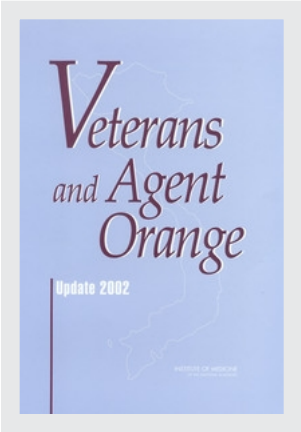

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Veterans and Agent Orange

Update 2002

Committee to Review the Health Effects in
Vietnam Veterans of Exposure to Herbicides
(Fourth Biennial Update)

Board on Health Promotion and Disease Prevention

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Willing is not enough; we must do."
—Goethe

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

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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 NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its 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 review of this report:

Linda S. Birnbaum, U.S. Environmental Protection Agency
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Stephen H. Safe, Texas A&M University
Joseph V. Simone, Simone Consulting
Bailus Walker, Jr., Howard University Cancer Center

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The

review of this report was overseen by **Robert B. Wallace**, University of Iowa. Appointed by the National Research Council and Institute of Medicine, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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 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 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) 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 TCDD 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 second, *Veterans and Agent Orange: Update 1998* (*Update 1998*) was published in 1999. The third, *Veterans and Agent Orange: Update 2000* (*Update 2000*) was published in 2001. The focus of this fourth updated review is on scientific studies published since the release of *Update 2000*. To conduct the review, the IOM established a committee of 10 members representing a wide range of expertise to take a fresh look at the studies reviewed in *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* along with the newest scientific evidence. In order to provide a link to the experience and expertise developed by the previous committees, seven of the members of the committee responsible for this report were recruited from the committee responsible for *Update 2000*. 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 TCDD 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 in April and September of 2002 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 oral presentations and written statements submitted to the committee are described in Appendix A. The committee thanks these individuals who provided valuable insights into the health problems experienced by Vietnam veterans.

In addition to its formal meetings, the committee actively and continuously sought information from, and explained its mission to, a broad array of individuals and organizations with interest or expertise in assessing the effects of exposure to herbicides. The committee also heard from the public through telephone calls, letters, and emails.

Michelle Catlin served as the study director for this project. The committee would also like to acknowledge the excellent work of IOM staff members and temporary staff members Jennifer Cohen, Anna Staton, Elizabeth Albrigo, Jakki

Sears, Jonathon Kossak, and David Butler. Thanks are also extended to Jim Banihashemi, who handled the finances for the project; Norman Grossblatt, who provided excellent editorial skills; William McLeod, who conducted database searches; Jennifer Bitticks, who supervised the report through the editorial and publication phases; and Rita Gaskins, who provided administrative support to the project.

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 Dr. Joel Michalek (Air Force Research Laboratory, Brooks Air Force Base, Texas) for presenting his most recent data at a public session.

Irva Hertz-Picciotto, *Chair*

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Veterans and Agent Orange

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Executive Summary

From 1962 to 1971, US military forces sprayed herbicides over Vietnam to strip the thick jungle canopy that helped conceal opposition forces, to destroy crops that enemy forces might depend on, and to clear tall grasses and bushes from the perimeters of US base camps and outlying fire-support bases. Mixtures of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, and cacodylic acid made up the majority of the herbicides sprayed. The herbicide mixtures used were named according to the color of an identification band painted on the storage drums; one of the main chemical mixtures sprayed was Agent Orange (a 50:50 mixture of 2,4-D and 2,4,5-T). At the time of the spraying, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, one form of dioxin) was an unintended contaminant from the production of 2,4,5-T and was present in Agent Orange and some other formulations sprayed in Vietnam.

In 1991, because of continuing uncertainty about the long-term health effects on Vietnam veterans of the herbicides sprayed, Congress passed Public Law 102-4 (PL 102-4), the Agent Orange Act of 1991. That legislation directed the secretary of veterans affairs to ask the National Academy of Sciences (NAS) to perform a comprehensive 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 those herbicides, including TCDD. The secretary was also to ask that NAS conduct updates at least every 2 years for 10 years from the date of the first report to review newly available literature and draw conclusions from the overall evidence.

In response to the request, the Institute of Medicine (IOM) of NAS convened a committee, whose conclusions IOM published in 1994 in *Veterans and Agent*

Orange: Health Effects of Herbicides Used in Vietnam (hereafter referred to as *VAO*). The work of later committees resulted in the publication of biennial updates (*Update 1996*, *Update 1998*, and *Update 2000*) and focused reports reviewing the scientific evidence regarding type 2 (non-insulin dependent) diabetes (*Type 2 Diabetes*), and acute myelogenous leukemia in children (*Acute Myelogenous Leukemia*). This report is the fourth review of recently published scientific evidence regarding associations between health outcomes and exposure to TCDD and other chemical compounds in herbicides used in Vietnam.

THE CHARGE TO THE COMMITTEE

In accordance with PL 102-4, the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Fourth Biennial Update) was asked "to determine (to the extent that available data permit meaningful determinations)" the following regarding associations between specific health outcomes and exposure to TCDD and other chemical compounds in herbicides:

> A) whether a statistical association with herbicide exposure exists, taking into account the strength of the scientific evidence and the appropriateness of the statistical and epidemiological methods used to detect the association;
>
> B) the increased risk of the disease among those exposed to herbicides during service in the Republic of Vietnam during the Vietnam era; and
>
> C) whether there exists a plausible biological mechanism or other evidence of a causal relationship between herbicide exposure and the disease.

In conducting its study, this committee operated independently of the Department of Veterans Affairs (VA) 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 report provides scientific information for the secretary of veterans affairs to consider as VA exercises its responsibilities to Vietnam veterans.

THE COMMITTEE'S APPROACH TO ITS CHARGE

To fulfill its charge of assessing whether a given human health effect is associated with exposure to at least one of the herbicides or TCDD, the committee concentrated on reviewing and interpreting epidemiologic studies. Experimental investigations that might contribute to biologic plausibility that the chemicals of interest might be related to a given effect were also reviewed. The committee began its evaluation presuming neither the presence nor the absence of associations.

To obtain all information potentially relevant to the evaluation of health effects related to herbicide exposure, the present committee, in addition to re-

viewing studies of Vietnam veterans, reviewed studies of other groups potentially exposed to the herbicides used in Vietnam (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram), other phenoxy herbicides, chlorophenols, and other compounds. Those groups include chemical production and agricultural workers, 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, and residents of Vietnam.

PL 102-4 did not provide a list of specific diseases and conditions suspected of being associated with herbicide exposure. Such a list was developed in *VAO* on the basis of diseases and conditions that had been mentioned in the scientific literature or in other documents identified through extensive literature searches. The *VAO* list has been augmented in response to developments in the literature, requests by VA, and concerns of Vietnam veterans.

The information that the present committee reviewed was identified through a comprehensive search of relevant databases, including public and commercial databases covering biologic, medical, toxicologic, chemical, historical, and regulatory information. Literature identification continued through July 1, 2002. More than 9,000 potentially relevant studies were identified in those searches, and more than 1,000 were reviewed. Input received from veterans and other interested persons at public hearings and in written submissions served as a valuable source of additional information.

To provide data on whether an association between an exposure and a health outcome 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. In deciding the strength of the evidence of an association between herbicide exposure and a particular outcome, the committee examined such estimates of risk and evaluated whether they might be due to error, bias, confounding, or chance, or were likely to represent true associations. The committee recognized that an absolute conclusion about the absence of association may never be attained, because, as is generally the case in science, studies of health outcomes after herbicide exposure are not capable of demonstrating that a purported effect is impossible.

THE COMMITTEE'S EVALUATION

Toxicology Data

Since *Update 2000*, many experimental studies have been published on the herbicides used in Vietnam or their contaminant TCDD. Some of those studies look at particular disease outcomes in animals after exposure to the chemicals, and others focus more on how the chemicals cause effects in cells, tissues, or

animals (the mechanism or mode of action of the chemicals). Toxicologic information on disease outcomes in animals can support a finding that an effect seen in an epidemiologic study is a true effect. Data on the mechanism of toxicity can also add information indicating that an effect is or is not plausible.

Many health effects have been seen in animals after exposure to the herbicides used in Vietnam or their contaminant TCDD. None of those chemicals is thought to act directly by mutating DNA to lead to cancer, but some animal experiments have shown that some of the chemicals can cause some kinds of cancers alone or in conjunction with other treatments. Those effects, and their relevance to human health outcomes, are discussed as part of the biologic plausibility of outcomes.

TCDD is thought to be the most toxic of the chemicals sprayed, and a large amount of recent experimental research has focused on how it causes its effects. Although a great deal is known about the cellular effects of TCDD, the exact mechanism by which it causes the various effects seen in humans and animals remains unknown.

Cacodylic acid is an organic form of arsenic; arsenic has two methyl groups attached to it. It is not known, however, whether the effects observed following exposure to inorganic arsenic are relevant to exposure to cacodylic acid. The present committee therefore did not consider the effects of inorganic arsenic relevant, and the literature on it was not reviewed.

Exposure Assessment

Assessment of human exposure to a chemical is a key element in determining whether specific health outcomes are linked to that chemical. Ideally exposure assessment would characterize the dose at the site of action of a chemical, but in human studies that is rarely possible. Exposure estimates, therefore, should be viewed as surrogates for dose. Many methods of estimating exposure are available for epidemiologic research; different methods have advantages and disadvantages that should be considered when evaluating the results of studies. Exposure can be characterized by measurement of chemical contact at various body barriers (for example, the lungs, skin, gastrointestinal tract). It can also be estimated through measurement of chemicals in biological samples. In epidemiologic studies exposure is often characterized by less quantitative methods such as questionnaires or job titles.

Update of the Scientific Literature

The health outcomes reviewed by the committee are categorized as cancer, reproductive and developmental effects, neurobehavioral disorders, and other health effects. This section briefly summarizes the relevant epidemiologic studies published on those health outcomes since *Update 2000*.

Cancer

Three major epidemiologic studies have been published since *Update 2000* that look at cancer outcomes: a study of residents of Chapaevsk, a Russian industrial community with documented contamination by TCDD and other chemicals; an update of a cohort of Dow Chemical Company workers; and a study of Swedish lumberjacks exposed to pesticides. The study in Russia showed some, mostly small, increases in lung and urinary bladder cancer in males, and nasal–nasopharyngeal, laryngeal, skin, lung, breast, and cervical cancer in females. It is difficult to draw conclusions regarding the association between exposure to the herbicides used in Vietnam and TCDD and health outcomes on the basis of that study because exposures to other chemicals in the Russian town could underlie any effects seen and because of the small number of cases. A small increase in deaths from lymphopoietic cancers was seen in the study of Dow workers, one case of skin cancer and a slight increase in non-Hodgkin's lymphoma were seen in the Swedish lumberjack study; again, the size of the study populations limits their usefulness.

In addition to those cohort studies that looked at numerous cancer end points, some smaller studies that are relevant to the exposures of interest and cancer were conducted. Effects were seen in two: an increase in soft-tissue sarcoma in a cohort living near a chemical factory in northern Italy, and an association between exposure to phenoxy herbicides (such as 2,4-D) and non-Hodgkin's lymphoma in a case–control study in Canada.

Reproductive and Developmental Effects

The study of residents of Chapaevsk, Russia, also looked at reproductive and developmental outcomes. An increase in congenital morphogenetic birth defects and a slight decrease in birth weight were seen. As discussed above, the usefulness of that study for drawing conclusions regarding the association between the exposures of interest and health outcomes is small. Evidence of an increase in birth defects was also seen in the study of US women Vietnam veterans (unspecified birth defects), in a study of residents of an area of Amsterdam thought to be contaminated with TCDD and other chemicals (unspecified birth defects), and in a study of infants in Baltimore and Washington, DC (effects on the developing cardiovascular system).

A study of Ontario farm families showed some indication of an increase incidence of spontaneous abortion in farm families. Some evidence of an increased risk of childhood cancer (neuroblastoma) was seen in a case–control study in New York City.

Other studies reviewed in this report, however, did not demonstrate reproductive or developmental effects of the herbicides used in Vietnam or TCDD. Thus, evidence regarding any effects of those chemicals on reproductive and developmental end points is inconsistent.

Neurobehavioral Disorders

An update of the Air Force Health Study (AFHS), which looked at cognitive effects on people involved in the aerial spraying of Agent Orange (the Ranch Hands) has been published, as has a study of cognitive effects on Czech workers exposed to TCDD during the production of 2,4,5-T. A case–control study of possible factors contributing to Alzheimer's disease, including pesticides, has also been published. Inconsistent effects on cognitive endpoints were seen in the AFHS and the Czech study. No significant relationship was seen between exposure to pesticides and Alzheimer's disease.

The relationship between exposure to pesticides and Parkinson's disease or parkinsonism has been investigated in two studies of agricultural cohorts, in both of which an association was seen: a study of agricultural workers in Iowa and North Carolina and a study of agricultural workers on sugarcane and pineapple plantations in Hawaii. No specific information on herbicide use is available in those studies.

Amyotrophic lateral sclerosis (ALS) was investigated in four case–control studies and in the update of the Dow chemical-plant cohort. An increased relative risk was seen in the Dow cohort on the basis of three cases of ALS, but no statistically significant associations were seen in the case–control studies.

No other consistent neurobehavioral or neurological effects were seen, and there was no consistent increase in chronic peripheral neuropathy in the AFHS.

Other Health Effects

A number of studies have investigated other health effects. The update of the Dow chemical-plant cohort looked at respiratory and circulatory disorders and saw no effects. A cross-sectional survey of residents of rural Saskatchewan did not indicate an increased prevalence of diabetes or circulatory disorders. The update of the AFHS showed an increase in hepatomegaly but no other liver effects. The study of residents of Chapaevsk, Russia, demonstrated an increase in mortality from cardiovascular disease, but the committee questioned the usefulness of that study for this report because of the use of different control populations, the likelihood of other exposures, the lack of individual confounding data, and demographic and socioeconomic confounders. A cross-sectional study that investigated diabetes, lipid and lipoprotein disorders, and circulatory disorders in people working at a municipal waste incinerator in Japan showed an increase in self-reported lipoprotein disorders.

Health-Outcome Conclusions

The present committee weighed the strengths and limitations of all the epidemiologic evidence reviewed in this report and in previous *Veterans and Agent*

Orange reports and reached its conclusions by interpreting the new evidence in the context of the entire body of literature. It assigned each health outcome being considered to one of four categories on the basis of that evidence. The definitions of the categories and the criteria for assigning particular health outcomes to them are described in Table ES-1; the health outcomes assigned to each category are also listed in the table.

The present committee made one change in the categorization of health outcomes in this report compared with the *Update 2000* and *Acute Myelogenous Leukemia* reports. Previously, the evidence of all forms of leukemia was considered together. In this update, however, the committee was asked by the VA to consider whether the evidence of chronic lymphocytic leukemia could be assessed separately from evidence of other forms of leukemia. Because of the available data and the etiology of chronic lymphocytic leukemia, the present committee made conclusions for that leukemia separately. On the basis of all the evidence reviewed, the committee concluded that there is sufficient evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chronic lymphocytic leukemia.

As mandated by PL 102-4, the distinctions between categories are based on statistical association, not on causality. It should be noted that the committee is charged with reviewing the scientific data, not with making recommendations regarding VA policy; therefore, conclusions reported in Table ES-1 are not intended to imply or suggest policy decisions. Furthermore, the conclusions are related to associations 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.

Increased Risk of Disease Among Vietnam Veterans

There have been numerous health studies of Vietnam veterans, but most have been hampered by relatively poor measures of exposure to herbicides or TCDD and by other methodologic problems. In light of those problems, many conclusions regarding associations between exposure to TCDD or herbicides and disease are based on studies of people exposed in occupational and environmental settings rather than on studies of Vietnam veterans. The committee believes that there is sufficient evidence to reach general conclusions about associations between herbicide exposure and the health outcomes, but the lack of adequate data on Vietnam veterans themselves makes it difficult to reach conclusions about increased risk of disease among Vietnam veterans. The lack of data on Vietnam veterans, the large uncertainties about the magnitude of potential risk posed by exposure to herbicides in epidemiologic studies, the inadequate control for other important risk factors in many epidemiologic studies, and the uncertainty about the nature and magnitude of exposure to herbicides in Vietnam add

TABLE ES-1 Summary of Findings in Occupational, Environmental, and Veterans Studies Regarding the Association Between Specific Health Outcomes and Exposure to Herbicides[a]

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 of an association. There is sufficient evidence of an association between exposure to herbicides and the following health outcomes:

- Chronic lymphocytic leukemia (CLL) (category change from *Update 2000*)
- Soft-tissue sarcoma
- Non-Hodgkin's lymphoma
- Hodgkin's disease
- Chloracne

Limited or 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 or suggestive evidence of an association between exposure to herbicides and the following health outcomes:

- Respiratory cancer (of lung and bronchus, larynx, and trachea)
- Prostate cancer
- Multiple myeloma
- Acute and subacute transient peripheral neuropathy
- Porphyria cutanea tarda
- Type 2 diabetes
- Spina bifida in the children of veterans

Inadequate or 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 cancer
- Nasal or nasopharyngeal cancer
- Bone cancer
- Skin cancers (melanoma, basal, and squamous cell)
- Breast cancer
- Female reproductive cancer (cervical, uterine, and ovarian)
- Testicular cancer
- Urinary bladder cancer
- Renal cancer
- Leukemia (other than CLL)
- Spontaneous abortion
- Birth defects (other than spina bifida)
- Neonatal or infant death and stillbirth

TABLE ES-1 *Continued*

Low birthweight
Childhood cancer in offspring, including acute myelogenous leukemia
Abnormal sperm characteristics and infertility
Cognitive and neuropsychiatric disorders
Motor or coordination dysfunction
Chronic peripheral nervous system disorders
Gastrointestinal, metabolic, and digestive disorders (changes in liver enzymes, lipid abnormalities, and ulcers)
Immune system disorders (immune suppression and autoimmunity)
Circulatory disorders
Respiratory disorders
AL-type primary amyloidosis
Endometriosis
Effects on thyroid homeostasis

Limited or Suggestive Evidence of *No* Association

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

Gastrointestinal tumors (stomach cancer, pancreatic cancer, colon cancer, and rectal cancer)
Brain tumors

[a]*Herbicides* refers to the major herbicides used in Vietnam: 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or 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.

up to the unavailability of the information that would be necessary to measure the risk to people exposed to herbicides during service in Vietnam during the Vietnam conflict.

Despite those limitations, some general conclusions can be drawn regarding the risks to Vietnam veterans, depending on the category of the association between exposure and a given health outcome. Even for outcomes for which there is "sufficient" or "limited or suggestive" evidence of an association with herbicide exposure, it is not possible to calculate precise estimates of risk, if any, among Vietnam veterans because of the lack of exposure information for this population. Such estimates are also not possible when there is "inadequate or insufficient" evidence of an association. But, when there is "limited or suggestive evidence of

no association" between herbicide exposure and a health outcome, the evidence suggests that there is no increased risk of the outcome among Vietnam veterans. That conclusion is limited to the conditions, exposures, and lengths of observation covered by the studies reviewed by the committee.

RESEARCH RECOMMENDATIONS

IOM has also been asked to make recommendations concerning the need, if any, for additional scientific studies to resolve continuing scientific uncertainties about the health effects of the herbicides used in Vietnam and their contaminants.

Progress had been made over the last several years in understanding the health effects of exposure to the herbicides used in Vietnam and TCDD and in elucidating the mechanisms underlying the effects, but there are still important gaps in our knowledge. On the basis of its review of the epidemiologic evidence and consideration of the quality of exposure information available in existing studies, especially of Vietnam veterans, the present committee concludes that continuation of epidemiologic studies of veterans could yield valuable information, especially as the Vietnam-veteran population ages and as a new exposure-reconstruction model is developed and validated. The committee sees value in continuing the AFHS, expanding studies of Army Chemical Corps veterans, and following the experience of Vietnam veterans as they age, with emphasis on diseases associated with aging. Continued study of other exposed cohorts (for example, the cohort studied by the National Institute for Occupational Safety and Health) could also provide information on diseases of aging.

The AFHS is an epidemiologic study whose purpose is to determine whether exposure to the herbicides used in Vietnam might be responsible for any adverse health conditions observed in a cohort of Air Force personnel responsible for conducting aerial spray missions (the Ranch Hands). Five health assessments have been conducted, and, in accordance with the study protocol, one additional assessment is under way and will be completed in April 2003. The AFHS is one of the few primary sources of information on the health of Vietnam veterans. An assessment of herbicide exposure of the AFHS participants and other Vietnam veterans is under revision, and more accurate and precise data are expected in the near future. In addition, the AFHS cohorts are now reaching the age where several health outcomes of interest may be expected to manifest, such as cancers and diseases related to aging. Therefore, the committee recommends continuing the study past its planned completion date. The committee further recommends retaining and maintaining medical records and samples on the AFHS cohort so that—with proper respect for the privacy of the study participants—they could be available for future research. It also recommends that the federal government examine whether and how the various forms of data and specimens collected in the course of the AFHS could be retained and maintained and what form of oversight should be established for their future use.

Members of the Army Chemical Corps (ACC) constitute the largest cohort of Vietnam veterans exposed directly to the herbicides and TCDD, and preliminary studies on this cohort have demonstrated increased TCDD concentrations in ACC veterans who reported spraying herbicides as part of their duties. Some research on the health effects in this population has been and is being conducted. The committee recommends continued and expanded long-term study of this cohort.

Veterans have raised concerns about glioblastomas and possibly astrocytomas. The committee considers those tumors worthy of further investigation despite previous evidence of *no* association. They are extremely rare tumors, and investigating them in epidemiologic studies is difficult. Recording or monitoring trends in those tumors, as well as other diseases of aging, in Vietnam veterans could be useful for indicating which diseases might warrant further study.

