard School of Public Health. He is certified in the comprehensive practice of industrial hygiene. His research interests are centered on the assessment of exposure as a cause of occupational and environmental disease. He has conducted research on the development of methods to measure the biologically active characteristics of reactive aerosols, and on studies of work processes in the construction and foundry industries to develop task-based models to identify and control the primary sources of worker exposures. Dr. Herrick is Past Chair of the American Conference of Governmental Hygienists (ACGIH), and Past President of the International Occupational Hygiene Association. He is active in the Association's mentor program which facilitates training for occupational hygienists in industrializing countries. Prior to joining the faculty at the Harvard School of Public Health, Dr. Herrick spent 17 years at the National Institute for Occupational Safety and Health (NIOSH) where he conducted occupational health research.

Irva Hertz-Picciotto, Ph.D., is Associate Professor in the Department of Epidemiology, School of Public Health, at the University of North Carolina, Chapel Hill. She received her Ph.D. in epidemiology from the University of California at Berkeley. She is a member of several professional societies, including the International Society for Environmental Epidemiology (ISEE), for which she hosted the 1994 Annual Meeting, and currently serves as Councillor. She also serves on the editorial boards of the *American Journal of Epidemiology*, *Epidemiology*, and

Human and Ecological Risk Assessment. She has published extensively on several topic areas, including risk assessment, occupationally related cancer, environmental exposures, reproductive outcomes, and methods for epidemiologic data analysis. Her primary research interests are in the area of environmental chemical exposures and their effects on pregnancy, young children, and other susceptible populations. She has also been involved in development of risk assessments using epidemiologic data, comparisons of reproductive toxicity and carcinogenic potency between animals and humans, and methodologic issues such as dose–response analysis and techniques for standardization.

David G. Hoel, Ph.D., received his Ph.D. from the University of North Carolina at Chapel Hill and has more than 25 years of experience as a biostatistician, toxicologist and environmental health researcher. Dr. Hoel currently holds the position of Distinguished University Professor and Associate Director of the Hollings Oncology Center at the Medical University of South Carolina. Before joining the Medical University of South Carolina, he held administrative positions at the National Institute of Environmental Health Sciences where he was most recently the Director of the Division of Biometry and Risk Assessment. Internationally, Dr. Hoel has been a member of the United States/Japan Cooperative Medical Science Program and also a member of numerous working groups of the International Agency for Cancer Research of the World Health Organization.

Andrew Olshan, Ph.D., is Associate Professor in the Department of Epidemiology, School of Public Health, at the University of North Carolina, Chapel Hill. He received his Ph.D. in epidemiology from the University of Washington. He was a postdoctoral fellow in medical genetics at the University of British Columbia from 1987 to 1989 and Assistant Professor in the Department of Clinical Epidemiology and Family Medicine, University of Pittsburgh, from 1989 to 1991. He is a member of several professional societies, including the Society for Epidemiologic Research, the American Society of Human Genetics, and the Teratology Society. His major areas of interest include cancer and perinatal health in relation to environmental, occupational and genetic factors. He has a particular interest in male-mediated effects on abnormal reproduction and development.

Trevor J. Orchard, MBBCh., MMSc., graduated from the Welsh National School of Medicine in 1974 and underwent further medical and epidemiological training at the university of Nottingham, gaining a Master of Medical Sciences (community health) degree in 1978. After a British Heart Foundation fellowship, he moved to the United States to work in the epidemiology department at the University of Pittsburgh, in 1979 where he is currently a professor of epidemiology, pediatrics, and medicine. His main interests have been in cardiovascular and diabetes epidemiology and the clinical management of lipid disorders.

Howard Ozer, M.D., Ph.D., is an Associate Professor of Medicine at MCP Hahnemann University and Director of the Allegheny Cancer Center, Philadelphia. Prior to accepting this appointment, he served as Chair and Director of the Winship Cancer Center at Emory University and as a member of the faculty at the University of North Carolina.

Kenneth S. Ramos, Ph.D., is Professor in the Department of Physiology and Pharmacology, College of Veterinary Medicine and Vice- Chairman of the Faculty of Toxicology at Texas A&M University. He also holds joint appointments in the Departments of Medical Physiology and Environmental and Occupational Health at the Texas A&M University Health Sciences Center. Dr. Ramos is a member of several professional societies, including the Society of Toxicology, Society for In Vitro Biology, American Society for Cell Biology, and American Society for Pharmacology and Experimental Therapeutics. He serves in the editorial boards of the *Journal of Biochemical Toxicology*, *Journal of Toxicology and Environmental Health*, *Annual Review of Pharmacology and Toxicology*, *Toxicology* In Vitro, *American Journal of Physiology, Toxicology and Applied Pharmacology*, *Chemico-Biological Interactions*, and *Cell Biology and Toxicology*. His primary research interests are in the area of cellular and molecular toxicology with emphasis on the study of chemically induced deregulation of gene expression, cell differentiation, and somatic growth control.