The committee is aware that an assessment of herbicide exposure of Vietnam veterans is nearing completion. That assessment should provide more accurate and precise data on the potential exposure of individuals to herbicides sprayed in Vietnam, and the data could be used in epidemiologic studies to increase their power to detect health effects associated with exposure to the herbicides in Vietnam. In light of the anticipated availability of this database, it is even more important to continue research into the health effects of the herbicides in Vietnam veterans themselves, making use of this potentially valuable tool. The federal government should consider the actions that might best facilitate such research and ensure the scientific validity of any such studies of Vietnam veterans.

Another population that has been understudied is the Vietnamese. Anecdotal evidence and studies published in non-English-language journals suggest an array of long-term health effects that are potentially related to the chemicals used by US troops in Vietnam. Although collaborative research by scientists in the two countries presents challenges, such research has the potential to fill a number of gaps in our understanding of the long-term health consequences of exposures to TCDD and herbicides used in Vietnam. The committee supports steps that would continue development of collaborative programs of research. The possibility of using the newly established exposure database for assessing exposures of the Vietnamese also warrants consideration.

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Introduction

Public Law 102-4, the Agent Orange Act of 1991, was enacted on February 6, 1991. That legislation, codified as 38 USC Sec. 1116, directed the secretary of veterans affairs to request that the National Academy of Sciences (NAS) conduct an independent, comprehensive review and evaluation of scientific and medical information regarding the health effects of exposure to Agent Orange (a 50:50 mixture of the herbicides 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid), and other chemical compounds in the herbicides, including the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, one form of dioxin). The legislation also called for reviews of newly available information to be completed every 2 years after the initial report for a period of 10 years. In addition, the NAS was asked to recommend, as appropriate, additional scientific studies to resolve continuing scientific uncertainties and to comment on particular programs mandated in the law.

In response to the request from the Department of Veterans Affairs (VA), the Institute of Medicine (IOM) of the NAS convened 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* (hereafter referred to as *VAO*; IOM, 1994). Successor committees were formed to fulfill the requirement for updated reviews. Those committees produced *Veterans and Agent Orange: Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), and *Update 2000* (IOM, 2001). In 1999, in response to a request from the VA, IOM convened a committee to conduct an interim review of type 2 diabetes. That effort resulted in the report *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (hereafter referred to as *Type 2 Diabetes*; IOM, 2000).

In 2001, VA requested that IOM convene a committee to conduct an interim review of acute myelogenous leukemia (AML). Its review of the literature, including literature available since its review for *Update 2000*, is published in *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Acute Myelogenous Leukemia in the Children of Vietnam Veterans* (hereafter, *Acute Myelogenous Leukemia*; IOM, 2002).

In conducting their work, the committees responsible for those reports operated independently of VA and other government agencies. They were not asked to and did not make judgments regarding specific cases in which individual Vietnam veterans have claimed injury from herbicide exposure. The reports are intended to provide scientific information for the secretary of veterans affairs to consider as VA exercises its responsibilities to Vietnam veterans.

CHARGE TO THE COMMITTEE

In accordance with PL 102-4, the committee was asked "to determine (to the extent that available data permit meaningful determinations)" the following regarding associations between specific health outcomes and exposure to TCDD and other chemical compounds in herbicides:

> A) whether a statistical association with herbicide exposure exists, taking into account the strength of the scientific evidence and the appropriateness of the statistical and epidemiological methods used to detect the association;
>
> B) the increased risk of the disease among those exposed to herbicides during service in the Republic of Vietnam during the Vietnam era; and
>
> C) whether there exists a plausible biological mechanism or other evidence of a causal relationship between herbicide exposure and the disease.

Details of how the committee approached its charge and the methods it used in reaching its conclusions are discussed in Chapter 2.

CONCLUSIONS OF PREVIOUS *VETERANS AND AGENT ORANGE* REPORTS

Health Outcome Conclusions

VAO, *Update 1996*, *Update 1998*, *Update 2000*, *Type 2 Diabetes*, and *Acute Myelogenous Leukemia* provide detailed reviews of the scientific studies evaluated by the committees and their implications for cancer, reproductive and developmental effects, neurobehavioral disorders, and other health effects.

The original committee addressed the statutory mandate to determine whether there is a statistical association between a given health effect and herbicide use by assigning each of the health outcomes under study to 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 in evaluating evidence of the carcinogenicity of various agents (IARC, 1977). Successor committees have adopted those categories in their evaluations. The categories, the criteria for assigning a particular health outcome to a category, and the health outcomes that have been assigned to the categories in past updates are discussed below. Table 1-1 summarizes the most recent categorization of the health effects based on the conclusions of *Update 2000* (IOM, 2001) and *AML* (IOM, 2001). It should be noted that the categories of association described are related to associations 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.

Health Outcomes with Sufficient Evidence of an Association

For effects 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. For example, the committee might regard evidence from several small studies that are free of bias and confounding and that show an association that is consistent in magnitude and direction to be sufficient evidence of an association.

The original committee found sufficient evidence of an association between herbicides and three cancers—soft-tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease—and two other health outcomes, chloracne and porphyria cutanea tarda (PCT) (IOM, 1994). After reviewing all the literature available in 1995, the committee responsible for *Update 1996* concluded that the statistical evidence still supported that classification for the three cancers and chloracne but that the evidence of an association with PCT warranted its being placed in the category of limited or suggestive evidence of an association with exposure; Chapter 11 of *Update 1996* details the decision. No changes were made in this category in *Update 1998* or *Update 2000*.

Health Outcomes with Limited or Suggestive Evidence of an Association

For effects in this category, the evidence must suggest an association between herbicides and the outcome considered, but the evidence of the association may be limited by the inability to rule out chance, bias, or confounding with confidence. Typically, at least one high-quality study indicates a positive association, but the results of other studies could be inconsistent.

The committee responsible for *VAO* found limited or suggestive evidence of an association between herbicides and three cancers: respiratory cancers, prostate cancer, and multiple myeloma. The *Update 1996* committee added three health outcomes to this list: PCT (explained above), acute and subacute transient periph-

TABLE 1-1 Summary of Combined Conclusions on Specific Health Outcomes and Exposure to Herbicides[a] from *Update 2000* and *AML*

Sufficient Evidence of an Association	
	Soft-tissue sarcoma
	Non-Hodgkin's lymphoma
	Hodgkin's disease
	Chloracne
Limited or Suggestive Evidence of an Association	
	Respiratory cancers (or lung and bronchus, larynx, and trachea)
	Prostate cancer
	Multiple myeloma
	Acute and subacute transient peripheral neuropathy
	Porphyria cutanea tarda
	Type 2 diabetes
	Spina bifida in children of veterans
Inadequate or Insufficient Evidence to Determine Whether an Association Exists	
	Hepatobiliary cancers
	Nasal or nasopharyngeal cancer
	Bone cancer
	Skin cancers (melanoma, basal, and squamous cell)
	Breast cancer
	Female reproductive cancers (cervical, uterine, and ovarian)
	Testicular cancer
	Urinary bladder cancer
	Renal cancer
	Leukemia
	Spontaneous abortion
	Birth defects (other than spina bifida)
	Neonatal or infant death and stillbirth
	Low birthweight
	Childhood cancer in offspring, including acute myelogenous leukemia
	Abnormal sperm characteristics and infertility
	Cognitive and neuropsychiatric disorders
	Motor or coordination dysfunction
	Chronic peripheral nervous system disorders
	Gastrointestinal, metabolic, and digestive disorders (changes in liver enzymes, lipid abnormalities, ulcers)
	Immune system disorders (immune suppression, autoimmunity)
	Circulatory disorders
	Respiratory disorders
	AL-type primary amyloidosis
Limited/Suggestive Evidence of *No* Association	
	Gastrointestinal tumors (stomach, pancreas, colon, rectum)
	Brain tumors

[a]*Herbicides* refers to the major herbicides used in Vietnam: 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or 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.

eral neuropathy, and spina bifida in children of veterans. Transient peripheral neuropathies had not been addressed in *VAO*—because, owing to their transient nature, they are not amenable to epidemiologic study—but in response to a request from VA, the committee responsible for *Update 1996* reviewed these neuropathies and based its determination on case histories (see Chapter 10 of *Update 1996*). A 1995 analysis of birth defects among the offspring of veterans of operation Ranch Hand, in combination with earlier studies of neural-tube defects in the children of Vietnam veterans published by the Centers for Disease Control and Prevention, led the *Update 1996* committee to distinguish spina bifida from other adverse reproductive outcomes and classify it in the limited or suggestive-evidence category (see Chapter 9 of *Update 1996*). No changes were made in this category in *Update 1998*.

Following the publication of *Update 1998*, on the basis of its evaluation of newly available scientific evidence and the cumulative findings of research reviewed in previous *Veterans and Agent Orange* reports, the committee responsible for *Type 2 Diabetes* found that there was limited or suggestive evidence of an association between exposure to the herbicides used in Vietnam or the contaminant TCDD and type 2 diabetes. The evidence reviewed in *Update 2000* supported that finding.

The committee responsible for *Update 2000* reviewed the material in earlier *Veterans and Agent Orange* reports and newly available published literature and determined that there was limited or suggestive evidence of an association between exposure to herbicides used in Vietnam or the contaminant TCDD and acute myelogenous leukemia in the children of Vietnam veterans. After release of that report, researchers from one of the studies reviewed in *Update 2000* discovered an error in their published data. After reconvening to reevaluate the previously reviewed and new literature regarding that illness, the *Acute Myelogenous Leukemia* report was produced; it reclassified acute myelogenous leukemia in children from "limited or suggestive evidence of an association" to "inadequate evidence to determine whether an association exists."

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

For outcomes 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 might fail to control for confounding or have inadequate exposure assessment.

Scientific data on many of the cancers and other health effects reviewed by the *VAO* and *Update 1996*, *1998*, and *2000* committees were inadequate or insufficient to determine whether any association exists between the exposures of interest and a health outcome (see Table 1-1). There was one change in the health outcomes in this category between the first 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 or suggestive evidence of *no* association.

On the basis of an evaluation of all the epidemiologic evidence, the *Update 1998* committee felt that urinary bladder cancer should be moved from the category of sufficient evidence of *no* association to this category. Although there was no evidence that exposure to herbicides or TCDD is related to this cancer, newly available evidence weakened the evidence of *no* association. No data reviewed in *Update 2000* resulted in a change in that classification. The committee responsible for *Acute Myelogenous Leukemia* concluded that there was inadequate or insufficient evidence to determine whether an association exists between the exposures and acute myelogenous leukemia in the offspring of Vietnam veterans.

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

For an outcome in this category, several well conducted studies covering the full range of exposure that human beings are known to encounter are consistent in not showing a positive association between exposure to herbicides and the outcome at any exposure and have relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, exposures, and length of observation covered by the available studies. The possibility of a very small increase in risk at the exposures studied can never be excluded.

The *VAO* committee found a sufficient number and variety of well-designed studies to conclude that there is limited or suggestive evidence of *no* association between a small group of cancers and TCDD or the herbicides under study. That group included gastrointestinal tumors (colon, rectum, stomach, and pancreas), skin cancer, brain tumors, and bladder cancer. As noted above, the *Update 1996* committee removed skin cancer from this category and the *Update 1998* committee removed urinary bladder cancer from this category because the evidence no longer supported a no-association classification. No further changes in this category were made in *Update 2000*.

Determining Increased Risk in Vietnam Veterans

The second part of the committee's charge is to determine, to the extent permitted by available scientific data, the increased risk of disease among people exposed to herbicides during service in Vietnam. As discussed in previous *VAO* reports, although there have been numerous health studies of Vietnam veterans, many of them were hampered by relatively poor measures of exposure to herbicides or TCDD and other methodologic problems. Most of the evidence on which the findings regarding associations are based, therefore, comes from studies of people exposed to TCDD or herbicides in occupational and environmental set-

tings rather than from studies of Vietnam veterans. The *VAO* and *Update 1996*, *1998*, and *2000* committees found that body of evidence sufficient for reaching conclusions about statistical associations between herbicides and health outcomes, but the lack of adequate data on Vietnam veterans themselves complicated their consideration of the second part of the charge.

Estimating the magnitude of risk of a particular health outcome among herbicide-exposed Vietnam veterans requires 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. The large uncertainties that remain regarding the magnitude of potential risk posed by 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 make quantitative risk assessments problematic. Therefore, the committees have found that, in general, it is 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 of herbicide exposure among various groups studied suggests 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. Individual veterans who had very high exposures to herbicides, however, could have risks approaching those described in the occupational and environmental studies. In their reports, the committees offer observations regarding increased risk in specific veteran populations on which relevant data are available.

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

Toxicologic data form the basis of the committee's response to the third part of its charge—to determine whether there is a plausible biologic mechanism or other evidence of a causal relationship between herbicide exposure and a health effect. That information is summarized in general terms in separate toxicology chapters in previous reports: Chapter 4 of *VAO* and Chapter 3 of *Update 1996*, *1998*, and *2000*. Specific findings on each health outcome are also given in the chapters that review the epidemiologic literature.

ORGANIZATION OF THIS REPORT

The remainder of this report is organized into nine chapters. Chapter 2 briefly describes the considerations that guided the committee's review and evaluation of the scientific evidence. Chapter 3 updates the toxicology data on the effects of 2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram; these

data contribute to the biologic plausibility of potential health effects in human populations. Chapter 4 provides an overview of the design of many of the epidemiologic studies reviewed by the committee. Chapter 5 addresses exposure-assessment issues and the exposure assessments conducted in the studies of the major cohorts. The committee's evaluation of the epidemiologic literature and its conclusions regarding associations between the exposures of interest and cancer, reproductive and developmental effects, neurobehavioral disorders, and other health effects are discussed in Chapters 6, 7, 8, and 9, respectively. The committee's research recommendations are presented in Chapter 10.

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Considerations in Evaluating the Evidence

This chapter outlines the approach that this and previous committees have used to evaluate the available scientific evidence. A more complete description of the committee's approach can be found in Chapter 5 of *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (hereafter referred to as *VAO*; IOM, 1994).

CHOICE OF HEALTH OUTCOMES

As discussed in Chapter 1, the committee was charged with 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. The legislation (PL 102-4) that led to the committee's work, however, did not provide a specific list of diseases and conditions suspected of being associated with herbicide exposure. *VAO* included a list of diseases and conditions developed on the basis of what had been mentioned in the scientific literature or in other documents; the list has been supplemented in response to developments in the literature, requests made by the Department of Veterans Affairs (VA), and concerns of Vietnam veterans.

IDENTIFICATION OF RELEVANT LITERATURE

The information used by the committee was developed through a comprehensive search of public and commercial databases covering biologic, medical,

toxicologic, chemical, historical, and regulatory information. The majority of those databases were bibliographic, providing citations to scientific literature. The reference lists of major review and research articles, books, and reports were examined. Literature identification continued through July 1, 2002. More than 9,000 potentially relevant studies were identified in those searches, and more than 1,000 were reviewed. Suggestions received from veterans and other interested persons at public hearings and in written submissions were a valuable source of additional information.

This report concentrates on the evidence published after the completion of work on *Veterans and Agent Orange: Update 2000* (IOM, 2001) and *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Acute Myelogenous Leukemia in the Children of Vietnam Veterans* (IOM, 2002). For each health outcome, the new evidence is reviewed in detail. Conclusions, however, are based on the totality of accumulated evidence, not just on recently published studies. That is, new evidence is interpreted not alone but in the context of evidence addressed in previous reports.

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 its findings. In offering that 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 later reports have adopted the original committee's approach.

As discussed in Chapter 3, cacodylic acid, or dimethylarsinic acid (DMA), in addition to being synthesized as a herbicide, is a metabolite of inorganic arsenic in humans. It is important, therefore, to consider the relationship between inorganic arsenic and DMA and the potential for similar adverse health effects after exposure to inorganic arsenic and to DMA. DMA was long thought to be a biologically inactive metabolite of inorganic arsenic, but evidence has been accumulating in recent years that one form of DMA (DMA^V) is an active metabolite of inorganic arsenic and might be responsible for some of the adverse effects observed after exposure to inorganic arsenic. It has yet to be determined, however, whether human exposure to DMA results in the same effects as exposure to toxic concentrations of inorganic arsenic (skin, bladder, and lung cancer and cardiovascular effects). Although some experimental evidence indicates that DMA induces effects similar to those of inorganic arsenic (see Chapter 3), it is insufficient to support a conclusion that exposure to inorganic arsenic is directly relevant to exposure to cacodylic acid. Therefore, the literature on the effects of inorganic arsenic is not considered in this report. Further details on the effects of inorganic arsenic are in *Arsenic in Drinking Water* (NRC, 1999) and *Arsenic in Drinking Water: 2001 Update* (NRC, 2001).

COMMITTEE'S APPROACH TO ITS CHARGE

As discussed in Chapter 1, the committee is charged with three specific tasks: determining whether a statistical association exists between exposure to the herbicides used in Vietnam and health outcomes, determining the increased risk of effects among Vietnam veterans, and determining whether a plausible biologic mechanism or other causal evidence of a given health outcome exists. This section discusses the committee's approach to each of those tasks.

Determining Whether a Statistical Association Exists

In trying to determine whether a statistical association exists between any of the herbicides used in Vietnam or the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and a health outcome, the committee found that the most helpful evidence came from epidemiologic studies—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. Epidemiologists estimate associations between an exposure and a disease in a defined population or group using measures such as relative risk, standardized mortality ratio, or odds ratio. Those terms describe the magnitude by which the risk or rate of disease is changed in a given population. For example, if the risk in an exposed population increases two-fold relative to an unexposed population, it can be said that the relative risk, or risk ratio, is 2.0. Similarly, if the odds of disease in one population are 1:20 and in another are 1:100, then the odds ratio is 5.0. Sometimes the use of terms such as relative risk, odds ratio, and estimate of relative risk is inconsistent, for instance when authors refer to an odds ratio as a relative risk. In this report *relative risk* refers to the results of cohort studies and *odds ratio* (an estimate of relative risk) refers to the results of case–control studies. An estimated relative risk greater than 1 could indicate a positive or direct association (that is, a harmful association), whereas values between zero and 1 could 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 unlikely to occur if there were no true difference and no biases.

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

herbicide exposure and a particular outcome exists, the committee examined the quantitative estimates of risk and evaluated their likelihood of being due to error, bias, confounding, or chance or of representing true associations.

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 after herbicide exposure cannot demonstrate that a purported effect is impossible or could never occur. Any instrument of observation, including epidemiologic studies, is limited in its resolving power. In a strict technical sense, therefore, the committee cannot prove the absolute absence of an association between a health outcome and exposure to the herbicides or TCDD.

Determining Increased Risk in Vietnam Veterans

Whether Vietnam veterans are at increased risk is relevant principally (but not exclusively) when there is evidence of a positive association between exposure and a health outcome. The best evidence for use in determining the risk is knowledge of the rate of occurrence of the outcome in Vietnam veterans who were exposed, the rate in those who were not exposed (the "background" rate in the population of Vietnam veterans), and the degree to which any other differences between exposed and unexposed veterans influence the difference in rates. When, as in most studies, exposure among Vietnam veterans has not been adequately determined, it is difficult to determine such an increased risk. Therefore, although the committees have found the available evidence (most of which is from studies of people exposed to dioxins or herbicides in occupational and environmental settings) sufficient for drawing conclusions about the association between herbicide exposure and a number of health outcomes, the lack of good data on Vietnam veterans, especially with regard to herbicide exposure, complicates the assessment of the increased risk of disease specifically among people exposed to herbicides during service in Vietnam.

Evaluating the Evidence of a Biologic Mechanism

Chapter 3 details the experimental evidence that provides the basis of the assessment of biologic plausibility, that is, the extent to which a statistical association is consistent with biologic or medical knowledge. As with the epidemiologic evidence, the chapter concentrates on studies published in 2000–2002 but considers all relevant studies in drawing conclusions. The issue of whether a relationship between a particular chemical exposure and a particular health outcome reflects a true association in humans is addressed in the context of research regarding the mechanism of interaction between the chemical and biologic systems, evidence from animal studies, and evidence of an association between exposure and the occurrence of a health outcome in humans, including evidence

from occupational and environmental chemical exposures. It must be recognized, however, that lack of data in support of a plausible biologic mechanism does not necessarily rule out the existence of an association.

ISSUES IN EVALUATING THE EVIDENCE

To assess whether a given human health effect is associated with any of the exposures of interest, the committees concentrated on reviewing and interpreting human epidemiologic studies and experimental investigations that might contribute to biologic plausibility, weighing the strengths and limitations of the available evidence. Their assessments have both quantitative and qualitative aspects and take into account the nature of the exposures, health outcomes, and populations exposed; the characteristics of the evidence examined; and the approach taken to evaluate that evidence. Some of the aspects the committees have considered in evaluating the evidence are addressed below.

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 humans. 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 associated with diseases in humans have been confirmed in experimental studies, data derived from such studies are generally accepted as a valuable guide in the assessment of biologic plausibility. Whether a given effect occurs in an animal species, however, cannot always be used to establish whether it occurs in humans.

As discussed in Chapter 3, TCDD, a contaminant of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), is thought to be responsible for many of the toxic effects of the herbicides used in Vietnam. Attempts to establish correlations between the effects of TCDD in experimental systems and in humans are particularly problematic because there are end-point, sex-, and species-specific differences in susceptibility to TCDD. Some data indicate that humans might be more resistant than other species to the toxic effects of this chemical (Dickson and Buzik, 1993), but other data suggest that, for some end points, humans may be at least as sensitive as some experimental animals (DeVito et al., 1995). Differences in susceptibility have a toxicokinetic component because elimination is slower in humans than in rodents (Geyer et al., 2002).

It also important, however, to consider TCDD's mode of action when considering species and strain differences. There is a 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.

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. That interaction results in DNA changes that alter the expression of genes involved in the regulation of cellular processes. The development of AhR-knockout mice (mice lacking the AhR) has helped to establish a definitive association between the AhR and TCDD-mediated toxicity. Toxicodynamic interactions are important because the affinity of TCDD for the AhR is species- and strain-specific (Lorenzen and Okey, 1991), and responses to occupancy of the receptor vary among cell types and developmental stages. The drug-metabolizing enzymes that are induced by TCDD in humans are different from those induced in rodents (Neubert, 1992); this suggests that the effect of different genetic backgrounds on AhR function is not completely understood. It is generally accepted that genetic susceptibility plays a key role in determining the adverse effects of environmental chemicals. Genetic susceptibility is central in the assessment of biologic plausibility because if polymorphisms of the gene encoding the AhR exist in humans as they do in laboratory animals, some people would be at greater risk or at lesser risk for the toxic and carcinogenic effects of TCDD.

Ultimately, however, the challenge in the assessment of the biologic plausibility of the toxicity of herbicides and TCDD is not restricted to understanding 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 many interactions between the AhR and other proteins. 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.

In addition, although studying AhR biology in transformed human cell lines minimizes 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.

Epidemiologic Studies

To obtain information relevant to the evaluation of health effects of exposure to the chemicals of interest, the committee reviewed studies of cohorts of people other than Vietnam veterans potentially exposed to the herbicides used in Vietnam (2,4,5-T, 2,4-dichlorophenoxyacetic acid [2,4-D], cacodylic acid, and picloram), TCDD, phenoxy herbicides, chlorophenols, and other compounds. The cohorts include chemical-production and agricultural workers, people thought to be exposed to large amounts of herbicides or dioxins as a result of residing near the site of an accident or near areas used to dispose of toxic waste, and residents of Vietnam. The committees felt that considering studies of cohorts other than

veterans could help address the issue of whether those 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 the 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 measured and often was determined. Furthermore, the general magnitudes and durations of exposure to the chemicals were greater, and the studies were large enough to examine the health risks among people with varied exposure.

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

Although its full potential has yet to be realized, the application of molecular and cellular end points to epidemiologic research promises to increase the understanding of the association between herbicide exposure and the occurrence of various health outcomes. Such information might provide an important advantage in the assessment of biologic plausibility because biologically based epidemiologic data allow more accurate identification and measurement of exposure. For instance, the analytic data available on people known to have been exposed to herbicides during the Vietnam War constitute a valuable resource for the study of TCDD-related disease; documented TCDD body burdens provide a quantitative bridge between experimental studies and 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 effects lies in data on human populations.

In recent years, the toxic equivalency factor (TEF) method of comparing the relative toxicity of dioxin-like chemicals has been used by several government agencies around the world. Although it is considered one of the best approaches to assessing the relative risk of complex mixtures of these contaminants, it is an interim approach, and it has several inherent uncertainties. TEFs are determined through inspection of the available congener-specific biologic and biochemical data on a compound and assignment of an order-of-magnitude estimate of relative toxicity in comparison with TCDD. TEF values are by no means precise; they are the result of scientific judgment and expert opinion taking into account all the available data form on the congeners. The scientific data on which they are based may vary considerably, often by several orders of magnitude depending on the different biologic end points chosen for a particular chemical. Therefore, considerable uncertainty exists about the use of these values, and it is often difficult to quantify the uncertainty. Although the recent World Health Organiza-

tion TEF values (Van den Berg et al., 1998) are most often cited and generally accepted, the values used can differ slightly among states, countries, and health organizations and with the classification scheme accepted by an agency. Nevertheless, most agencies in the United States, including the Environmental Protection Agency, support the basic approach as a "reasonable estimate" of relative toxicity. Furthermore, numerous countries and several international organizations have adopted it although, again, the accepted values may differ.

The TEF concept is based on the premise that the toxic and biologic responses of all the chemicals in question are mediated through the AhR. Although all the available data support the concept, the set of data on each particular chemical considered to be dioxin-like is incomplete. One possible limitation of the approach is that it does not consider synergistic or antagonistic interactions among the chemicals. In addition, it does not consider possible actions or interactions of these chemicals that are not mediated by the AhR. Indeed, little research has been done on this. For a chemical mixture like PCBs, another limitation of the TEF method is that the risk posed by non-dioxin-like chemicals (that is, noncoplanar PCBs) is not assessed, and some noncoplanar PCBs can act as antagonists (Safe, 1997-1998). Furthermore, the kinetics and metabolism of each dioxin-like chemical differ considerably. Data are often available only on tissue concentrations at any given time and not necessarily on the original exposure of the organism. Sometimes, tissue concentrations are not available. Extrapolation to a meaningful dose may add considerable uncertainty to calculation of the TCDD toxicity equivalent (TEQ) to which a person was exposed. In vivo, there also is exposure to dietary flavonoids and other phytochemicals that are AhR antagonists that is not taken into account with the TEQ method (Ashida et al., 2000; Ciolino et al., 1999; Quadri et al., 2000).

As discussed in *Update 1998* (IOM, 1999), quantitative structure–activity relationship (QSAR) models have been used to estimate the binding affinity of multiple chemical classes. Prediction with these models has been largely unsuccessful because of a focus on minimal energy conformations to predict the activity of molecules. Some QSAR models have been useful across classes of halogenated aromatic compounds.

With the exception of acute and subacute transient peripheral neuropathy, the committee did not specifically consider case studies or other published studies that lacked control or comparison groups. The committee elected to consider case histories when evaluating the association between exposure and those conditions because their transience precluded using case–control and other types of studies with comparison populations.

Publication Bias

It has been documented in biomedical research that studies with statistically significant findings are more likely to be published than studies with nonsignifi-

cant results (Song et al., 2000). Evaluations of disease–exposure associations that are based solely on the published literature, therefore, could be biased in favor of positive associations. In reviewing reports of overall associations with exposure, however, the committee did not consider the risk of publication bias to be high among studies of herbicide exposure and health risks; because numerous published studies show no positive association, it examined a substantial amount of unpublished material, and it felt that publicity surrounding exposure to herbicides, particularly of Vietnam veterans, has been so intense that studies that show no association would be unlikely to be viewed as unimportant by the investigators—that is, the pressure to publish such "negative" findings would be considerable.

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 between diverse fields of research; and formulating the conclusions themselves. Those aspects of the committee's review required thoughtful consideration of alternative approaches at several points and could not be accomplished by adherence to a narrowly prescribed formula.

The approach described here therefore evolved throughout the committee process and was determined, to a large extent, by the nature of the evidence, exposures, and health outcomes examined. Although the quantitative and 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.

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3

Toxicology

As in *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (hereafter referred to as *VAO;* IOM, 1994), *Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996;* IOM, 1996), *Veterans and Agent Orange: Update 1998* (hereafter, *Update 1998;* IOM, 1999), and *Veterans and Agent Orange: Update 2000* (hereafter, *Update 2000;* IOM, 2001), this review summarizes the recent experimental data that serve as a scientific basis of assessment of the biologic plausibility of health outcomes reported in epidemiologic studies. Efforts to establish the biologic plausibility of effects of herbicide exposure in the laboratory strengthen the evidence of the herbicide effects suspected to occur in humans. Toxic outcomes are influenced by differences in dosage (magnitude and frequency of administration); by exposure to other chemicals, including chemicals other than herbicides; by pre-existing health status; by genetic factors; and by the route and rate of absorption, distribution, metabolism, and excretion. Any attempt to extrapolate from experimental studies to human exposure must therefore carefully consider such variables before conclusions are made.

Multiple chemicals were used for various purposes in Vietnam. The chemical nature of the substances themselves is discussed in more detail in Chapter 6 of *VAO*. 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), 4-amino-3,5,6-trichloropicolinic acid (picloram), and cacodylic acid (dimethylarsenic acid, DMA). In addition, this chapter focuses to a large extent on a contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, or dioxin) because its potential toxicity is of concern and considerably more information is available on it than on the herbicides. Most of the

experimental studies of those chemicals, unless otherwise noted, were conducted with pure chemicals, in contrast with the epidemiologic studies discussed in later chapters, in which exposures were often to mixtures of chemicals.

This chapter begins with a brief summary of major conclusions presented in previous Veterans and Agent Orange reports regarding the toxicology of the compounds of interest. That summary is followed by what makes up the majority of the chapter, overviews and discussions of the relevant experimental studies that have been published on 2,4-D, 2,4,5-T, picloram, cacodylic acid, and TCDD since *Update 2000*. Within the update for each of the chemicals, the experimental studies investigating the toxicokinetics, mechanisms of action, and disease outcomes of exposure to the chemical are discussed. Where appropriate, the mechanisms of action are discussed as they relate to a particular endpoint. Estimating potential human health risks on the basis of the animal data is then discussed.

HIGHLIGHTS OF PREVIOUS REPORTS

Chapter 4 of *VAO* and Chapter 3 of *Update 1996*, *Update 1998*, and *Update 2000* review the results of animal and in vitro studies published through 2000 that investigate the toxicokinetics, mechanism of action, and disease outcomes of the herbicides used in Vietnam, and the contaminant TCDD. The toxicity of the four herbicides has not been studied extensively, but in general they are not considered particularly toxic because high concentrations are usually required to modulate cellular and biochemical processes. In contrast, the toxicity of TCDD has been studied extensively. On the basis of the experimental data reviewed in previous Agent Orange reports, the committees concluded that TCDD elicits a diverse spectrum of sex-, strain-, age-, and species-specific effects, including carcinogenesis, immunotoxicity, reproductive and developmental toxicity, hepatotoxicity, neurotoxicity, chloracne, and loss of body weight. The scientific consensus is that TCDD is not directly genotoxic and that its ability to influence the carcinogenic process is mediated by epigenetic events, such as enzyme induction, cell proliferation, apoptosis, and intracellular communication. Most, if not all, of TCDD's effects are mediated through the aryl hydrocarbon receptor (AhR), which interacts with other proteins, binds to DNA and results in biochemical effects, including enzyme induction.

TOXICITY PROFILE UPDATE OF 2,4-D

Toxicokinetics

Toxicokinetics (also referred to as pharmacokinetics) pertains to the routes and rates of uptake, tissue distribution, transformation, and elimination of a toxicant. Those processes, in part, determine the amount of a particular chemical that reaches potential target organs or cells and thereby influences toxicity to organs

or cells. Understanding the toxicokinetics of a compound is important for valid reconstruction of exposure to it.

Since *Update 2000*, several studies have examined the pharmacokinetics and metabolism of 2,4-D in animal species. Recent data support the conclusions of previous updates that metabolism and elimination of 2,4-D are relatively rapid and that tissue uptake is small. Kim et al. (2001) constructed a physiologically based pharmacokinetic (PBPK) model to describe and predict the kinetic behavior of 2,4-D in rats after long-term exposures to low doses. The model was tested with experimental data from rats that were given 2,4-D at 1 or 10 mg/kg body weight per day by subcutaneous infusion for 7, 14, and 28 days. In general, the experimental data fell within the range of 2,4-D concentrations predicted by the PBPK model for the blood and different brain regions. The model supports the concept that uptake of the chemical into brain was limited primarily by the membrane components of the blood–brain barrier. In another study (Barnekow et al., 2001) the elimination and metabolism of 2,4-D following oral administration were evaluated in laying hens dosed with 2,4-D at 18 mg/kg body weight for 7 days and in lactating goats dosed with 2,4-D at 483 mg/kg body weight for 3 days. More than 90% of the total dose was eliminated within 24 h of the final dose. Individual tissue residues accounted for less than 0.1% of the dose. The most abundant residue was 2,4-D; a minor metabolite, 2,4-dichlorophenol, was also present. Overall, those studies suggest that in the species used 2,4-D is eliminated relatively rapidly and that uptake and metabolism by most tissues are low.

A study by Dickow et al. (2000) attempted to correlate plasma concentrations with observed clinical effects in dogs after a dose of twice the reported LD_{50} (the lowest dose that kills half the animals that receive it), 2,4-D at 100 mg/kg body weight. All dogs survived, but vomiting and diarrhea were observed. The mean total and unbound plasma 2,4-D concentrations were 511 mg/L and 129 mg/L, respectively.

As discussed in previous updates, studies suggest that although 2,4-D is relatively nontoxic, the developing nervous system might be a target after exposure to high concentrations. Sturtz et al. (2000) therefore investigated the lactational transfer of 2,4-D by measuring it in tissues of rats whose dams received 2,4-D at 50, 70, and 100 mg/kg body weight during nursing. 2,4-D residues in tissues depended on dose and exposure time. At the highest dose, there was impaired body growth, low tissue weights, and diminished stomach contents of the offspring. The analysis of tissues indicated that 2,4-D was transferred to the neonates during nursing and that, at least at the highest maternal dose, the toxicity might be explained by diminished milk intake or direct toxic effects on the neonate. When 2,4-D treatment was discontinued, the residues remained in the stomach contents of the neonates for at least a week.

Mechanisms of Toxic Action

Mechanisms Related to Genotoxic Effects

Several studies reviewed in *Update 2000* indicate a relatively weak or no genotoxic potential of 2,4-D. Two studies published since then are consistent with a lack of genotoxicity. A study by Venkov et al. (2000) demonstrated a lack of mutagenic action of 2,4-D by using tests in yeast, transformed hematopoietic cells, and mouse bone marrow cells. Charles et al. (2000) also demonstated a lack of genotoxicity after exposure to 4-(2,4-dichlorophenoxy) butyric acid, of which 2,4-D is a metabolite, by looking at gene mutation in bacteria and cultured mammalian cells, cytogenetic abnormalities in mammalian cells, and induction of DNA damage and repair in rat hepatocytes. A study by Amer and Aly (2001), however, observed increased genotoxity after oral exposure to 2,4-D at 3.3 mg/kg body weight for 3 and 5 consecutive days; a significant increase in the percentage of chromosomal aberrations in bone marrow and spermatocytes was observed with both regimens. The genotoxic effects of 2,4-dichlorophenol, a metabolite of 2,4-D, were also investigated in that study and were much weaker. Only the highest concentration tested, 2,4-D at 180 mg/kg body weight, induced a significant percentage of effects after intraperitoneal injection (Amer and Aly, 2001).

Mechanisms Related to Effects on Energy Metabolism or Mitochondrial Function

Several reports cited in previous updates suggest that the toxicity of 2,4-D might be related, at least in part, to its effect at relatively high concentrations on calcium homeostasis and energy metabolism. Those actions might be mediated by a direct action on mitochondria. A study discussed in *Update 2000* indicated that the mitochondrial effects of some herbicide preparations, including those containing 2,4-D, might be due primarily to the surfactant in the formulations and not to 2,4-D itself. A similar study by Oakes and Pollak (2000) confirmed that as much as 50% of the effects of several formulations, including Agent Orange, on oxidative functions of submitochondrial particles is due to "inert" components. A molecular study by Di Paolo et al. (2001) isolated a single protein contained in rat liver mitochondria to which radiolabeled 2,4-D or one of its metabolites was covalently bound. Although the identity of the protein is not known, the investigators suggest that the alteration of its function may be related to known alterations in mitochondrial function produced by 2,4-D.

Previous updates noted that 2,4-D is a peroxisome proliferator, that is, it causes an increase in the number and size of peroxisomes in several tissues of susceptible species. Such chemicals are nonmutagenic carcinogens in the livers of rodents. Humans and hamsters are considered to be relatively resistant to the effects of peroxisome proliferators. A study by Ozaki et al. (2001) observed distinct morphologic changes in the kidneys of rats and mice chronically exposed

to 2,4-D and WY-14643 (a known peroxisome proliferator) for up to 3 months. The changes were characterized by alteration in tubule structures, long brush borders of tubule cells, and reduced volume and number of mitochondria. Those changes were not observed in hamsters. The authors indicate that although 2,4-D is considered a weak peroxisome proliferator in the rodent liver, it appeared to be more effective in inducing renal changes. Kaioumova et al. (2001) determined that the dimethylammonium salt of 2,4-D (up to 3 mM) caused concentration- and time-dependent apoptosis in peripheral lymphocytes of healthy people and in vitro in Jurkat T cells. Further examination of the mechanism indicated that those effects were mediated by direct action of the chemical on mitochondria. Hepatocyte ultrastructural changes were observed in rats whose mothers received the sodium salt of 2,4-D in drinking water (at a daily dose of 2,4-D at 250 mg/kg body weight) before fertilization and during pregnancy and lactation; the changes were consistent with effects of 2,4-D on mitochondria and energy metabolism (Pilat-Marcinkiewicz et al., 2000).

Mechanisms Related to Effects on Thyroid Hormones

Effects of 2,4-D on serum concentrations of thyroid hormones, particularly decreases in thyroxine, were noted in previous updates. A recent report by Kobal et al. (2000) likewise observed decreased serum concentrations of thyroxine and triiodothyronine after oral exposure of male and female rats to 2,4-D at 11 and 110 mg/kg body weight per day for 10 days. Chemical-induced alterations in thyroid homeostasis can adversely affect the development of many organ systems including the nervous and reproductive systems. Most of these effects are caused by lack of thyroid hormone alone rather than by increases in TSH.

Mechanisms Related to Effects on Cell Stress Responses

Stress proteins (for example, heat-shock proteins) are most often induced in a variety of cells in response to environmental and chemical stressors and have been proposed as markers of the presence of stressors. Two studies examined the ability of 2,4-D to increase heat-shock proteins in bacteria and a human cell line. 2,4-D exposure induced several heat-shock proteins in bacteria (Cho et al., 2000), but did not induce the *hsp70* promoter in a HeLa cell line (Ait-Aissa et al., 2000). An additional study determined that a single exposure to 1 mM 2,4-D diminished growth and total protein in all *E. coli* strains tested; successive exposures to 0.01 mM 2,4-D also had a toxic effect on cell growth (Balague et al., 2001).

Disease Outcomes

Studies of disease outcomes published since *Update 2000* are consistent with the previous conclusion that 2,4-D is relatively nontoxic and has weak oncogenic

potential. Also as previously indicated, the developing fetus appears to be most sensitive to the effects of 2,4-D for a number of toxic end points. One recent investigation yielded no evidence that paternal exposure to a herbicide formulation containing 2,4-D and picloram caused birth defects or any other adverse reproductive outcome (Oakes et al., 2002a). Recent animal studies of disease outcomes of 2,4-D exposure are discussed below.

Neurotoxicity

Bortolozzi et al. (2001) studied the effects of nonphysiologic, direct, intracebral administration of 2,4-D (2,4-D at 50 or 100 µg/rat) on behavior and neurochemical alterations in the rat brain. 2,4-D induced a regionally specific neurotoxicity in the basal ganglia, but the neurotoxic effects depended on the location of injection, the dose, and the length of time since the injection. Those data suggest that 2,4-D has the ability to produce direct effects on the brain if high enough concentrations can be achieved. In another study, 2–4 mM 2,4-D directly affected the viability of isolated frog sciatic nerve (Kouri and Theophilidis, 2002). Garabrant and Philbert (2002) reviewed the scientific evidence relevant to neurologic effects of 2,4-D. Although high doses in experimental animals have been found to produce myotonia and alterations in gait and behavioral indexes, there is no evidence of effects on the neurologic system at doses in the microgram-per-kilogram-per-day range. That information is consistent with the conclusion of this and previous Agent Orange updates.

Reproductive and Developmental Toxicity

Several studies have examined the developmental toxicity of 2,4-D. Charles et al. (2001) examine the potential for 2,4-D and its salts and esters to induce developmental toxicity in rats and rabbits. In both species, effects on maternal body weight manifest with 2,4-D at 30 mg/kg maternal body weight per day. At higher doses, body weights and feed consumption were more severely affected. The no-observed-adverse-effect level (NOAEL) for maternal toxicity was about 10 mg 2,4-D/kg body weight per day. Significantly decreased fetal body weights and fetal variations were seen in rats only at doses greater than 90 mg 2,4-D/kg body weight per day. At maternally toxic doses in rabbits, embryonal and fetal development were unaffected. Those data suggest that those end points in the developing rat and rabbit are not uniquely sensitive to 2,4-D or its salt and ester forms. Postnatal measures were not examined in that study. A study by Fofana et al. (2000) examined maternal and fetal toxicity after exposure of pregnant dams to 2,4-D at daily doses of 50, 70, 110, or 150 mg/kg maternal body weight on gestational days 6–10, 6–15, or 11–15. There was significant maternal weight loss in all experimental groups and a dose-related embryolethality. Kidney and urogenital malformations were found in the fetuses. A later study by Fofana et al.

(2002) reported similar findings except that impaired growth of the unaffected offspring was not observed.

Two studies examined brain development in rats and concluded that exposure to 2,4-D may produce alterations in brain development. Ferri et al. (2000) injected dams with 2,4-D (at 100 mg/kg body weight per day) or vehicle during lactation (on postnatal day 9–15 or 9–25). No overt signs of toxicity were observed in the dams, but significant differences were observed in the development of the brain monoaminergic system of neonates exposed to 2,4-D through mother's milk. There was an increase in 5-hydroxyindolacetic acid and serotonin in brains of 25-day-old pups. Alterations in serotonin, dopamine, and norepinephrine were also seen in several brain areas. Rosso et al. (2000) exposed neonatal rats to 2,4-D at 100 mg/kg body weight per day on postnatal days 7–25 or 2,4-D at 70 mg/kg body weight per day on postnatal days 12–25. Decreased body and brain weights were noted only at the higher dose regimen, but both regimens decreased the amount of brain myelin gangliosides and myelin deposition. Alterations in muscular force and motor activity were also seen. Another study investigated whether 2,4-D alters sensitivity to amphetamine by altering the number of D2-like receptors, a subtype of dopamine receptors in the brain (Bortolozzi et al., 2002). Rats exposed to 2,4-D at 70 mg/kg body weight per day from gestational day 16 to postnatal day 23 and acutely challenged with amphetamine exhibited increased sensitivity to amphetamine and an increase in D2-like receptor density. The increased density depended more on the particular brain region and the sex of the animal than on the timing of the 2,4-D exposure. A reversal to basal density of D2-like receptors did not occur after cessation of 2,4-D exposure.

A recent study investigated the male-mediated reproductive toxicity of a mixture of 2,4-D and picloram similar to Agent White called Tordon 75D® (Oakes et al., 2002b). Male rats were exposed by gavage (5 days/week for 9 weeks) to Tordon 75D® (2.5%, 5%, and 10% solutions, corresponding to approximate Tordon 75D® doses of 37, 75, and 150 mg/kg body weight per day) and then mated with untreated females at various times during the treatment and after an 11-week recovery period. On gestational day 20, pregnant females were killed, and fetuses were weighed and examined for malformations. The positive control, cyclophosphamide, increased postimplantation fetal death, but no effects on fetal survival or malformations were observed in the herbicide-treated groups.