Noel R. Rose, M.D., Ph.D., is Professor of Pathology and of Molecular Microbiology and Immunology at the Johns Hopkins University and holds joint appointments in the Departments of Medicine and of Environmental Health Sciences. He is also Director of the World Health Organization Collaborating Center for Autoimmune Diseases and of the Johns Hopkins Reference Laboratory. Dr. Rose directs the University's training program in immunotoxicology and is active as a consultant in immunotoxicology. He has also served on panels of the National Institutes of Health, the Food and Drug Administration, the National Center for Toxicological Research, the National Research Council, and other governmental agencies. He is past-president of the Clinical Immunology Society and Editor-in-Chief of the journal *Clinical Immunology and Immunopathology.* Dr. Rose's main area of research is autoimmune disease.

Susan Woskie, Ph.D., C.I.H., is Associate Professor in the Department of Work Environment at the University of Massachusetts, Lowell. She holds a doctoral degree in biomedical science (industrial hygiene) from Clark University and a master's in environmental health from the Harvard School of Public Health. Her research has focused on assessing exposures for epidemiological studies, including exposure assessments in the metalworking and semiconductor industries. She has also studied diesel exhaust exposures among railroad and construction workers, lead exposures in bridge painting, and silica exposures in construction. Dr.

Woskie currently serves on the editorial review board of the *American Industrial Hygiene Association Journal* and is a scientific advisor on the NIOSH/NCI Lung Cancer Mortality Study of Diesel Exposure in Non-Metal Mines.

STAFF

Kathleen Stratton, Ph.D., is the Director of the Division of Health Promotion and Disease Prevention of the Institute of Medicine. She received a Bachelor of Arts degree in Natural Sciences from Johns Hopkins University and Ph.D. from the University of Maryland at Baltimore. After completing a post-doctoral fellowship in neuropharmacology of phencyclidine compounds at the University of Maryland School of Medicine and in neurophysiology of second-messenger systems at the Johns Hopkins University School of Medicine, she joined the staff of the Institute of Medicine in 1990. Dr. Stratton has worked on projects in environmental risk assessment, neurotoxicology, the organization of research and services in the Public Health Service, vaccine safety, fetal alcohol syndrome, and vaccine development. She has had primary responsibility for the reports *Adverse Events Associated with Childhood Vaccines: Evidence Bearing on Causality, DPT Vaccine and Chronic Nervous System Dysfunction; Fetal Alcohol Syndrome: Diagnosis, Epidemiology, Prevention, and Treatment;* and *Vaccines for the 21st Century: A Tool for Decisionmaking*.

David A. Butler, Ph.D., is a Senior Project Officer in the Division of Health Promotion and Disease Prevention of the Institute of Medicine. He received B.S. and M.S. degrees in engineering from the University of Rochester, and a Ph.D. in public policy analysis from Carnegie-Mellon University. Prior to joining the IOM, Dr. Butler served as an analyst for the United States Congress Office of Technology Assessment and was Research Associate in the Department of Environmental Health at the Harvard School of Public Health. He is on the Editorial Advisory Board of the journal *Risk: Health, Safety & Environment.* His research interests include exposure assessment and risk analysis.

Sanjay S. Baliga, M.P.H., was a Research Associate in IOM's Division of Health Promotion and Disease Prevention. He received undergraduate degrees in Biology and Economic Development from Stanford University and an M.P.H. from the University of Michigan. Before joining IOM, Mr. Baliga worked with the policy research group, World Resources Institute, Washington, DC, and with the Naval consulting group, Designers and Planners, Inc., Arlington, VA. His interests focus on risk assessment and management in the context of sustainable development.

James A. Bowers is a Research/Project Assistant in the Division of Health Promotion and Disease Prevention of the Institute of Medicine (IOM). He re-

ceived his undergraduate degree in environmental studies from Binghamton University. He has also been involved with the IOM committees that produced *Characterizing Exposure of Veterans to Agent Orange and Other Herbicides Used in Vietnam*, and *Adequacy of the Comprehensive Clinical Evaluation Program: Nerve Agents*.

Index

Note to the reader: This index contains entries for each of the three volumes of the *Veterans and Agent Orange* series released to date: *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (I)*, Veterans and Agent Orange: Update 1996* (II)*,* and *Veterans and Agent Orange: Update 1998* (III)*.* Page numbers for the discussions of topics in specific volumes follow the roman numerals denoted above. Thus, for example, the entry "Agent Blue, I: 27, 89-90, 93, 97, 100; III: 136, 137" first refers to material found on pages 27, 89-90, 93, 97, and 100 in *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam,* then to material found on pages 136 and 137 of *Veterans and Agent Orange: Update 1998.*

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y