Garabrant and Philbert (2002) reviewed the scientific evidence relevant to reproductive risks posed by 2,4-D exposure. They conclude that there is a lack of reproductive and developmental toxicity by any route of administration at 2,4-D doses that do not exceed 50 mg/kg body weight, a dose that saturates renal clearance mechanisms, and that offspring of treated pregnant animals show mild to moderate alterations in skeletal development only in the presence of overt maternal toxicity. Those conclusions are consistent with the data presented in this and previous updates.

Immunotoxicity

Lee et al. (2001) examined the effect of exposure to a commercial 2,4-D formulation during gestation on the immune response in mice. Pregnant mice were given the formulation in drinking water (0–1.0%, equivalent to 2,4-D at 0–650 mg/kg body weight per day) on gestational days 6–16. Immune function in the offspring was evaluated 7 weeks after birth. Decreased body weights and minor reductions in kidney weights were seen in the two highest-dose groups (0.1 and 1.0%). Immune alterations were observed only in the highest-dose group. Suppression of the lymphocyte response to mitogens, an increase in relative B-cell counts, and reduction in the number of cytotoxic and suppressor T cells were seen. The humoral immune response, as measured by antibody production against sheep red blood cells, and peritoneal macrophage phagocytic function were not altered. The authors conclude that the effect on human and animal immune function would probably be minimal when 2,4-D is encountered after normal application in the environment. Garabrant and Philbert (2002) reviewed the scientific evidence relevant to possible effects of 2,4-D on the immune system and concluded that there is little evidence of a significant effect at any dose. That conclusion is consistent with the conclusion of this and previous updates,which note that 2,4-D has at most a weak effect on the immune system.

Carcinogenicity

Using a protocal similar to that discussed above, Lee et al. (2000) examined the effect of exposure to a commercial 2,4-D formulation during gestation on urethan-induced lung adenoma in mice. Female offspring of dams exposed to 2,4-D (0–1.0%) on gestational days 6–16 were given urethan (1.5 mg/g) at the age of 7 weeks to induce pulmonary adenoma. Offspring were examined at the age of 12 weeks for formation of pulmonary adenomas. Gestational 2,4-D exposure did not affect the number of tumors produced, but it did reduce the mean tumor diameter in the highest-dose group. The authors concluded that gestational 2,4-D exposure had no persistent effect on immune cells involved in cell-mediated immunosurveillance mechanisms. Garabrant and Philbert (2002) reviewed the scientific evidence relevant to cancer risks posed by 2,4-D exposure and concluded that there was no experimental evidence that 2,4-D or any of its salts or esters damages DNA and that studies in experimental animals had demonstrated a lack of carcinogenic effects of 2,4-D. Those conclusions are consistent with the conclusions of the present and previous updates.

TOXICITY PROFILE UPDATE OF 2,4,5-T

No relevant studies on the toxicokinetics of 2,4,5-T or the disease outcomes seen in experimental animals after exposure to 2,4,5-T have been published since *Update 2000*.

Previous updates reviewed several possible mechanisms by which 2,4,5-T may affect biologic systems. Much of the available information suggests that 2,4,5-T may disrupt cellular pathways involving acetylcoenzyme A. Several reports suggested that 2,4,5-T has only weak mutagenic potential but that it may alter the profile of enzymes involved in the metabolism of procarcinogens.

Two recent studies have investigated the mechanisms underlying the cellular effects of 2,4,5-T. A study by Kaya et al. (2000) examined the ability of several herbicides, including 2,4,5-T, to produce genotoxicity in the wing-spot test of *Drosophila melanogaster*. It was found to increase slightly the frequency of small single spots but not other types of mutant clones. Furthermore, the slight effect was observed only in a particular type of cross. Those data are consistent with a weak mutagenic potential of 2,4,5-T. A study by Yamanoshita et al. (2001) investigated whether low concentrations of 2,4,5-T affect apoptosis in PC12 cells, a cell line of rat pheochromocytoma cells. Exposure to 2,4,5-T concentrations as low as 10^{-12} g/L increased cell viability and inhibited DNA fragmentation induced by serum deprivation. The authors concluded that because the physiologic mechanisms leading to cell death are necessary for the normal development of tissues, the inhibitory effect of 2,4,5-T on those mechanisms might cause damage by interrupting normal cell homeostasis and differentiation.

TOXICITY PROFILE UPDATE OF CACODYLIC ACID

Cacodylic acid was present (at 4.7%) in a herbicide that was used in Vietnam in defoliation and crop-destruction missions. The active ingredient in cacodylic acid is dimethylarsinic acid (DMA), which is a metabolite of inorganic arsenic in humans; inorganic arsenic is known to cause cancers in humans. Because of possible concerns that the health effects seen following exposure to inorganic arsenic might be seen after exposure to cacodylic acid, the committee discussed whether studies of inorganic arsenic are relevant to its conclusions. Dimethylarsinic acid is resistant to hydrolysis, and is not demethylated to inorganic arsenic. Although dimethylarsinic acid is formed and is an active metobolite in humans following inorganic arsenic exposure, as discussed in Chapter 2, it has not been established and cannot be inferred that the effects seen following exposure to inorganic arsenic occur following exposure to cacodylic acid. Therefore, in general, the literature on inorganic arsenic is not considered in this report. The reader is referred to *Arsenic in Drinking Water* (NRC, 1999) and *Arsenic in Drinking Water: 2001 Update* (NRC, 2001) for further details on the effects of inorganic arsenic. The toxicokinetics of inorganic arsenic as they relate to cacodylic acid formation are discussed below.

Toxicokinetics

Arsenic forms reactive metabolites that affect cellular respiration in nearly every organ system in the body. It was thought for years that methylation of

inorganic arsenic was a detoxification process, but recent studies have disputed that theory. The initial methylation of arsenic yields pentavalent monomethylarsonic acid (MMA^{V}), which is reduced to trivalent monomethylarsonous acid (MMA^{III}) and further methylated to pentavalent dimethylarsinic acid (DMA^{V}). DMA is further reduced to dimethylarsinous acid (DMA^{III}), which is methylated to form trimethylarsine oxide (Styblo et al., 2000). The route of excretion is primarily the urinary system. As discussed in *Arsenic in Drinking Water* (NRC 1999), in most animals the DMA that is formed is rapidly excreted in the urine, but in rats DMA accumulates in the red cells and tissues. The pentavalent arsenic species (MMA^{V} and DMA^{V}) are less toxic than the trivalent ones. MMA^{III} is about 4 times more toxic than inorganic arsenic following acute exposure; the toxicity of DMA^{III} is similar to that of arsenic III (NRC, 2001).

Mechanisms of Toxic Action

A primary mechanism of the acute toxicity of arsenic is interference with cellular respiration, but recent attention has been devoted mostly to understanding the carcinogenic properties and pathways of arsenic. Inorganic arsenic, a known human carcinogen, does not induce neoplasia in laboratory animals, but cancer has been induced in the urinary bladder, kidneys, liver, thyroid glands, and lungs of laboratory animals by exposure to high concentrations of the metabolite DMA (IOM, 2001; Kenyon and Hughes, 2001; NRC, 2001). The mechanisms responsible for those neoplasms remain unknown. Recent studies have suggested that DMA may act through induction of oxidative damage (Yamanaka et al., 2001) or damage to DNA (Kenyon and Hughes, 2001; Mass et al., 2001; Noda et al., 2002; Sordo et al., 2001). Another recent study demonstrated that DMA caused necrosis of the epithelium of the urinary bladder followed by regenerative hyperplasia (Cohen et al., 2001).

Disease Outcomes

Few animal studies are available on the noncancer health effects of cacodylic acid. Previous reports indicate that cacodylic acid is fetotoxic and teratogenic in rats and mice but only at high, maternally toxic doses (Kenyon and Hughes, 2001). Cacodylic acid acts as a tumor promotor in several organ systems. In a recent initiation-promotion study, however, cacodylic acid given in the drinking water at 220 ppm for 29 weeks did not act as a promotor of kidney tumors in male NCI-Black Reiter rats initiated with *N*-ethyl-*N*-hydroxyethylnitrosamine (Vijayaraghavan et al., 2000). In another study, a dose-dependent increase in the incidence of transitional-cell carcinoma occurred in the urinary bladder of male rats given cacodylic acid at 50 or 200 ppm in the drinking water for 104 weeks starting at the age of 10 weeks (Wei et al., 1999). The authors conclude that cacodylic acid is a weak carcinogen. In another study by Seike et al. (2002), oral

administration of cacodylic acid at 400 ppm did not exert promoting effects in the lungs of male F344 rats initiated with *N*-bis (2-hydroxypropyl) nitrosamine.

TOXICITY PROFILE UPDATE OF PICLORAM

Picloram and 2,4-D are components of Agent White, a herbicide formulation used in Vietnam. Studies reviewed in previous updates and in *VAO* reported a fairly rapid elimination of picloram and suggest carcinogenic and some neurologic effects of exposure but only at extremely high doses. Some cellular abnormalities in liver and inconsistent developmental effects have also been reported.

Two relevant studies of picloram have been published since *Update 2000*, both focusing on its potential reproductive effects. A study by Oakes et al. (2002b) investigated the possibility of male-mediated reproductive toxicity of a mixture of 2,4-D and picloram similar to Agent White, called Tordon 75D®. As discussed earlier, male rats were exposed to Tordon 75D® by gavage for 5 days per week for 9 weeks at 37 (low dose), 75 (medium dose), or 150 mg/kg of body weight per day (high dose). The 9-week treatment caused a reduction in testicular weight in some animals treated with the highest dose. The small testes had shrunken tubules and germ-cell depletion that was still evident in some rats after a 21-week recovery period. There were no significant differences in the serum concentration of testosterone between control animals and treated animals. In a related study by the same investigators (Oakes et al., 2002a), each of the males exposed to the three doses of herbicide was mated with two untreated females during weeks 2 and 3, 4 and 5, and 8 and 9 of treatment and with four untreated females after an 11-week recovery period. Negative control males were treated with distilled water, and positive controls with cyclophosphamide. On day 20 of gestation, litter size, fetal weight, and fetal malformation rate were all unaffected by herbicide treatment. The positive and negative controls showed the expected results. The results of those studies suggest that exposure to herbicide formulation containing 2,4-D and picloram can cause male-mediated birth defects or other adverse reproductive outcomes.

TOXICITY PROFILE UPDATE OF TCDD

Toxicokinetics

The distribution of TCDD and other chlorodibenzo-*p*-dioxin congeners has been examined extensively in animal models and to a smaller extent in humans over the last two decades. Similar planar halogenated aromatic hydrocarbons (PHAHs), especially the polychlorinated dibenzofurans and non-*ortho*-polychlorinated biphenyls, have also been examined extensively. As discussed in numerous papers reviewed in previous reports (*VAO* and *Updates 1996, 1998, 2000*), those chemicals are hydrophobic and tend to be readily absorbed across cell membranes.

Properties of the chemicals, properties of the organs and cells, and the route of exposure affect the partitioning, absorption, and accumulation of chemicals. TCDD is distributed to all compartments of the body in amounts that differ from organ to organ. Lipid content is a major factor in the accumulation of TCDD and other PHAHs in different organs and in the body as a whole. Biologic processes, especially metabolism, can affect the distribution and elimination of the chemicals. The concentration of a chemical in a given organ or tissue therefore depends on the dose, absorption, lipid content, and metabolism in the organ of concern. TCDD metabolism can be influenced by processes in other organs. For example, the capacity of the binding protein cytochrome P450 1A2 (CYP1A2), which is prominently expressed in the liver, can alter the accumulation of TCDD in extrahepatic organs.

Since *Update 2000*, several refinements have been made in the understanding of processes that affect the distribution and elimination of TCDD, including efforts to refine PBPK models in animals and humans. Those models are important in exposure assessment because they can be used to extrapolate from measured concentrations, usually in serum, to estimate initial doses in humans.

Animal Studies

Studies in rodent models continue to support the value of PBPK models to predict the disposition of TCDD. Wang et al. (2000) showed that a PBPK model developed for female Sprague-Dawley rats could accurately predict the disposition of TCDD in male Sprague-Dawley rats and in different strains of rats and mice. Evans and Andersen (2000) refined a "steady-state" PBPK model that successfully described the dose-dependent distribution of TCDD in adipose and liver tissues of rats. The analyses showed further that the induction of CYP1A2 affects the maximal accumulation in the liver but that an inflection point in the curve describing the accumulation was affected by conditions associated with TCDD binding to the AhR and to the binding of the activated receptor complex to DNA.

Diliberto et al. (2001) described the distribution of TCDD in mice after a subchronic low-dose exposure. They used repeated dosing at multiple times and doses to examine the disposition of TCDD and used the induction of CYP1A1 as a marker of an effective dose. The dosing regimen was thought to emulate the most likely mode of exposure in humans, by diet. The dosing regimens resulted in nearly steady-state concentrations of TCDD. Hepatic CYP1A1 activity was measurably induced by TCDD at 0.15 ng/kg body weight per day (a body burden of TCDD of 2.8 ng/kg of body weight). The study emphasized the cumulative effects of daily dosing and the importance of determining tissue dosimetry or body burden for chemicals with long half-lives such as TCDD.

Hurst et al. (2000) examined the distribution of TCDD in exposed dams and rat pups exposed to TCDD in utero by using a low-dose subchronic exposure; the regimen achieved the desired steady-state distribution. The objective of the study

was to obtain information to aid in developing models to assess the potential risk associated with such exposure in humans. The authors determined that low-dose maternal exposure results in low concentrations of TCDD in the fetus and that the subchronic exposure produced concentrations in the fetus similar to those seen after a single higher dose during gestation. The study also adds to the information about relationships between maternal and fetal TCDD; individual embryo concentrations of TCDD were 1.6, 7, and 16 pg/g of body weight after maternal exposure to TCDD at 1, 10, and 30 ng/kg body weight per day. On day 16, TCDD concentrations were similar in fetal liver, urogenital tract, head, and all other parts of the body together; this suggests that there was no preferential sequestration in the fetal liver. There was evidence, however, of sequestration in liver as soon as 4 days after birth.

Chen CY et al. (2001) further examined the disposition of polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated biphenyl (PCB), and polychlorinated dibenzofuran (PCDF) congeners in female rats and their offspring. Mixtures of nine dioxin-like compounds were given to female Long-Evans rats at various doses to determine transfer to the placenta, fetuses, and pups. Pharmacokinetic differences among the chemicals influenced the transfer of TCDD from dam to offspring. Those differences were the result of preferential sequestration of planar dioxins and furans by CYP1A2. The authors emphasize, however, that uncertainties remain regarding the sequestration of such compounds by CYP1A2 in humans and that the uncertainties need to be clarified for human risk assessment.

A study of rats exposed during gestation and through lactation (Iba and Fung, 2001) showed that the effects of TCDD, as indicated by CYP1A induction, in the offspring persisted longer than might be expected on the basis of the half-life of TCDD in rats; the effects were more prolonged in lung than in liver. The study also revealed a pronounced induction of CYP1A2 protein in lung, supporting earlier suggestions that CYP1A2 can be induced in some extrahepatic organs, especially the lungs of rodents. Whether CYP1A2 is induced in human lung is not clear, although there have been reports of CYP1A2 transcript in human lung (Mace et al., 1998). There is also evidence suggesting CYP1A2 expression in human bronchial mucosa and peripheral lung tissue (Wei et al., 2001).

Studies modeling the disposition and effects of TCDD in rodents provide evidence that supports the development and use of PBPK models in humans. Comparisons of humans and laboratory animals, however, may not be as accurate as comparisons among rodent species. Van Birgelen and van den Berg (2000) point out that the well-known differences in pharmacokinetic behavior of TCDD in humans and rodents (principally the rates of metabolism, which is slower in humans, and the possible differences in the role of CYP1A2) indicate that a lower daily intake would be needed in humans to reach tissue burdens similar to those in rats. The authors emphasize that estimation of daily intake will be important in applying PBPK models to humans.

Human Studies

Human populations of concern in assessing exposure and risk include those directly exposed to TCDD at high concentrations by accident, in the workplace, or in Vietnam. Comparison populations exposed at background concentrations are also important for assessing the risk posed by higher exposures. In a study by Arfi et al. (2001) of 16 people in France exposed only to background TCDD, the TCDD in benign lipomas of patients was evaluated in relation to their mean daily dietary TCDD intake. The mean daily dietary TCDD intake in France in TCDD equivalents (TEQs) is 1–4 pg TEQ/kg body weight per day—similar to the intake in other industrialized countries. The authors did not find a correlation between the daily dietary dioxin intake and TCDD in the lipomas.

The possibility that TCDD residues might be transferred from more highly exposed to less highly exposed people was examined in a study by Manz et al. (2001) of 14 workers in Germany exposed to TCDD occupationally and their female partners who were not occupationally exposed. Analysis of blood and fatty tissue showed that the female partners accumulated substantial TCDD—as much as 10% of the concentrations in the tissues of their male partners. The underlying mechanism or pathway of transfer reflected in those results is not clear. The authors did not report on TCDD in semen, a possible route of transfer to the female partners. Earlier studies have measured TCDD in semen samples from Vietnam veterans (Schecter et al., 1996); the findings suggest a possible mechanism of male-mediated adverse reproductive outcomes after Agent Orange or other dioxin exposure.

The elimination of TCDD from highly exposed people has been examined in a study of two patients who were exposed to and had accumulated extremely high concentrations of TCDD—at 144,000 pg/g of blood fat in one patient and 26,000 pg/g of blood fat in the other (Geusau et al., 2001). A route of elimination seldom studied is through the skin, and cutaneous elimination of TCDD was examined in those patients. Elimination via skin was assessed in one of the patients by comparing TCDD concentrations in material collected at various times from the skin surface and in cerumen and epithelial cysts with blood concentrations. The authors conclude that elimination via the skin, most probably in cellular material, accounted for 1–2% of the daily elimination. But it is possible that skin is one avenue by which nonexposed partners of highly exposed people could be exposed to TCDD concentrations somewhat greater than in the general population.

Various attempts to accelerate the elimination of TCDD were made in the two patients of Geusau et al. (2002), principally by continuous administration of Olestra. Olestra had been shown to enhance TCDD elimination (discussed in *Update 2000*). It was given at three different doses; at the highest dose, 66 g/day, fecal excretion of TCDD was increased by factors of 10 and 8 for the more and less severely contaminated patients, respectively. At Olestra treatments of 33 g/day, fecal excretion was increased by factors of 4.9 and 6.7, respectively. Efforts

were made to remove lipids from the blood (LDL-apheresis) of the more severely contaminated patient. Although the binding of TCDD to VLDL (see Dalton et al., 2001) suggests that such a treatment might be effective, it did not have a major impact on TCDD body burden.

The relationship between concentrations of chemical residues in blood to those in other tissues is important for assessing tissue dose and elimination rates based on blood concentrations. Elimination of TCDD residues is generally through fecal excretion, which may include residues not absorbed and residues eliminated in bile. The concentrations of 20 dioxin-like compounds (seven PCDDs, 10 PCDFs, and three PCBs) in bile were compared with concentrations in blood and liver in 27 autopsy cases (Kitamura et al., 2001). Total TEQs were the same in bile and blood but were higher in liver. Correlation between blood and bile TEQs was high: a correlation coefficient of 0.89 among the 27 autopsy cases. The study estimated that the rate of accumulation of dioxins, in TEQs, was 0.99, 0.70, and 1.91 pg/g of lipid per year in bile, blood, and liver, respectively.

Half-Life Studies

A number of recent studies are revealing substantial variation in the half-life of TCDD in humans that is associated with a number of variables. Dose and time after exposure have been identified as important. A summary of half-lives estimated in studies in humans and animals is presented in Table 3-1.

Miniero et al. (2001) reviewed data concerning the half-life of TCDD and how it correlates with body weight. The major determinants of TCDD half-life are thought to be lipophilicity, metabolism, and sequestration in the liver, but it seems to correlate empirically with the body weight of mammals. To evaluate that correlation, Miniero et al. (2001) regressed half-life measures with body weight and found a significant correlation between them. Other factors did not seem to influence the dispersion of points about the regression line. That suggests that although uncertainties about the role of CYP1A2 in TCDD distributions in humans remain, metabolism might not affect the half-life substantially. The data may be relevant to a minimal physiologic toxicokinetic model being developed by Salvan and colleagues to describe the long-term behavior of TCDD at the individual level (Bortot et al., 2002; Salvan et al., 2001). The model principally describes the variations of TCDD in serum lipids in terms of body mass, not tissue distribution or sequestration of TCDD in the liver, and it has been used for exposure assessment in studies to identify TCDD effects (Schnoor et al., 2001).

The two highly exposed patients studied by Geusau et al. (2001) showed overall TCDD half-lives of 1.5 and 2.9 years in the more and less severely contaminated patients, respectively. Those half-lives are considerably shorter than values commonly reported: 7.2 years in the Boehringer cohort (Flesch-Janys et al., 1996) and 6.9 and 9.8 years in men and women, respectively, in the Seveso cohort (Michalek et al., 2002) (see below and Table 3-1).

TABLE 3-1 Estimates of TCDD Half-Life in Humans and Animals

Reference	Half-life[a]	Confidence Interval	Comment
Human Studies			
Pirkle et al., 1989	7.1 yr	5.8–9.6 yr	Adult males, Ranch Hands, 9–23 yr PE
Michalek et al., 2002	7.5 yr		Adult males, Ranch Hands 9–33 yr PE
Flesch-Janys et al., 1996	7.2 yr		Adult males, Boehringer cohort
Needham et al., 1994	7.8 yr	7.2–9.7 yr	Adults, Seveso cohort
Michalek et al., 2002	6.9 yr		Adult males, Seveso cohort, 3–16 yr PE
	9.8 yr		Adult females, Seveso cohort, 3–16 yr PE
	0.34 yr[b]		Adult males, Seveso cohort, 0–3 mo PE
Geusau et al., 2002	1.5 yr[b]		Adult female, severe exposure 0–3 yr PE
	2.9 yr[b]		Adult female, severe exposure 0–3 yr PE
Animal Studies			
Viluksela et al., 1996	20.2 days		Rats, Long-Evans TurkuAB strain
	28.9 days[c]		Rats, Long-Evans Charles River strain
Weber et al., 1993	16.3 ± 3.0 days		Rats, male Sprague-Dawley
Pohjanvirta et al., 1990	21.9 days		Rats, male Han/Wistar resistant strain
Neubert et al., 1990	73.7 days	60.9–93.8	Monkey, Marmoset, single injection

[a]Half-lives of TCDD in humans based on measurement of TCDD in serum samples.
[b]Shorter half-lives measured in humans during first months after exposure or in severely contaminated persons consistent with nonlinear elimination predicted by PBPK modeling (e.g., by Carrier et al., 1995). Greater half-life in females attributed to greater body mass index.
[c]Attributed to differences in dilution due to different growth rates.
ABBREVIATION: PE, postexposure.

The toxicokinetics of TCDD were examined in adults exposed to TCDD in the Seveso accident and were compared with data on TCDD elimination in the veterans of Operation Ranch Hand (Michalek et al., 2002). Serum TCDD concentrations in the Seveso cohort were available from samples obtained within days of exposure, providing a measured initial dose, whereas the earliest serum measurements in the Ranch Hand population were obtained in 1982 and at 5-year intervals thereafter to 1997, or 9–33 years after initial exposure. The mean half-life in the Seveso males during the first 3 months after exposure was 0.34 year. The mean half-life in males during the period from 3 to just over 16 years after exposure was 6.9 years. The mean half-life in the Ranch Hands 9–33 years after

exposure was 7.5 years, which is barely significantly different from the latter rate in Seveso males. In the Seveso females, the half-life during the slower phase of elimination, 9.8 years, was longer than that in males. The authors point out that the fast initial elimination in the Seveso cohort is consistent with the pattern expected on the basis of a two-compartment model and with temporal changes in whole-body elimination observed in rodents. The slower elimination in females has been observed in other studies as well (see *Update 1998* and *Update 2000* for discussion of studies).

In a related study, Jackson and Michalek (2001) presented follow-up temporal changes in TCDD concentrations in the Vietnam-era veterans who were not occupationally exposed to herbicides and from whom serum samples were available from 1987, 1992, and 1997. Among those veterans, serum TCDD concentrations decreased by 0.25 parts per trillion (ppt) per year. The decrease in the comparison veteran group from 1987 to 1992 was similar to the decline in serum TCDD observed in Germany from 1989 to 1994 and is considered to reflect a decline in background TCDD.

Van der Molen et al. (2000) used a pharmacokinetic model described in an earlier study (Van der Molen et al., 1996) to assess elimination in longitudinal and cross-sectional studies on the basis of previously published data (Flesch-Janys et al., 1996; Schrey et al., 1993). In the Van der Molen model, body composition, body weight, and intake rate are assumed to depend on age, and elimination rate is assumed to depend on body composition. The model also treats background intake rates as an input, avoiding the subtraction of background concentrations from observed concentration in persons temporarily exposed to higher doses. It does not consider sequestration in the liver. The model's prediction that elimination rate must change with age is consistent with other observations. Differences in half-life could be due to differences in a number of variables, including body mass index, weight, initial dose, time after exposure, and age. Differing half-lives and a biphasic elimination of TCDD could confound the extrapolation from serum measurements to initial exposure. Whether a biphasic curve like that which might be inferred from the Seveso cohort could be used in exposure reconstruction is not certain, but the possibility should be tested.

There have been continuing efforts to identify substances that can aid in the elimination of TCDD and similar compounds from the body. Morita et al. (2001) determined that chlorophyll can inhibit absorption of dioxin and dibenzofuran in rats and enhance their excretion; fecal excretion of seven PCDD congeners and 10 PCDF congeners was increased with increasing dietary chlorophyll. In a related study, Morita and Nakano (2002) observed that seaweed, common in the Japanese diet, also accelerated the elimination of PCDD and PCDF congeners. But they observed that the elimination was principally of metabolites and that the amount of parent compound eliminated was less than 10% of the total; in humans, parent compounds of the congeners constituted 37–90% of the total eliminated. Morita and Nakano (2002) did see an increased elimination of parent

compound in rats when fed seaweed, but whether seaweed would have a similar effect in humans is not known.

To summarize the information on toxicokinetics, there is now considerable understanding of the toxicokinetic behavior of TCDD. The data continue to show that body composition (that is, percentage of fat) is a key determinant of disposition and half-life at low body burdens (background exposures). At higher exposures, CYP1A2 binding holds the dioxins in the liver and results in faster elimination. Therefore, there is both dose-dependent tissue distribution, with the amount in liver relative to that in fat increasing as the dose goes up, and dose-dependent elimination. New data on individuals exposed to very high concentrations of TCDD have shown that at such high doses, the half-life can be quite short relative to that in individuals exposed to background or only moderate concentrations. This is graphically illustrated in the two highly exposed Viennese women (Geusau et al., 2001) and in the new analysis of Seveso exposure data (Michalek et al., 2002). Despite the substantial knowledge, however, it still is quite difficult to reconstruct initial exposure levels from blood levels taken much later.

Mechanisms of Toxic Action

Studies published since *Update 2000* are consistent with the hypothesis that TCDD produces its biologic and toxic effects by binding to a gene regulatory protein, the aryl hydrocarbon receptor (AhR). The mechanistic model indicates that binding of TCDD to the AhR, dimerization of the AhR with a nuclear protein (AhR nuclear transport protein, or Arnt), and interaction of this complex with specific DNA sequences (Ah-responsive elements, or AhREs, and dioxin-responsive elements, or DREs) present in the 5'-promoter regions of responsive genes lead to the inappropriate modulation of gene expression. Those molecular changes are the initial steps in a series of biochemical, cellular, and tissue changes that result in the toxicity observed. That hypothesis is supported by numerous studies that have evaluated structure-activity relationships of various chemicals that bind to the AhR, the genetics of mutant genes that express the AhR, AhR-deficient mice, and the molecular events contributing to and regulating AhR expression and its activity. Additional details of the events have been uncovered since *Update 2000*. The exact relationships between the modulated expression of known regulated or modulated genes (Table 3-2) and the diversity of toxic effects elicited by TCDD in humans and numerous animal species, however, have yet to be uncovered.

The finding that many AhR-regulated genes are modulated in a species-, cell-, and developmental stage-specific pattern suggests that the molecular and cellular pathways leading to a particular toxic event are complex. Many of the data are consistent with the notion that cellular processes involving growth, maturation, and differentiation are most sensitive to TCDD-induced modulation as mediated by the AhR. The findings in animals continue to indicate that reproduc-

TABLE 3-2 Genes and Proteins Known to Be Modulated by TCDD and/or Dioxin-like Chemicals

Reference	Genes
Genes and Proteins Directly Regulated by AhR	
Poland and Knutson, 1982	CYP1A1
Tukey and Nebert, 1984	CYP1A2
Sutter et al., 1994	CYP1B1
Pimental et al., 1993	glutathione-*S*-transferase Ya
Takimoto et al., 1992	aldehyde dehydrogenase 4
Favreau and Pickett, 1991	NAD(P)H-menadione oxidoreductase 1
Lamb et al., 1994	UDP glucuronosyltransferase1
Krishnan et al., 1995	cathepsin D (inhibition); Sp1 (inhibition)
Gillesby et al., 1997	pS2 (inhibition)
Porter et al., 2001	heat shock protein 27 (inhibition)
Gaido and Maness, 1994	plasminogen activator inhibitor-2
Jeon and Esser, 2000	interleukin-2
Kraemer et al., 1996	cyclooxygenase-2
Gao et al., 1998	ecto-ATPase
Genes and Proteins Suspected to Be Directly Regulated by AhR	
Rivera et al., 2002	CYP2S1
Masten and Shiverick, 1995	BSAP
Lai et al., 1996	transforming growth factor-beta (TGF-β)
Matikainen et al., 2002	Bax
Lai et al., 1996	interleukin-6; interferon-gamma
Kim et al., 2000	c-myc
Sugawara et al., 2001	steroidogenic acute regulatory protein
Ogi et al., 2001	polκ
Ohbayashi et al., 2001	DIF-3
Genes and Proteins Modulated by Posttranscriptional Mechanisms	
Gaido et al., 1992	transforming growth factor-alpha (TGF-α); urokinase plasminogen activator
Dong et al., 1997	MHC Q1
Puga et al., 1992	c-fos; c-jun
Genes and Proteins Reported to Be Altered by AhR Ligand Exposure	
Vogel and Abel, 1995	tumor necrosis factor-alpha (TNF-α)
Shridhar et al., 2001	corticotrophin-releasing hormone
Park and Lee, 2002	Hrk; interleukin-3 receptor-beta (IL-3β)
Svensson and Lundberg, 2001	adseverin
Kolluri et al., 1999	p27Kip1
Ohsako et al., 2001	5-alpha reductase 2
Nukaya et al., 2001	low molecular weight prekinonogen
Nishimura et al., 2001	metallothionein
Ma et al., 2001	poly(ADP-ribose) polymerase
Kakeyama et al., 2001	NMDA receptor
Yang et al., 2001	carboxylesterase
Oikawa et al., 2002	IgE-dependent histamine-releasing factor

TABLE 3-2 *Continued*

Reference	Genes
Ishimura et al., 2002	glucose transporter 3
Tian et al., 1998	estrogen receptor
Roth et al., 1988	malic enzyme
Sewall et al., 1995	epidermal growth factor receptor (EGFR)
Poland and Glover, 1973	ALA synthetase
Sparrow et al., 1994	pyruvate carboxylase
Kolluri et al., 2001	*N*-myristoyltransferase 2
Mathieu et al., 2001	multidrug resistance 1
Sugihara et al., 2001	xanthine oxidase/dehydrogenase
Sutter et al., 1991	interleukin-1beta (IL-1β)
Krig and Rice, 2000	transglutaminase
Puga et al., 2000a	guanine nucleotide exchange factor; Ki-ras2 proto-oncogene; semaphorin; inositol 1,4,5-triphosphate receptor; MEK5; advillin; casein kinase 1 delta; RAY1; SHP-2 tyrosine phosphatase; MST2 serine/threonine kinase; phosphoinositide 3-kinase; phospholipase A2; calcium-modulating cyclophilin; calmodulin; neurogranin; phosphatidic acid phosphatase; S100 calcium-binding protein A4, A7, and A12; FRK, fyn-related kinase; calcineurin A; visinin-like factor 1; phospholamban; calbindin 1; spindle pole body protein; p56 Lck; FLT1, fms-related tyrosine kinase; protein kinase C-beta and zeta; IkB-alpha; PKA regulatory subunit; phosphatidylinositol 4-kinase; serine/threonine kinase 2; Thy-1 cell surface antigen; A-kinase anchor protein; CD3E; E74-like Ets-domain transcription factor; Erg-1 and Erg-2 transcription factors; MAD; MADS box transcription enhancer; frizzled homolog; FREAC-2 forkhead-like protein; troponin C; inducible NO synthase; G protein-coupled endothelin; endothelial NO synthase; follistatin; FGL2, prothombinase; arginine vasopressin receptor 1A; 5-lipoxygenase-activating protein; midkine; very low density lipoprotein receptor; coagulation factor XII; neuropeptide Y receptor Y1; vascular endothelium growth factor; multiple exostoses 2; dermatan sulphate proteoglycan; decorin; thrombomodulin; granzyme A; cyclin B2; human RACH1; matrilin 2; lamin B receptor; caspase-1 and 4; PDCD2; P107, RB-related protein; glycogen synthase kinase 3 beta; tumor necrosis factor members 3, 6, 8, 9 and 10; CC3; growth arrest-specific GAS-1; anti-mullerian hormone receptor; heat-shock protein hsp-40; NEK-2 serine/threonine kinase; breakpoint cluster region; matrilin 2; LIM domain; alpha (1,3) fucosyltransferase; integrin beta 1 and 3; CD63 antigen; translation initiation factor 4; homeobox HB9 and Pax 3; bagpipe homeobox homolog; epimorphin; CD47 antigen; phosphatidylinositol glycan, class C, H, and K;

TABLE 3-2 *Continued*

Reference	Genes
Puga et al., 2000a *continued*	dystrophin-associated glycoprotein 1; mannosidase alpha type II; VAMP 8; VAMP-associated protein; ankyrin 2, neuronal; gamma-butyrobetaine hydroxylase; epimorphin; membrane fatty acid desaturase; sulfotransferase 2B family member; *N*-methylpurine-DNA glycosylase; thioredoxin peroxidase; 3-hydroxybutyrate dehydrogenase; suppressor of Ty homolog; estrogen sulfotransferase; mammalian mutS homolog; endonuclease G; cytochrome c-1
Frueh et al., 2001	G protein-coupled receptor HM74; agrin precursor-like protein; enhancer of filamentation; XMP; cytochrome b5; DNA-binding protein inhibitor ID-2; aquaporin 3-like protein; mannose-binding protein C precursor; coagulation factor XI; arylacetamide deacetylase; cytochrome P450 subfamily XIX; calponin; endothelial actin-binding protein; phospholipase A2, membrane-associated precursor; keratin 17; apolipoprotein C-1 precursor; lectin galactdoside-binding soluble 3 (galectin 3); phospholipase D; ATP synthase lipid-binding protein P1 precursor; glutaminyl-tRNA synthetase; hybrid receptor gp250 precursor; glutamate-cysteine ligase regulatory subunit; inwardly rectifying potassium channel Kir3.2; aminopeptidase N; aminoacylase-1; B2-bradykinin receptor, 3; histidine ammonia-lyase; L-myc-1-proto-oncogene protein; YL-1 protein; fibrogen alpha chain precursor; thyroxin-binding globulin and globulin precursor; SPARC/osteonectin; alcohol dehydrogenase 1 alpha polypeptide; fibrinogen gamma-B chain
Kurachi et al., 2002	RAB11a and RAB3D (members of RAS oncogene family); Ral-A protein; interferon-inducible GTPase; insulin-like growth factor binding protein 3 and ALS; calcium binding protein A11; regucalcin; S100 calcium binding protein A1; cyclin-dependent kinase inhibitor 1A; proteasome 26S subunit; proprotein convertase subtilison/kexin type 6; metaxin 2; ubiquitin-like 1; tyrosine 3-monooxygenase activation protein; Sin3-associated polypeptide; eukaryotic translation initiation factor s 2 and 3; eukaryotic translation elongation factors 1 and 2; nucleobindin; heterogeneous nuclear ribonucleoproteins C and K; high mobility group protein 1; histone gene complex 1; YY1 transcription factor; zinc-finger protein 207; upstream transcription factor 2; histone deacetylase 5; nuclear RNA export factor 1 homolog; homeodomain interacting protein kinase 1; RNA polymerase 1-3; basic transcription factor 3; ribosomal proteins S6, L9, S8, L7a, S3; RNase A family 4;

TABLE 3-2 *Continued*

Reference	Genes
Kurachi et al., 2002 *continued*	splicing factor, arginine/serine-rich 2 (SC-35); endothelial monocyte activating polypeptide 2; protein that interacts with C kinase 1; GrpE-like 1, mitochondrial; chaperonin subunits 3 and 4; enoyl coenzyme A hydratase, short chain 1; phosphatidylcholine transfer protein; organic anion transporter member 10; FXYD domain-containing ion transport regulator 1; ATPase-like vacuolar proton channel;ATP-binding cassette, sub-family G; neurophilin; tenomodulin; CD82 antigen; poliovirus sensitivity; LDL receptor related protein, associated protein 1; integral membrane protein 3; natural killer tumor recognition sequence; syndecan 4; pigment epithelium-derived factor; argininosuccinate synthetase 1; cytochrome P450, 2d10; protein phosphatase 1, catalytic subunit; tryptophan-2,3-dioxygenase; sialyltransferase 9; acetyl-coenzyme A dehydrogenase, long chain; ornithine decarboxylase; ketohexokinase; plasmin inhibitor alpha 2; phosphoenolpyruvate carboxykinase 1; stearoyl-coenzyme A desaturase 1; sorbitol dehydrogenase 1; phosphoglycerate kinase; ornithine transcarbamylase; galactokinase; lactate deyhydrogenase 1, A chain; lysosomal acid lipase 1; carbonyl reductase 1; carbonic anhydrase 3 and 5; cytochrome c oxidase, subunits Va and VIIb; glucose regulated protein, 58 kDa; heat shock protein cognate 70; cell death-inducing DNA fragmentation factor; heat shock 10kDa protein 1 (chaperonin 10); clusterin; fatty acid binding protein 1, liver; presenilin 2; GM2 ganglioside activator protein; peroxisome biogenesis factor 16; male enhanced antigen 1; mucolipin 1; gene trap ankyrin repeat; apolipoproteins E and CI; serum albumin variant;major urinary protein; alpha-2-HS-glycoprotein; ferritin heavy chain; serine protease inhibitors 1-3; beta-2 microglobulin;hepcidin antimicrobrial peptide; complement components 1, 3, and 4; plasminogen; transthyretin; haptoglobin; alpha-2-glycoprotein 1, zinc; kininogen; alpha-2-macroglobulin; glutathione peroxidase; profilin 1; melanoma X-actin; destrin; gelsolin; keratin complex 2, basic gene 1; prefoldin 2

tive, developmental, and oncogenic end points are very sensitive to TCDD. The data support the biologic plausibility of similar end points of toxicity in exposed humans. However, many of the responses are tissue- and species-specific, and the exact mechanistic basis of the differences is not known.

The conclusions indicated above are similar to those in *Update 2000*. Since that update, many interactions of the AhR at the cell and molecular levels have been reported. However, in many cases it is not clear how these might be related to a particular toxic end point. Therefore, although the text below references all related work published since *Update 2000* that was identified by the committee, closer attention is given only to studies that added substantial new information, particularly as it might be relevant to the exposure of veterans in Vietnam. As discussed in *Update 2000*, it is important to consider exposure and species sensitivity when discussing animal data and their relevance to humans.

Structural and Functional Aspects of the AhR

The AhR Gene and Protein Several studies have characterized AhR structure, expression, and function in different animal species, including zebrafish (Andreasen et al., 2002a,b), rainbow trout (Pollenz et al., 2002), Atlantic killifish (Karchner et al., 2002), chick embryos (Walker MK et al., 2000), and guinea pigs (Korkalainen et al., 2001). In general, the published data are consistent with the conservation of AhR structure and function among species. Zebrafish, however, express two AhR-like molecules; one is not responsive to TCDD, and the other appears to be active in mediating cardiovascular toxicity in developing animals (Andreasen et al., 2002b). The closest homologue of the human AhR is the guinea pig receptor (Korkalainen et al., 2001); this is of interest because the guinea pig is one of the species most susceptible to TCDD-induced lethality.

There are many data on the ability of various regions of the AhR protein to function in ligand binding, DNA binding, nuclear localization, and interaction with other proteins, including Arnt. New information has added to our understanding of those regions (Kronenberg et al., 2000; Andreasen et al., 2002c; Berg and Pongratz, 2001; Elbi et al., 2002; Ikuta et al., 2002; Jones and Whitlock, 2001; Kumar et al., 2001; Levine et al., 2000). Exposure of cells and animals to relatively high TCDD concentrations has been shown to stimulate pathways that mediate the degradation of the AhR, leading to a subsequent decrease in AhR-mediated gene alterations. Additional data have also improved our understanding of those pathways (Ma and Baldwin, 2000; Pollenz and Barbour, 2000; Santiago-Josefat et al., 2001). Notably, recent data indicate that environmentally relevant concentrations do not appear to change AhR concentrations in the rat (Franc et al., 2001). It is of interest that nuclear localization and transcriptional activation of the AhR have been found to occur in the absence of an exogenous ligand, such as TCDD (Richter et al., 2001). That finding might indicate the existence of an endogenous ligand for the AhR whose identification would greatly increase our

knowledge of AhR function and of how the inappropriate stimulation of the AhR by TCDD may lead to toxic effects.

A recent publication characterizes the regulatory regions of the *Ahr* gene (Garrison and Denison, 2000). The data suggest that the expression of the gene depends on acetylation of nuclear histone components (Garrison and Denison, 2000; Garrison et al., 2000). Three polymorphisms in the human *Ahr* gene have been reported at codons 517, 554, and 570 in exon 10 (Smart and Daly, 2000; Wong et al., 2001a,b). Exon 10 is the major region that is responsible for the transactivation of other genes. In vitro assays using expressed variant forms of the AhR indicate that none of those individual polymorphisms has an important effect on the ability of the AhR to induce the *CYP1A1* gene in a TCDD-dependent manner (Wong et al., 2001b). A combination of the lysine_{554} + isoleucine_{570} variant and the lysine_{554} + isoleucine_{570} + serine_{517} variant, however, was unable to support induction of *CYP1A1* by TCDD. That suggests that people with that combined polymorphism might be less susceptible to some of the effects of TCDD. Cauchi et al. (2001) identified several human Ahr polymorphisms, but none was found to be associated with altered *CYP1A1* inducibility or with susceptibility to lung cancer. Roberts et al. (2000) reported that TCDD failed to induce the CYP genes in a human hepatoma cell line and that this was probably because of the presence of a defective AhR protein.

Interaction of the AhR with Other Proteins The function and regulation of the AhR depends on the presence of several other intracellular proteins. Additional studies have been published on the interactions of the AhR with a protein known as X-associated protein 2 (XAP2; also called ARA9 and AhR-interacting protein, or AIP) (Bell and Poland, 2000; Kazlauskas et al., 2000, 2002; LaPres et al., 2000; Petrulis et al., 2000), with 90-kilodalton (90-kDa) heat-shock protein (hsp90) (Heid et al., 2000; Kazlauskas et al., 2001), and with p23 (Cox and Miller, 2002). Those proteins have been shown to function in stabilizing the AhR in cells and in regulating the intracellular localization of the AhR. An additional study indicates that tyrosine phosphorylation of the AhR is required for its DNA-binding activity (Park et al., 2000). Interactions of the AhR with the retinoblastoma protein (Elferink et al., 2001), tyrosine kinases (Dieter et al., 2001), the orphan receptor COUP-TF (Klinge et al., 2000), the short heterodimer partner (SHP) orphan nuclear receptor (Klinge et al., 2001), myb-binding protein 1a (Jones et al., 2002), the Brahms/SWI2 gene 1 protein (Wang and Hankinson, 2002), the silencing mediator of retinoic acid and thyroid hormone receptors (SMRT) (Rushing and Denison, 2002), and the NcoA/SRC-1/p160 family of transcriptional coactivator proteins (Beischlag et al., 2002) may also modulate the ability of the AhR to regulate genes.

Update 2000 noted the identification of an AhR repressor (AhRR) protein that inhibits AhR function by competing with the AhR for dimerization with Arnt. Additional data on this protein from human, mouse, and killifish (Karchner

et al., 2002) are consistent with previous information. They also indicate that AhRR is evolutionarily conserved. Notably, recent data indicate the association of an AhRR polymorphism (proline to leucine at the 185 position) with micropenis in humans (Fujita et al., 2002); this is particularly relevant because TCDD exposure to animals is known to affect the development of reproductive tissue.

Chemicals Other Than TCDD That Affect AhR Function As indicated in previous updates, data on the ability of various dioxin-like chemicals to bind to the AhR and cause toxicity support a role of the AhR in mediating the toxicity of those chemicals; newer information is consistent with that role (Simanainen et al., 2002). Recent data, however, also indicate that relative potency values derived from one end point of toxicity, in this case lethality, are not necessarily valid for other end points of toxicity.

Since *Update 2000*, a number of chemicals have been found to modulate AhR function by directly binding to the AhR. Several of those chemicals—such as indirubin and indigo (Adachi et al., 2001), some prostaglandins (Seidel et al., 2001), and 7-ketocholesterol (Savouret et al., 2001)—appear to act as AhR agonists. Notably, these molecules are found in human and animal tissues. Other chemicals, such as the naturally occurring flavonoids (Ashida et al., 2000a; Ciolino et al., 1999; Quadri et al., 2000) and synthetic flavonoids (Nazarenko et al., 2001), also bind to the AhR, but some of them act as antagonists. The ability of some PCBs to have agonist or antagonist activity appears to depend on their relative affinity for the AhR and properties related to their intrinsic efficacy, that is, their ability to produce a response once bound to the AhR (Hestermann et al., 2000). The ability of PCBs to bind to the AhR and elicit a conformation that would bind to DREs in vitro and induce *CYP1A1* in whole cells has been used to detect AhR agonist and antagonist activity (Petrulis and Bunce, 2000). The work by Pohjanvirta et al. (2002) emphasizes the point that although a chemical like indolo[3,2-*b*]carbazole can bind to the AhR and act as an AhR agonist, it might not elicit the same toxic responses as TCDD. That is probably because of the rapid metabolism of the chemical, and it further suggests that prolonged occupation of the AhR and persistent modulated gene expression may be necessary for some toxic end points. Additional work has been done to understand the molecular constraints responsible for the ability of the dioxin-like chemicals to bind to the AhR (Arulmozhiraja et al., 2000; Mhin et al., 2002).

Other chemicals—such as resveratrol (Lee and Safe, 2001), some heavy metals (Maier et al., 2000), and bisphenol A (Jeong et al., 2000)—appear to block TCDD- and AhR-dependent gene transcription by mechanisms not related to their ability to bind the AhR.

AhR-Mediated Alterations of Gene Expression

Much of our current understanding of the mechanism of TCDD action is based on analysis of the induction of particular genes. Several genes known to be modulated by TCDD and dioxin-like chemicals in a variety of biologic systems, including human cells, are listed in Table 3-2, which includes several genes published since *Update 2000*. Genes in which either mRNA or protein concentrations have been shown to be altered are included, but enzymes or proteins whose biologic activities are altered by some other mechanisms are not. Several genes are known to be modulated by direct interaction of the AhR–Arnt complex with DREs in the promoter region; recent data indicate that the induction of some of these genes failed to occur when mice in which the expression of Arnt was conditionally disrupted were treated with TCDD (Tomita et al., 2000). Other genes are suspected, but not yet proved, to be induced by this mechanism. The expression of several genes—such as cathepsin D, Sp1, pS2, and heat-shock protein 27—has been shown to be inhibited by the ability of the AhR to bind to DREs near the DNA-binding sites for the estrogen receptor. The expression of other genes, including transforming growth factor-alpha (TGF-α) and MHC Q1, is thought to be altered by posttranscriptional mechanisms. Finally, Table 3-2 includes genes modulated in a variety of cells or tissues in several species, including humans, but of which the mechanisms of alteration are not yet understood; it is quite likely that the induction or repression of many of these genes might be secondary to the ability of the AhR–Arnt complex to act directly on other genes. The size of the latter category emphasizes the ability of the ligand-bound AhR to initiate a cascade of molecular and biochemical events that eventually leads to cell and tissue alterations. That these events are known to be tissue-, species-, and developmental stage-specific also emphasizes the complex nature of the biochemical events that lead to some particular toxic response. Several recent investigations of those gene changes have been carried out in human cells (Frueh et al., 2001; Puga et al., 2000a).

Data on several individual genes are discussed below in the context of particular tissue systems or toxic end points that might be affected by TCDD. However, this should not be interpreted to indicate that the ability of TCDD to modulate the expression of a particular gene is limited to that particular tissue; although the effects of TCDD are very tissue- and cell-specific, genes or biochemical pathways modulated in one tissue are often found to be modulated in several other tissues.

Mechanisms Related to Particular Toxic End Points

As indicated in previous updates, an accumulation of studies in experimental animals indicates that TCDD affects a variety of tissues, and the type of effect observed is often tissue-specific. In addition, effects are most often dose-depen-

dent, that is, some toxic end points appear to be most sensitive to low exposures, and others may occur only at high concentrations. Furthermore, effects have been found to depend on the species examined and often on the age and sex of the animal. There is no reason to suspect that humans would be different in that respect. Findings in animals suggest that reproductive, developmental, and oncogenic end points are the most sensitive to TCDD and are consistent with the notion that growth, maturation, and differentiation are the most sensitive cellular processes. Those data support the biologic plausibility of similar toxic end points in humans. Although the exact biochemical mechanisms of those end points and the observed differences are not yet understood, recent data have emphasized the possibility that at least some of the effects are mediated by TCDD's ability, through the AhR, to modulate cell-cycle control, signaling pathways that lead to cell death, hormones and growth factors and the responses to them, or the biochemical pathways that lead to oxidative stress (Barouki and Morel, 2001; Nebert et al., 2000). Those mechanisms are implicated in many of the toxic end points discussed below.

Mechanisms Related to Wasting Syndrome Exposure of most animal species to relatively high doses of TCDD elicits a wasting syndrome characterized by decreased food consumption and loss of body weight. The biochemical pathways affected by TCDD that lead to the syndrome have not been identified. Tuomisto et al. (2000) observed that TCDD treatment alters food intake and food selection in rats. A study by Dunlap et al. (2002) demonstrated that the absence of c-src protein kinase expression affects the development of several TCDD-elicited toxic end points—such as decreased body-weight gain, adipose tissue to liver weight ratio, decreased weight of pancreas, glycogen depletion, and phosphoenolpyruvate carboxykinase downregulation—that are related to the syndrome. Previous updates discuss the effect of TCDD on vitamin stores in animals. Two additional studies suggest that altered retinoid homeostasis, particularly an increased mobilization of vitamin A from storage sites mainly in the liver, leading to increased serum and kidney retinoic acid may contribute to the syndrome (Fletcher et al., 2001; Kelley et al., 2000; Nilsson et al., 2000). Glover et al. (2000) observed that rats treated with TCDD had increased sensitivity to endotoxin, which resulted in increased nitric oxide. Previous studies had suggested that endotoxin is a contributing factor in TCDD-induced wasting syndrome, possibly through its effects on the stimulation of cytokine production, which can suppress appetite.

Mechanisms Related to Effects on Skin and Adipose Tissue Skin lesions, including chloracne, are often reported in animals and humans after exposure to TCDD and related chemicals. Loertscher et al. (2001a) observed that TCDD exposure reduces the number of normal human keratinocytes grown under culture conditions, but not because of increased cellular apoptosis. Using an immortalized human keratinocyte line, those investigators also observed that TCDD

caused alterations in the pattern of terminal differentiation without increased apoptosis or altered cellular proliferation (Loertscher et al., 2001b). Krig et al. (2002) investigated the mechanism whereby TCDD suppresses retinoid induction of the transglutaminase gene in human keratinocytes. They found that the suppressive action of TCDD is at the transcriptional level but that it occurs indirectly through a DNA site outside a 5-kilobase region of the TGM2 promoter and does not directly interfere with retinoid action or at the retinoid response element in this gene.

As indicated in previous updates, TCDD has been shown to inhibit the differentiation of some preadipocyte cell lines to adipocytes, and this process is AhR-dependent. Several research groups have examined the mechanism of that effect because it may be relevant to how TCDD acts in various tissues. Shimba et al. (2001) demonstrated that overexpression of the AhR in fibroblast cells in the absence of TCDD exposure suppresses morphologic differentiation and induction of adipocyte-related genes, whereas underexpression of the AhR induces differentiation and expression of the genes. The authors conclude that the AhR is a negative regulator of adipose differentiation and that regulation can occur independently of TCDD exposure. Liu et al. (2002) present data suggesting that the antiadipogenic action of TCDD is related to the expression of C/EBPα, a factor believed to coordinate genes involved in lipogenesis. Kern et al. (2002a) observed a decrease in catalase activity in the adipose tissue of TCDD-treated rats. Nagashima and Matsumura (2002) established that TCDD causes downregulation of glucose uptake activity in preadipocytes. Kern et al. (2002b) observed a decrease in glucose transport in a TCDD-treated adipocyte line; in addition, TCDD stimulated secretion of TNF and decreased lipoprotein lipase activity. The authors conclude that this may indicate a physiologic mechanism for epidemiologic studies linking dioxin exposure to diabetes.

Mechanisms Related to Effects on Bone and Teeth Possible effects of TCDD on bone have not been thoroughly investigated. Jamsa et al. (2001) studied effects of 19 weekly TCDD treatments amounting to total TCDD doses of 0.17–170 μg/kg body weight in young adult rats. Tibial growth was inhibited in a dose-dependent manner, but there was no effect on bone mineral density. Breaking force and stiffness were reduced by TCDD at 17 μg/kg body weight. Resistance of the H/W rat strain to those effects was associated with an altered transcription domain of the AhR. Singh et al. (2000) observed that TCDD inhibits osteogenesis in a chicken periosteal osteogenesis model and that this effect is restricted primarily to the osteoblastic differentiation phase. Collagen type I, osteopontin, bone sialoprotein, and alkaline phosphatase mRNA content were decreased. Similar results were observed with a rat stromal bone cell line. And TCDD induced a reduction in bone mineralization. All those effects were antagonized by co-treatment with resveratrol, which is found at high concentrations in red wine. The authors postulated that the high concentrations of AhR ligands in cigarette smoke,

might be important in linking smoking to osteoporosis and periodontal disease. Partridge et al. (2000) noted that low concentrations of TCDD and estrogen affected synthesis or secretion of parathyroid-stimulated collagenase-3 in a rat osteoblast osteosarcoma cell line.

Previous reports have suggested that defects in children's first molars may be associated with environmental exposure to dioxins. Render et al. (2000) observed proliferation of periodontal squamous epithelium in mink fed TCDD at 5 ppb for 6 months. Sahlberg et al. (2002) reported that AhR and Arnt are coexpressed in developing mouse tooth buds, especially in secretory odontoblasts and ameloblasts. Allen and Leamy (2001) found that in utero exposure to TCDD did not affect the fluctuating asymmetry of mandibles in mice, but did decrease mandible size and affect their overall shape.

Mechanisms Related to Cardiovascular Toxicity There is a paucity of information on the potential for TCDD to exert toxic effects on the mammalian cardiovascular system. However, a recent study by Riecke et al. (2002) noted a dose-dependent increase in the incidence of myocardial fibrosis in marmosets 2 or 4 weeks after a single dose of TCDD at 1, 10, or 100 ng/kg body weight. Further analysis indicated increased transforming growth factor β1 (TGFβ1) and TGF-β receptor type I in heart tissue. The authors suggested a relation to the myocardial fibrosis in that this factor is known to cause fibrosis in many tissues of experimental animals. Additional studies in chicken embryos, which appear to be very susceptible to cardiac effects after TCDD exposure, noted that the activation of the AhR signaling pathway correlates with the ability of various dioxin-like chemicals to induce cardiotoxicity (Heid et al., 2001) and that TCDD-induced apoptosis may contribute to the observed changes in myocyte proliferation, coronary development, and structural malformations (Ivnitski et al., 2001).

Mechanisms Related to Pulmonary Toxicity Iba and Fung (2001) observed that gestational and lactational exposure of rats to TCDD caused long-lasting and sex-dependent induction of CYP1A1 and CYP1A2 protein in the lungs. Wei et al. (2001) observed both CYP1A1 and CYP1A2 in lung biopsy specimens from human subjects.

Genter et al. (2001) indicated that TCDD can induce many metabolic enzymes—including CYP1A1, CYP1A2, CYP2B1, CYP2C11, and epoxide hydrolase—in rat olfactory mucosa and that the pattern of the changes was different from that in liver. The increase in nasal mucosal enzymes resulted in enhanced metabolism of lidocaine. The authors concluded that environmental exposure to TCDD may affect drugs administered nasally.

Mechanisms Related to Hepatotoxicity The liver is a primary target organ of TCDD and related chemicals, but the severity of effects can vary considerably

among species. The liver and its cells are often used to study the effects of TCDD on biochemical pathways that may be responsible for toxic end points.

Porphyria cutanea tarda, the most common clinical form of porphyria, has been reported in humans exposed to hexachlorobenzene and TCDD. A study in CYP1A2-null mice by Smith et al. (2001) demonstrated that the presence and inducibility of this gene are necessary for the uroporphyrinogenic effects of TCDD and contribute to TCDD-induced hepatocellular injury. Injury probably occurs through the metabolism of uroporphyrinogen III to uroporphyrin III, which cannot be used for heme synthesis. Data published by Robinson et al. (2002), however, suggest that a gene in addition to *Ahr* may modulate hepatic porphyria and injury caused by TCDD in mice.

Since the last update, the ability of TCDD or dioxin-like compounds to elicit changes in the expression of several genes or change the activity of proteins has been observed in liver cells from a number of species. They include the multidrug-resistance gene (Mathieu et al., 2001), the *N*-myristoyltransferase 2 gene (Kolluri et al., 2001), carboxylesterase (Yang et al., 2001), the low-molecular-weight prekininogen gene (Nukaya et al., 2001), poly (ADP-ribose) polymerase (Ma et al., 2001), and CYP2S1 (Rivera et al., 2002). Additional data on the abilty of TCDD to modulate the activity of the transcription factors AP-1, c-myc, and NF-κB (Ashida et al., 2000b; Puga et al., 2000b) and to induce CYP1A1 (Kono et al., 2001; Korner et al., 2002), CYP1B1 (Shehin et al., 2000), and an ecto-ATPase gene (Gao and Whitlock, 2001) have been published since *Update 2000*. Santini et al. (2001) observed that the induction of CYP1A1 in mouse hepatoma cells depends heavily on the stage in the cell cycle, and is markedly suppressed in G2/M cells. However, Muller et al. (2000) noted that TGF-β1 inhibits TCDD-induced CYP1 activities in primary rat hepatocytes. Ashida et al. (2000b) suggested that changes in activity of c-myc and AP-1 may be affected by TCDD-induced alterations in protein kinase and phosphatase activities. Shimba et al. (2000) also reported that TCDD treatment may upregulate the urokinase-type plasminogen activator gene by inducing binding of a 50-kDa protein to the mRNA. Several recent publications reported the use of advancing technologies, including microarrays, to analyze TCDD-induced gene changes in mouse (Kurachi et al., 2002) and human liver-tumor cells (Frueh et al., 2001; Puga et al., 2000a). Holman et al. (2000) used synchrotron infrared spectromicroscopy to determine that TCDD exposure to human liver-tumor cells results in a relative increase in the number of methyl–methylene groups in single cells; this suggests an increase in DNA methylation and altered gene expression. A publication by Kohn et al. (2001) described a model to characterize the relationships between rat liver concentrations of TCDD and enzyme induction.

Several research groups have suggested the induction of cellular oxidative stress as a mechanism by which TCDD could elicit damage via the AhR and lead to many of the toxic end points observed, including liver injury. Since *Update 2000*, several publications have examined the mechanism whereby that may

occur. Subchronic or acute exposure of rats and mice to TCDD has been shown to increase production of liver superoxide anion, thiobarbituric acid-reactive substances (evidence of lipid peroxidation), and DNA single-strand breaks (Bagchi et al., 2002; Hassoun et al., 2001, 2002; Slezak et al., 2000). Several other investigations suggested that changes in tissue catalase and glutathione peroxidase activities (Kern et al., 2002a), suppression of carbonic anhydrase III (Ikeda et al., 2000), or induction of xanthine oxidase–dehydrogenase activity (Sugihara et al., 2001) may contribute to the oxidative damage observed after TCDD exposure. TCDD treatment of mice was found to increase reactive-oxygen production by liver mitochondria, and liver ATP concentrations were significantly decreased at the peak times of reactive-oxygen production (Senft et al., 2002). In contrast, the induction of metallothionein by TCDD may play a protective role in TCDD-elicited oxidative stress responses (Nishimura et al., 2001). Notably, Arnt protein is also required by hypoxia-inducible factor-1α (HIF-1α) to enhance expression of various genes in response to hypoxia. The data provided by Nie et al. (2001) suggest that exposure to TCDD, via the recruitment of Arnt by the AhR, may repress HIF-1α-inducible responses. Hirai et al. (2002) found that α-tocopherol and L-dehydroascorbic acid, but not vitamin C, protect human cells in culture from toxicity induced by very high concentrations of TCDD.

Update 2000 reported that TCDD, via the AhR, blocks many estrogen-induced responses (see "Mechanisms Related to Effects on the Mammary Gland" below). Stanton et al. (2001a,b) demonstrated that TCDD treatment strongly blocked several responses of male chickens to estrogen, including hepatic lipid synthesis and metabolism and weight gain.

Mechanisms Related to Gastrointestinal Tract Effects Sterling and Cutroneo (2002) observed that TCDD and benzo[a]pryene induce CYP1A1 in rat small intestinal epithelial cells and human colon carcinoma cells. It cannot be concluded from those data alone, however, that human colon cells are responsive to TCDD; the authors noted that induction depended heavily on the presence of extracellular matrix and the differentiation state of those cells.

Mechanisms Related to Neurotoxicity Few studies have examined the possibility of nervous system damage in adult animals exposed to TCDD; the developing brain appears to be more sensitive (see "Developmental Toxicity" below). However, observed behavioral alterations—such as anorexia, weight loss, changes in circadian rhythm, and altered reproductive behavior—suggest that the nervous system may be affected even in adult animals. Huang et al. (2000) demonstrated that the AhR and Arnt are expressed in rat brain and pituitary and that CYP1A1 is induced in these tissues after TCDD exposure. Chronic exposure of *Cynomolgus* monkeys to TCDD was found to increase corticotropin-releasing hormone mRNA in the paraventricular nucleus of the hypothalamus (Shridhar et al., 2001). As in the liver, exposure to TCDD was shown to increase brain

oxidative stress as determined by increased superoxide anion, lipid peroxidation, and DNA single-strand breaks (Bagchi et al., 2002; Hassoun et al., 2001, 2002). The AhR was expressed and CYP1A1 was induced by TCDD in a rat glial cell line. In addition, cyclic-AMP-induced differentiation, as measured by extension of astrocyte processes and the induction of glial fibrillary acidic protein, was inhibited by TCDD; TCDD's inhibiting effect was blocked by an AhR antagonist (Takanaga et al., 2001). The AhR and Arnt have been found to be widely expressed throughout the brain and brainstem (Petersen et al., 2000).

Mechanisms Related to Immunotoxicity The animal immune system is highly sensitive to the toxic effects of TCDD, but the primary cell targets remain unclear. Multiple cell types make up this system, and many undergo rapid proliferation and differentiation in response to stress or a foreign antigen. It is possible, and indeed highly likely, that many cell types at different stages of development might be affected by TCDD.

In a recent review, Kerkvliet (2002) suggested that TCDD may cause the inappropriate activation of cells and lead to anergy or cell death and the premature termination of the immune response. Recent data on T-cell responses (Camacho et al., 2001; Fujimaki et al., 2002; Nohara et al., 2002a) and dendritic cell function (Vorderstrasse and Kerkvliet, 2001) in mice after TCDD exposure are consistent with that hypothesis. Hematopoietic stem cells also have been suggested as sensitive targets for TCDD (Murante and Gasiewicz, 2000). That effect in particular may contribute to a previously demonstrated reduction in the capacity of bone marrow from TCDD-treated mice to generate pro-T lymphocytes associated with elicited thymic atrophy. TCDD may also target thymocytes directly by inducing cell-cycle arrest (Lai et al., 2000).

The exact genes or biochemical alterations induced by TCDD leading to those effects are not known. Since *Update 2000*, TCDD has been shown to increase the production of TNF-1 by peripheral lymphocytes from exposed rhesus monkeys (Rier et al., 2001a), inhibit AP-1 activity in activated B cells (Suh et al., 2002), induce the expression of adseverin in mouse thymus (Svensson and Lundberg, 2001; Svensson et al., 2002), and induce the expression of the genes encoding Hrk and interleukin-3 receptor in Jurkat T cells (Park and Lee, 2002). TCDD has been shown not to affect the complement system in the guinea pig (Wagner et al., 2001). The role of the AhR in TCDD-induced immunotoxicity has been described in the previous updates, and additional data consistent with a role of the AhR have been published recently (Vorderstrasse et al., 2001).

Mechanisms Related to Carcinogenesis As indicated in previous updates, TCDD has been demonstrated to be a potent tumor promoter in several model systems. Its ability to induce cell proliferation and to alter differentiation is believed to be an important factor in the mechanism of TCDD-induced carcinogenesis. A study by Thornton et al. (2001) used Big Blue® lacI transgenic rats to

assess the mutagenicity of TCDD. After 6 weeks of exposure to TCDD at 2 μg/kg body weight, there was no increase in mutation frequency or change in mutation spectrum; this is consistent with many previous reports indicating the low mutagenic potential of this chemical. Also consistent with earlier data is the observation by Ramakrishna et al. (2002) of an increase in tumor multiplicity in mouse *N*-nitrosodimethlyamine-initiated lung tumors after treatment with a single dose of TCDD at 1.6 μg/kg body weight. The authors observed a significant decrease in membrane-associated K-ras protein p21 but an increase in raf-1; they hypothesized that these biochemical alterations constitute one mechanism by which TCDD may promote tumors.

Chronic bioassays have shown TCDD to increase the incidence of hepatic tumors in female, but not male, rats. A recent study by Wyde et al. (2002) indicated that this may be explained by the weaker potency of tumor promotion and DNA damage in male rats compared with females and lack of induction of cell replication in females. The investigators also found that hepatic 8-oxo-deoxyguanosine adduct formation, a measure of oxidative DNA damage, was significantly higher in TCDD-treated female rats and depended on the presence of 17-β estradiol (Wyde et al., 2001a). However, when diethylnitrosamine-treated ovariectomized rats were treated for 20 or 30 weeks with TCDD in the presence and absence of 17-β estradiol supplementation, estradiol supplementation did not appear to increase hepatotoxicity, as determined histopathologically and with analysis of serum characteristics (Wyde et al., 2000).

Several of the enzymes induced by TCDD, including CYP1A1 and CYP1A2, are responsible for the metabolic activation of many promutagens, so the activation of the AhR is considered to be important for the carcinogenic activity of many chemicals. Machala et al. (2001) determined that the ability of several polycyclic aromatic hydrocarbons to induce AhR-mediated gene expression could contribute to their mutagenic potential. Similarly, Jeffrey et al. (2002) observed that both activation of the AhR pathway and metabolism of benzo[a]pyrene are necessary for the transcriptional repression of the BRCA-1 gene by benzo-[a]pyrene. Repression of this gene may be a predisposing event in the onset of sporadic breast cancer. However, Uno et al. (2001) observed that although the lack of the CYP1A1 gene in CYP1A1-knockout mice protected the animal from benzo[a]pyrene-mediated liver toxicity and death by decreasing the formation of large amounts of toxic metabolites, there was a paradoxic increase in liver benzo[a]pyrene–DNA adduct formation because of the slower metabolic clearance of this chemical.

All available data suggest that the AhR plays a role in TCDD-induced tumor promotion. An interesting study by Andersson et al. (2002) found that transgenic mice that had a constitutively active AhR had reduced life span and induced tumors of the glandular part of the stomach. Up to the age of 6 months, the animals also had decreased thymic weights and increased liver weights, which were consistent with a simulation of low-dose exposure to TCDD. Notably, sev-

eral species of animals treated with AhR ligands have previously been reported to develop lesions of the glandular stomach. A recent study showed that TCDD and four AhR antagonists inhibited the growth of human pancreatic cell lines in a dose-dependent manner (Koliopanos et al., 2002).

Greenlee et al. (2001) observed that the proliferation of tumor cells and their ability to invade normal tissue are inhibited in the presence of TCDD. The authors suggest that that might be the basis of some rodent bioassays that show a decrease in the incidence of mammary tumors after TCDD exposure. On the other hand, there is data cited in this and earlier updates to suggest that under some conditions TCDD exposure may increase the risk for the development of breast tumors (see below and "Biologic Plausibility" for Breast Cancer in Chapter 6). Clearly, additional work is needed to determine how different exposure factors may affect breast tumor development.

Mechanisms Related to Effects on the Testis Many effects of TCDD in male rodents have been reported previously, including decreases in the size of accessory sex organs and in daily sperm production. Other investigations suggest that TCDD may cause tissue damage by induction of oxidative stress. Those findings are consistent with results of studies by Latchoumycandane et al. (2002a,b) in rat testis. Subchronic treatment of adult male rats with TCDD at 1–100 ng/kg of body weight per day resulted in a dose-dependent decrease in epididymal sperm counts and a decline in the activities of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase. There was also an increase in hydrogen peroxide and lipid peroxidation in testis. Similar changes were seen in the mitochondrial and microsomal fractions of testis. AhR activation was found by Ogi et al. (2001) to enhance the expression of the mouse Polκ gene. That gene is a low-fidelity DNA polymerase that appears to be developmentally regulated in the mouse testis. TCDD was also found to induce expression of DIF-3, a gene implicated in spermatogenesis in mouse embryonic stem cells (Ohbayashi et al., 2001). TCDD treatment had no effect on the production of testosterone by isolated mouse testicular cells, primary cultures of rat Leydig cells, or mouse Leydig tumor cells (Mandal et al., 2001; Uchida et al., 2002), although a single dose of TCDD at 50 μg/kg body weight resulted in a 75% decrease in serum testosterone in adult male rats. Mandal et al. (2001) concluded that the effect of TCDD on testosterone in rats may not be a direct result of decreased capacity of Leydig cells to synthesize steroids.

Mechanisms Related to Effects on the Prostate TCDD has been shown to induce CYP1A1 in three human prostate cancer cell lines (Jana et al., 2000; Schaufler et al., 2002). In the study by Jana et al. (2000), although expression of the CYP1B1 and PA1-2 genes were induced by TCDD in breast and uterine human cell lines, they were not induced in LNCaP prostate cells. TCDD also inhibited testosterone-induced reporter gene activation in all three cell lines,

suggesting that TCDD and the target steroid hormones negatively regulate each other's activity. No effects on cell-cycle distribution and cell growth were observed. CYP1B1 was induced in the PC3 and DU145 human prostate cell lines (Schaufler et al., 2002). Using human prostate-carcinoma cells, Schrader and Cooke (2000) observed that TCDD partially inhibited 5α-dihydrotestosterone-induced gene induction.

Mechanisms Related to Effects on the Ovary Immature female rats treated with TCDD before gonadotropin-induced follicular development and ovulation have been shown to produce significantly fewer ova than untreated control animals. A review by Petroff et al. (2001) indicated that a blockade of ovulation by TCDD probably involves actions at several target tissues and on multiple intracellular pathways. For example, they found an effect on matrix metalloproteinase in the follicular wall, altered hypothalamohypophyseal mechanisms responsible for the ovulatory luteinizing hormone surge, and effects on the expression of several genes including those that encode for cyclooxygenase-2, plasminogen activator inhibitor-2, and the progesterone receptor.

Data from Roby (2001) indicate that TCDD treatment alters the ability of the ovary to respond to gonadotropin and results in the abnormal development of follicles as assessed by lower gonadotropin binding, lower estradiol production, and lower concentrations of cAMP. Treatment of rats with gonadotropin-releasing hormone was able to partially restore ovulation inhibited by TCDD. The authors interpreted those data as confirming a direct effect of TCDD on the hypothalamic–pituitary axis and the ovary (Gao et al., 2000). A later study confirmed that TCDD decreased the responsiveness of the hypothalamus to estrogen as a feedback inducer of gonadotropin secretion (Gao et al., 2001). Blockade of ovulation by TCDD was alleviated by treatment with estradiol, although the systemic toxicity, as determined by weight loss, was increased (Petroff et al., 2000). A study by Petroff et al. (2002) suggested that inhibin, a member of the TGF superfamily, did not have a role in TCDD-elicited effects on the ovary. Recent investigations have suggested that the AhR-dependent alteration of Bax expression may have a role in developing fetal ovarian germ cells (Matikainen et al., 2002).

The AhR and Arnt were found to be expressed in rat ovary and liver, but the relative expression depended on the estrous cycle (Chaffin et al., 2000). Human uterine tissue and ovarian tissue were also found to express the AhR and Arnt (Khorram et al., 2002), but the relative expression was altered in some pathologic conditions, such as endometriosis and uterine leiomyoma.

TCDD has been reported to have a number of biochemical effects on cultured granulosa cells, including CYP1A1 and CYP1B1 induction and an increase in estrogen receptor-β mRNA (Dasmahapatra et al., 2001), reduction in expression of mRNA for P450scc and P450arom involved in the steroid biosynthetic pathway (Dasmahapatra et al., 2000), increases in luteinizing hormone receptor

protein and mRNA (Hirakawa et al., 2000), and alerations in the secretion of estradiol and progesterone (Pieklo et al., 2000). Gregoraszczuk et al. (2001) also observed that TCDD treatment decreased progesterone secretion of luteal cells. Studies by Rogers and Denison (2002) suggest that TCDD induces a factor that inhibits estrogen-dependent gene expression in human ovarian-carcinoma cells.

Mechanisms Related to Effects on the Uterus TCDD has been shown to decrease uterine weights in rodents and alter endometrial structure. Buchanan et al. (2000) examined the relative contributions of uterine stromal and epithelial cells to TCDD-induced effects. TCDD inhibited estradiol-induced epithelial-cell mitogenic and secretory activity, but the effects appeared to be mediated indirectly through the AhR in stromal cells. The authors postulated that TCDD impairs uterine epithelial function by altering normal stromal–epithelial interactions. Additional work by Buchanan et al. (2002) indicated that changes in epithelial-cell mitogenesis might involve changes in cell cycle and in cyclin and TGF-β expression.

Bulun et al. (2000) found transcripts of the AhR, Arnt, and several AhR target genes—including CYP1A1, CYP1A2, and CYP1B1—to be expressed in human endometriotic tissues. Pitt et al. (2001) found that expression of AhR mRNA in cultured human endometrial explants increases with age and that Arnt mRNA expression is tissue-phase- and age-dependent. The presence of endometriosis did not change the expression of the AhR and Arnt. Both CYP1A1 and CYP1B1 were induced by TCDD in those explant cultures (Bofinger et al., 2001). Hasan and Fischer (2001), however, observed that a change in AhR localization in rabbit uterus occurred in pregnancy and shortly before the expected time of implantation. Those data suggest that maternal steroid hormones may regulate AhR expression in this tissue. Neither estrogen nor progesterone inhibited induction of CYP1A1 activity by TCDD in human Ishikawa endometrial-cancer cells, although TCDD blocked estradiol-induced responses. However, estrogen was shown to inhibit induction of CYP1A1-dependent enzyme activity in ECC-1 endometrial cells (Wormke et al., 2000a). Both CYP1A1 and plasminogen activator inhibitor-2 were induced in the human RL95-2 uterine cell line (Jana et al., 2000). Coexpression of the estrogen receptor increased responsiveness of these cells to TCDD.

Some concern exists about the possibility that TCDD increases the prevalence of endometriosis in humans. Some of the evidence of such an effect comes from animal studies that have been summarized by Birnbaum and Cummings (2002). Although the mechanisms remain unclear, the authors suggest that effects of the dioxin-like compounds on growth factors, cytokines, and hormones may mediate the promotion of endometriosis.

Mechanisms Related to Effects on the Mammary Gland Human breast-cancer cells have been useful for investigating the mechanisms of AhR signaling

and the effects of TCDD on hormonally induced responses, especially responses to estrogen. *Update 2000* reported that TCDD has been shown to block many estrogen-induced responses in human breast-cancer cells. Wang et al. (2001) reported additional data on the mechanism whereby TCDD inhibits estradiol-mediated induction of the cathepsin D gene via the AhR. TCDD was shown by Porter et al. (2001) to inhibit estradiol-induced heat-shock protein 27 (hsp27) gene by a similar mechanism, that is, the binding of the AhR to a DRE present in the gene-promoter region. Using suppression subtractive hybridization, Chen I et al. (2001) identified 33 genes in human breast-cancer MCF-7 cells that are induced by estradiol and inhibited by AhR agonists and that may be important for mediating the antiestrogenic activity of TCDD. Additional work by Wormke et al. (2000b) also suggested that TCDD-induced activation of proteosomes and later increased degradation of estrogen receptor-α may contribute to the noted antiestrogenic activity of TCDD. Notably, expression of a constitutively active AhR in human MCF-7 cells enhanced expression of CYP1A1, inhibited estrogen-dependent cathepsin D expression, and inhibited growth of these cells (Kohle et al., 2002).

Angus et al. (2000) found that exposure of human breast epithelial cells to TCDD results in a time-dependent increase in membrane protein kinase erbB2 and erbB3 and leads to cell proliferation. The authors suggest that TCDD might facilitate the transition of breast cells from being estrogen-dependent to being estrogen-independent. That has been shown to be a key step in the progression of breast cancer and the overexpression of erbB2 and erbB3, which are negative prognostic indicators for survival. Studies by Davis et al. (2001) suggest that TCDD inhibits apoptosis in a human mammary epithelial cell line by stimulating the production of TGF-α, which results in the activation of the epidermal growth-factor receptor pathway. Kim et al. (2000) found that TCDD was able to induce the c-myc promoter in MCF-10F cells by a mechanism that required the interaction of the AhR with RelA and the binding to NF-κB elements in the promoter; the author suggested this may be a novel mechanism by which the AhR can stimulate proliferation and tumorigenesis of mammary cells.

TCDD was shown to induce the expression of CYP1A1, CYP1B1, and plasminogen activator inhibitor-2 in MCF-7 cells (Jana et al., 2000). Guo et al. (2001) found that the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) suppresses TCDD-induced CYP1A1 through a protein kinase C-dependent process in MCF-10A cells; alteration of TGF-β activity may also contribute to this suppression.

Mechanisms Related to Other Endocrine Effects As indicated in previous updates, TCDD has been shown to affect the thyroid and thyroid hormones in several animal species. TCDD treatment of rats increased thyroid-stimulating hormone concentrations in the anterior pituitary, changed thyroid structure in a way consistent with accelerated biosynthesis of T4 in the thyroid, but signifi-

cantly decreased both serum T4 and free T4 concentrations. The UDP-glucuronosyltransferase-1 gene was induced in the liver at TCDD doses as low as 1 μg/kg body weight. The authors suggest that induction of UGT1 is the main event that triggers the alterations in thyroid-hormone homeostasis. Additional studies by Craft et al. (2002) suggest that species differences in the response to chemicals that induce alterations in thyroid-hormone status may be due to differential induction of hepatic UGT enzymes.

Work by Pitt et al. (2000) indicates that a single exposure to TCDD during pregnancy does not appear sufficient to induce maternally mediated developmental, reproductive, and behavioral toxicity through an effect on the hypothalamic–pituitary–adrenal axis.

Mechanisms Related to Developmental Toxicity Extensive data from studies in experimental animals suggest that developing tissues are highly sensitive to the toxic effects of TCDD as mediated by the AhR and that tissue growth and differentiation processes are affected.

Matthews et al. (2001) examined the effect of TCDD treatment of mouse embryos in culture on the number of embryos developing from the morula to the blastocyst stage and the number of apoptotic blastomeres; no effects of 3 μM TCDD were observed. And Wu et al. (2002) did not observe CYP1A1 mRNA in mouse embryos at the one-, two-, and eight-cell stages after exposure to TCDD, whereas CYP1A1 mRNA was significantly increased at the blastocyst stage. TCDD exposure had no effect on the rate of development of preimplantation embryos, the cell number of blastocyst embryos, or apoptotic indexes.

Bryant et al. (2001a) examined the effects of loss of EGF and TGF-α expression on the incidence of hydronephrosis and cleft palate in developing mice. Animals expressing no EGF did not develop cleft palate after maternal TCDD exposure. The lack of the EGF or TGF-α genes, however, increased the incidence and severity of TCDD-induced hydronephrosis. Previous studies have found that TCDD produces hydronephrosis by altering the differentiation and proliferation of ureteric epithelial cells in fetal mouse urinary tract. Bryant et al. (2001b) found that late-gestational ureteric cells in vitro respond to TCDD by a stimulation of epithelial growth and differentiation, but that effect was not observed in the presence of EGF over longer culture periods. Notably, immunostimulation was found to decrease the incidence of cleft palate in mice significantly in response to TCDD; the decrease is thought to be due to a modulation of growth factor or cytokine production in developing tissues (Holladay et al., 2000). TCDD was also found to retard lower jaw development and circulation in the zebrafish embryo; however, the reduction in perfusion rate occurred well after the inhibition of jaw development (Teraoka et al., 2002).

Update 2000 cited several reports indicating that development of the male reproductive system is exceptionally sensitive to in utero and lactational TCDD exposure. Impaired prostate growth has been shown consistently, and recent

studies confirm it. Ohsako et al. (2001) reported that low-dose administration of TCDD in rats affected the development of the external genital organs and ventral prostate more than the testis and other internal genital organs. They also suggested that the effect of TCDD on the prostate may be due to decreased responsiveness of the prostate to androgen and decreased expression of androgen receptor. Theobald et al. (2000) found that the ability of the ventral prostate to form 5α-dihydrotestosterone was not significantly altered in rats after in utero and lactational exposure. However, the androgen-induced expression of prostate-binding protein subunit C3 was transiently decreased, and the formation of androgen-responsive luminal epithelial cells was inhibited. Altered prostate epithelial-cell differentiation was hypothesized as the major effect leading to impaired prostate growth. Timms et al. (2002) demonstrated that intrauterine position and individual differences in gonadal steroid concentrations influenced the response of the developing prostate to TCDD and that these effects may be mediated by a decrease in serum estradiol. Additional studies by Ohsako et al. (2002) indicate that there is a critical and narrow window of exposure—about gestation day 15 in the rat—during which the fetus is sensitive to these TCDD effects. Using *Ahr* null-allele mice, Lin et al. (2002) demonstrated that effects depend on the AhR. Notably, they also found that development of several tissues—including liver, heart, spleen, thymus, lung, submandibular gland, testis, epididymis, and kidney—are affected by absence of the AhR (Lin et al., 2001). Haavisto et al. (2001) reported that exposure of male rats in utero caused a stimulatory effect on testicular testosterone synthesis and increased circulating testosterone; these effects were caused by stimulation of pituitary luteinizing-hormone production and enhanced sensitivity of the fetal testis to luteinizing hormone. Slezak et al. (2002) reported that male rat pups exposed perinatally to TCDD showed increased production of reactive oxygen species in the liver, although no alteration in lipid peroxidation or total glutathione was observed.

Lewis et al. (2001) reported that in utero and lactational exposure of female fetuses to TCDD impairs mammary gland differentiation as determined by the distribution of terminal ductal structures and increased expression of estrogen receptor-α. The ability of those tissues to differentiate in response to estrogen, however, was not affected. That result is consistent with data published by Fenton et al. (2002), who also observed reduced lactation and milk production by the offspring when they reach adulthood. Exposure at gestation day 15 appeared to be critical for the inhibition of breast epithelial development.

As indicated in the previous updates, several reports of studies in animals and exposed humans suggest that perinatal exposure to TCDD or dioxin-like chemicals may impair brain development. Recent publications by Markowski et al. (2002) and Hojo et al. (2002) indicate that low-dose prenatal TCDD exposure (less than 0.54 μg/kg body weight) of rats results in subtle behavioral effects, including altered sexually dimorphic behavior and impaired ability to inhibit or delay voluntary behavior. Several publications have documented specific bio-

chemical changes in the brains of animals after transplacental exposure to TCDD, including altered hippocampal astroglia–neuronal gap junction communication (Legare et al., 2000), changes in NMDA receptor subunit mRNA expression (Kakeyama et al., 2001), alterations in fetal brain aromatase activity (Ikeda et al., 2002), a decrease in serotonin-immunoreactive neurons in raphe nuclei of male offspring that lasted up to the age of 42 days (Kuchiiwa et al., 2002), and altered Sp1 DNA binding in the developing cerebral cortex and cerebellum (Nayyar et al., 2002).

Ishimura et al. (2002) observed that treatment of pregnant rats with a single oral dose of TCDD at 0.8 or 1.6 μg/kg body weight on gestation day 15 increased glycogen content and glucose transporter 3 mRNA. The authors hypothesized that altered glucose kinetics in the placenta contribute to TCDD-induced fetal toxicity.

Disease Outcomes

Lethality and Defects

An unusual characteristic of TCDD is the large variation among species in susceptibility to its lethal effects. Both intraspecies variations and interspecies variation can occur; some of the difference in sensitivity is related to differences in the expression and primary amino acid sequences of the AhR protein. Regardless, most species develop a wasting syndrome when exposed to acute toxic doses of TCDD.

Mink in particular appear to be highly sensitive to the toxic effects of TCDD. In a recent study (Hochstein et al., 2001), mink fed diets containing TCDD at 0.016–1.40 ppb exhibited several dose-dependent adverse health effects, including the wasting syndrome. Theirs was the first report of thickened, deformed, and elongated toenails in TCDD-exposed mink. The effects of TCDD on toenails in mink are reminiscent of metaplasias seen in nail beds of rhesus macaques treated with PCB mixtures (McNulty, 1985) and pigmented or deformed nails in children exposed to PCBs in utero following a poisoning in Taiwan from PCB contaminated cooking oil (Rogan et al., 1988). In another study (Render et al., 2001), loose and displaced incisor teeth were observed in mink kits fed TCDD at 2.4 ppb; radiographs of the jaw showed maxillary and mandibular osteolysis with lysis of the lamina dura.

Kattainen et al. (2001) exposed three lines of F10 generation rats from Hans/Wistar and Long/Evans crossings to TCDD in utero and via lactation; TCDD at 1 μg/kg body weight prevented the development of the third lower molars in the most sensitive lines. The authors acknowledge that tooth development appears to be a highly sensitive end point of TCDD-induced toxicity. A large proportion of the pups from mother rats exposed 1 day after birth to TCDD at 1,000 μg/kg body weight lacked their third molars (Lukinmaa et al., 2001). However, in contrast

with prevention of development of the lower third molars after in utero exposure, the molars missing after neonatal (lactational) exposure were most frequently the upper third molars. It has been proposed that coexpression of the AhR and ARNT during early tooth development and during the formation and mineralization of the dental matrix suggests that the AhR–ARNT pathway is a mediator of dental toxicity of TCDD (Sahlberg et al., 2002).

Cardiovascular Toxicity

There is a paucity of information on the potential cardiovascular toxicity of TCDD. In a study in which TCDD in corn oil was injected into chicken eggs at 0.40 pmol/g egg, TCDD reduced the size and number of coronary arteries (Ivinitski et al., 2001). The effect was associated with a reduction in proliferation of monocytes that was preceded by an increase in apoptosis in structures in which cell death normally occurs. Others have suggested that TCDD induces developmental defects in the cardiovascular system—that the cells that line the blood vessels could be a target of TCDD toxicity (IOM, 1999). A study by Dong et al. (2002) demonstrated that a decrease in blood flow in the mesencephalic vein is one of the earliest adverse effects of TCDD in the zebrafish embryo; they hypothesized that the decrease is a result of endothelial damage caused by oxidative stress associated with CYP1A induction. Dalton et al. (2001) observed that daily treatments of mice with 5 μg of TCDD for 3 days resulted in an increase in urinary excretion of vasoactive eicosanoids and serum triglycerides and an increase in mean tail-cuff blood pressure.

Subchronic treatment of hyperlipidemic apolipoprotein E (ApoE)-null mice with TCDD at 150 ng/kg body weight three times a week for 7 or 26 weeks caused a trend toward earlier onset and greater severity of atherosclerotic lesions than in vehicle-treated mice. Notably, ApoE-deficient mice have a lipoprotein profile similar to that of humans with type III hyperlipoproteinemia, and they develop extensive aortic and coronary atherosclerosis with lesions that are similar to those observed in humans. In a recent study (Riecke et al., 2002), male marmosets treated with a single subcutaneous injection of TCDD at 100 ng/kg body weight showed no overt signs of toxicity or effects on heart weights. Histologic examination, however, revealed an increase in picrosirius red-positive areas in the hearts of the TCDD-treated marmosets compared with controls. Western blotting confirmed an increase in collagen, fibronectin, and laminin in the hearts of the TCDD-treated marmosets.

Although the cardiovascular system appears to be affected predominantly in developing animals, there is only minimal evidence that it is a primary target of TCDD toxicity in adult animals. Further research is needed to determine whether exposure to TCDD might exacerbate lesions in blood vessels and be a risk factor for atherosclerosis.

Pulmonary Toxicity

Very little information is available to incriminate TCDD as a pulmonary toxicant in acute exposures. When rats were treated with TCDD at 125 ng/kg body weight for 60 weeks, there was a significant increase in alveolar–bronchiolar metaplasia (Tritscher et al., 2000). In the same study, when rats were initiated with diethylnitrosamine and then exposed to TCDD at 125 ng/kg body weight for 60 weeks, bronchiolar epithelial hyperplasia was noted. But the lesions were not observed in rats treated with TCDD for 30 weeks and then with corn oil for 30 weeks; continuous TCDD exposure might be required for their continued development. The study suggests that chronic oral exposure to TCDD can induce metaplasia and proliferative changes in the lung.

Hepatotoxicity

The liver is a primary target organ of halogenated aromatic hydrocarbons, but the severity of lesions varies considerably among species (IOM, 2001). It has been reported that a single oral dose of TCDD can inhibit normal hepatic accumulation of dietary vitamin A (Kelley et al., 1998); this effect has recently been confirmed in four species: hamster (TCDD at 0.9 μg/kg body weight), guinea pigs (0.1 μg/kg body weight), rats (1.1 μg/kg body weight), and mice (3.6 μg/kg body weight) (Fletcher et al., 2001).

Neurotoxicity

Some studies have implicated TCDD as a neurotoxin, but others have been unable to show neurotoxic activity (IOM, 1999, 2001). Hans/Wistar rats given TCDD intraperitoneally at 1,000 μg/kg body weight exhibited weight loss but no neurologic impairment (IOM, 2001). However, male and female Sprague-Dawley rats exposed in utero to TCDD at 1 μg/kg maternal body weight showed a deficit in learning in the spatial discrimination-reversal learning (RL) task, whereas the male progeny also showed a facilitation of task-specific spatial learning and memory (IOM, 2001). A study in primates showed that prenatal exposure to TCDD facilitated some spatial tasks but impaired visual RL tasks (IOM, 2001). In a more recent study in which Holtzman rats were exposed to TCDD at 20–180 ng/kg body weight on gestation day 18, the offspring had dose-dependent reductions in the number of earned opportunities to run on specifically designed running wheels, in lever response rates, and in total number of revolutions on the wheel (Markowski et al., 2001). Another study by the same group (Markowski et al., 2002) showed that female Holtzman rats exposed in utero to a single dose of TCDD at 0.18 μg/kg maternal body weight on gestation day 15 had significantly less accuracy and committed more errors in lever chambers than did the non-exposed controls. It was shown recently that GABA neurons in the brain are

targets of TCDD (Hays et al., 2002). In 3-day-old pups exposed in utero to TCDD at 1 μg/kg maternal body weight on gestation day 15, virtually all GABA neurons expressed the AhR gene. The noted effects of in utero exposure to TCDD have not been observed when animals have been exposed postnatally. In some species, the neurobehavorial development of the fetus seems to be sensitive to the toxic effects of TCDD.

Reproductive and Developmental Toxicity

It has been discussed in previous updates that low doses of TCDD can affect reproductive development and fertility of progeny. In *Update 2000*, one study demonstrated decreased sperm production and an increased number of abnormal sperm in male offspring of female rats treated with TCDD from before mating through lactation. All TCDD-exposed males, however, were able to impregnate females and produce viable fetuses in them. Other reproductive indexes evaluated in the study were also unaffected by TCDD.

TCDD has been reported to decrease seminal vesicle growth and prostate weight in male rats (IOM, 2001). Recent studies have confirmed that in utero exposure to TCDD impairs prostate growth. One study suggested that low-dose (TCDD at 800 ng/kg body weight) administration of TCDD to pregnant Holtzman dams had a greater effect on development of the external genital organs and ventral prostate than on development of the testis and other internal genital organs in the male offspring (Ohsako et al., 2001). A continuation of those studies (Ohsako et al., 2002) showed that TCDD exposure at 1 μg/kg maternal body weight on gestation day 18 resulted in significant decreases in the urogenital complex and ventral prostate weight and in urogenital–glans penis length (the length between the anterior end of the urethra and the glans penis) of male offspring. In utero TCDD exposure (1 μg/kg body weight) in Holtzman rats impaired prostate growth and androgen responsiveness by inhibiting prostate epithelial-cell differentiation (Theobald et al., 2000).

Administration of TCDD at 1.0 μg/kg maternal body weight on gestation day 13.5 resulted in increased pituitary luteinizing hormone concentrations and testicular testosterone synthesis in Han/Wistar fetuses but not in Long/Evans fetuses (Haavisto et al., 2001). Testosterone concentrations, however, were not affected in offspring of Holtzman rats given a single oral dose of TCDD at 1 μg/kg body weight or when primary murine testicular cells were cultured with TCDD (Timms et al., 2002; Uchida et al., 2002). Male Sprague-Dawley rats treated with TCDD at 10 μg/kg body weight had a reduced adrenocorticotropin-to-corticosterone ratio, suggesting that TCDD disturbs the hypothalamic–pituitary–adrenal axis (Pitt et al., 2000); this effect, however, was not detected in the pregnant females. It has been suggested that dioxin inducible factor-3 may be a target gene for TCDD during spermatogenesis (Ohbayashi et al., 2001).

TCDD exposure can also affect female reproduction. Female Sprague-Dawley rats given TCDD at 32 μg/kg body weight had reduced numbers of ova in

their oviducts, which affected ovulation (Gao et al., 2000). It was also noted that gonadotropin-releasing hormone induced surges in luteinizing hormone and follicle stimulating hormone, but the increases only partially restored the inhibitory effects of TCDD on ovulation. The female fetuses and offspring of pregnant Long-Evans rats treated with TCDD at 1 μg/kg maternal body weight on gestation day 15 were evaluated for developmental effects (Fenton et al., 2002; Hurst et al., 2002). In the fetus, the mesenchyme that separates the Müllerian ducts was widened, and the zone of unfused ducts was increased (Hurst et al., 2002). The postnatal effects included delayed vaginal opening and persistent vaginal threads but no altered estrous cycle (Fenton et al., 2002). There was also a reduction of the primary branches, decreased epithelial elongation, and fewer alveolar buds and lateral branches in the mammary glands of the TCDD-exposed rats.

Nonhuman primates (rhesus monkeys) had increased serum TCDD and endometriosis 13 years after treatment with TCDD at 25 ng/kg body weight (Rier et al., 2001b). They also exhibited an increase in TNF-α secretion from peripheral-blood mononuclear cells and a decrease in cytolytic activity of natural killer cells (Rier et al., 2001a). In a study in which cynomolgus macaques were treated with a single oral dose of TCDD at 4 μg/kg body weight, the menstrual cycle was eliminated and mean follicle-stimulating hormone concentrations were increased (Moran et al., 2001); the endometria of the noncycling monkeys were inactive. In another study in which cynomolgus monkeys were treated with TCDD at 25 ng/kg body weight, progesterone concentrations and menstruation data indicated that TCDD did not interfere with ovulation (Shridhar et al., 2001). TCDD stimulated production of the corticotropin-releasing hormone, however, suggesting that the hypothalamic–pituitary–adrenal pathway is stimulated. TCDD (4 μg/kg body weight) has also been shown to induce epithelial transdifferentiation in the cynomolgus cervix 1–2 years after treatment (Scott et al., 2001). When human endometrial explants were cultured in medium containing estradiol or estradiol and progesterone, TCDD significantly increased the expression of the AhR. TCDD did not affect ARNT mRNA or endometriosis (Bofinger et al., 2001; Pitt et al., 2001).

In a teratogenic study in which pregnant female mice were treated with TCDD at 24 μg/kg body weight on gestation day 12, cleft palate and hydronephrosis were induced in the offspring (Bryant et al., 2001a). An evaluation of the role of epidermal growth factor (EGF) using wild-type mice and knockout mice (mice that do not express EGF) revealed that EGF influences the induction of cleft palate by TCDD but that EGF is not required for formation of hydronephrosis.

Endocrine Effects

TCDD has been reported to affect concentrations of thyroid hormone, but contrasting results confuse interpretation. In a recent study (van der Plas et al., 2001), Sprague-Dawley rats exposed to TCDD at 1 μg/kg body weight per week

exhibited a decrease in total thyroid hormone but an increase in plasma retinal. Most TCDD investigations, however, have focused on assessing the effects of hormonal changes on reproduction. Most of those effects are mentioned in the preceding section. Estradiol produced by mature ovarian follicles triggers increased secretion of gonadotropin-releasing hormone. A recent study reported that TCDD at 32 µg/kg body weight decreased the responsiveness of the hypothalamus to estradiol, which normally acts as a feedback inducer of preovulatory gonadotropin secretion in Sprague-Dawley rats (Gao et al., 2001). In another study, it was determined that estradiol was not associated with the hepatotoxicity commonly observed in TCDD-treated rats (Wyde et al., 2000).

Immunotoxicity

The immune system of animals is highly sensitive to the toxic effects of TCDD and is a primary target of TCDD toxicity. For that reason, many investigations have focused on the immunotoxic effects of TCDD and the mechanisms responsible for them. TCDD is a potent immunosuppressant in laboratory animals at extremely low doses (0.1 µg TCDD/kg body weight or lower). There are, however, considerable differences in the immune response between rats and mice and strain differences within those species. Both resistant and sensitive strains have been identified for the numerous immunotoxic end points of TCDD toxicity.

TCDD has been shown to alter host resistance to infectious disease. It was reported that a single dose of TCDD increased the mortality of mice infected with influenza A (Burleson et al., 1996). That effect, however, was not confirmed in a more recent study using a similar protocol in four strains of mice (B6C3F1, BALB/c, C57Bl.6N, and DBA2) (Nohara et al., 2002b). The reasons for the discrepancy between the studies are not clear. Another group reported that in mice treated with TCDD (10 µg/kg body weight) and influenza virus there was no increase in the pulmonary virus burden; that suggested that TCDD impaired viral replication in lung epithelial cells (Lawrence et al., 2000). In another disease-susceptibility investigation, brain lesions and number of brain cysts after *Toxoplasma gondi* infection were not altered in C57Bl/6 mice dosed with TCDD at 50 µg/kg body weight (King et al., 2000). But in male A/J mice given TCDD intraperitoneally at 5 µg/kg body weight followed by three weekly doses at 1.4 µg/kg body weight, an increase in mortality from myocardial coxsackievirus B3 infection was seen 7 days after virus inoculation; no effect on the inflammatory lesions in the myocardium was seen (Funseth et al., 2002). When Brown Norway rats were exposed concurrently to TCDD (30 µg/kg bw) and house mites, TCDD exposure suppressed, rather than enhanced, immune response, as measured by immunoglobulin E (IgE) synthesis, and decreased immune-mediated lung disease (Luebke et al., 2001). TCDD has also been shown to stimulate the expression of IgE-dependent histamine-releasing factor mRNA via the AhR-dependent pathway (Oikawa et al., 2002).

In thymocytes obtained from C57BL/6N mice and treated in culture, TCDD skewed differentiation of thymocytes toward CD8 T cells, which require activation of the extracellular signal-related kinase pathway (Tsukumo et al., 2002). NC/Nga mice given TCDD intraperitoneally at 5.0 or 20 μg/kg body weight markedly suppressed the concentrations of interleukin-4 (IL-4) and interleukin-5 (IL-5) in culture supernatants of spleen cells (Fujimaki et al., 2002). TCDD exposure also reduced anti-ova antibody and total IgE antibody titers in the plasma of those mice. A recent study indicates that the maturing $B220^+$ B cells are not the direct target for TCDD-induced toxicity but that hematopoietic progenitor cells are most likely a direct target for TCDD-mediated effects (Wyman et al., 2002).

Dendritic cells play a major role in activation of naive T cells. When dendritic cells from C57Bl/6 mice treated with TCDD at 15 μg/kg body weight were cocultured with allogeneic T cells, the proliferative response and production of IL-2 and interferon-δ were increased, as was the production of IL-12 (Vorderstrasse and Kerkvliet, 2001). The total number of dendritic cells recovered from the TCDD-treated mice, however, was significantly decreased. It was suggested that TCDD leads to premature deletion of dendritic cells. TCDD (15 μg/kg bw) has also been shown to interfere with survival and differentiation of OVA-specific T-helper cells; that interference could prevent expansion and differentiation of those cells into effector T-helper cells (Shephard et al., 2000).

In utero exposure of rats on gestation day 15 to TCDD at 12.5–800 ng/kg maternal body weight had no significant effect on the thymic or splenic weights of the offspring (Nohara et al., 2000). A dose-dependent induction of CYP1A1 mRNA occurred in the thymus of the offspring, and a very weak induction occurred in the spleen of the offspring. In contrast, there was no effect of TCDD on cell numbers or populations (CD4 and CD8 markers) in the thymus, but splenocyte numbers decreased in a dose-dependent manner with no effect on splenic cellularity.

The complement system affords a defense against microbial infections. Administration of TCDD to guinea pigs at 0.5 μg/kg of body weight did not induce any significant change in complement activity (Wagner et al., 2001). That suggests that the complement system is resistant to the toxic effects of low doses of TCDD.

Carcinogenicity

TCDD is a known hepatocarcinogen in rats and mice and is considered to be a carcinogen in humans. It is not genotoxic but acts as a promoter involving multiple pathways in regulatory cell proliferation and differentiation. TCDD is known to promote hepatic neoplasia in laboratory rats at doses as low as 0.01 ng/kg body weight per day (IOM, 2001). Recent multistage carcinogen models have used a variety of initiators—including diethylnitrosamine, azaserine, and methylnitrosourea—to investigate the tumor-promoting activities of TCDD (Desaulniers

et al., 2001; Oztas, 2000; Wyde et al., 2001a, b). They showed that TCDD promoted hepatocellular foci formation, pancreatic acinar cell foci, and mammary-tumor development. In another experiment using diethylnitrosamine as an initiator, TCDD (1.75 µg/kg bw) administered continuously (biweekly) for 60 weeks, but not 30 weeks, to Sprague-Dawley rats promoted development of hepatocellular adenomas and carcinomas (Walker NJ et al., 2000). In fact, there were considerably fewer hepatocellular adenomas and carcinomas combined in the 30-week TCDD-treated group (17%) than in the vehicle control group (55%). Those data indicate that liver promotion by TCDD in female rats depends on long-term continuous exposure to TCDD. Another feature of that study is that the mean focus volume of preneoplastic altered hepatic foci (AHF) continued to increase after the 30-week treatment with TCDD.

Conclusions

TCDD is one of the most highly toxic chemicals known to affect animals, but there is an extreme range (of a factor of 1,000) in lethal effects among species and even within strains of animals. Oral intake is the primary route of exposure, and doses in nanogram amounts can elicit toxic effects in the most sensitive strains and species. The most sensitive time of exposure to TCDD is exposure of the fetus during pregnancy; toxicity results primarily in the nervous, immune, and reproductive systems. Structural abnormalities can also be a result of prenatal exposure to TCDD. TCDD is a potent promoter of hepatocarcinogenesis and is a hepatotoxin in animals. The immune system of animals is particularly sensitive to the toxic effects of TCDD; TCDD is immunosuppressive at doses lower than 0.1 µg/kg body weight. The endocrine system also appears to be a sensitive target of TCDD. Endocrine effects on the male and female reproductive tract have been reported at extremely low doses of TCDD. As mentioned above, the most toxic effects occur when animals are exposed in utero, but TCDD also interferes with reproduction when sexually mature animals are exposed. The cardiovascular and pulmonary systems appear more resistant to the toxicity of TCDD than the aforementioned organ systems.

SUMMARY OF TOXICITY PROFILES

This section synthesizes the experimental data on 2,4-D, 2,4,5-T, picloram, cacodylic acid, and TCDD reviewed in this and previous *VAO* reports, with a focus on recent data.

2,4-D

The majority of the studies of 2,4-D have found it to be relatively nontoxic, with health effects in animals observed only at high doses. Three studies pub-

lished since *Update 2000* support earlier findings demonstrating that tissue uptake of 2,4-D is poor and metabolism fairly rapid, which could partially explain its low toxicity.

Earlier studies demonstrated the potential of high doses of 2,4-D to cause behavioral effects, muscle weakness, and incoordination in animals. Recent studies have demonstrated the ability of 2,4-D to damage nerve cells and produce neurotoxicity, but they tested high concentrations of the chemical after in vitro exposure or direct injection into the brain and therefore might not be relevant to physiologic exposures. The reproductive and developmental effects of 2,4-D have been examined recently. No developmental toxicity was seen in one study, but recent studies suggest that neonatal exposure to 2,4-D (100 mg/kg body weight per day) can result in alterations in brain development. A study of the effects of paternal exposure to 2,4-D did not demonstrate any effects on fetal survival or malformations. The effects of in utero exposure to 2,4-D on immune function in the offspring demonstrated effects only at the highest dose tested (650 mg/kg body weight per day). Carcinogenicity tests of 2,4-D have generally been negative; a recent study looking at the effects of 2,4-D on urethan-induced lung adenomas did not show an increase in adenomas with 2,4-D treatment, and thus supported 2,4-D's lack of carcinogenicity. Previous studies have suggested that 2,4-D might affect thyroid hormones (more specifically serum thyroxine). A recent study found that both thyroxine and triiodothyronine concentrations decreased after oral exposure to 2,4-D.

Genotoxicity tests and mechanistic studies have also been conducted on 2,4-D. It is either nongenotoxic or only weakly mutagenic in the many assays used. 2,4-D has been shown to have a number of effects on cells or biochemical measures, including effects on some hormones, on cellular components involved in the development and functioning of brain cells, and on some enzymes and transporters. Effects on calcium metabolism and energy metabolism, possibly through direct effects on mitochondrial function, have also been seen in response to 2,4-D treatment, as have effects on stress proteins. The relationship of any of those effects to any disease outcomes in animals or humans, however, is unknown.

Taken all together, the experimental data reviewed in this and previous reports indicate that 2,4-D is relatively nontoxic, with neurodevelopmental effects after neonatal exposure (at 100 mg/kg body weight per day) being one of the few effects seen.

2,4,5-T

Although not a great deal of research has been conducted recently on 2,4,5-T, the available data indicate that 2,4,5-T itself is relatively nontoxic. Studies indicate that 2,4,5-T is absorbed into the body after oral exposure, but absorption after dermal exposure is much slower. No recent toxicokinetic studies have been conducted. Studies of the reproductive effects of 2,4,5-T have demonstrated that

it can be fetotoxic in rodents at doses greater than 20 mg/kg body weight per day on days 6-15 of pregnancy, retarding growth and causing increased embryolethality and cleft palate. No such effects were seen in rabbits, sheep, or monkeys, and evidence suggests that TCDD contamination of the 2,4,5-T might underlie the reproductive effects seen in rodents. The carcinogenicity of 2,4,5-T has also been investigated; no indications of carcinogenicity were seen. Studies of the genotoxicity of 2,4,5-T show it to have weak genotoxic potential. Little is known regarding the cellular effects of 2,4,5-T, but it has been shown to alter cellular metabolism (for example, on the acetylcoenzyme A system), affect cholinergic transmission and the tyrosine kinase receptor, and disrupt apoptosis. As in the case of 2,4-D, the relevance of those effects to human diseases is not known, and the data consistently indicate that 2,4,5-T is relatively nontoxic.

Cacodylic Acid

Cacodylic acid, or DMA, is a metabolite of inorganic arsenic. As discussed in Chapter 2, because the relevance of studies of inorganic-arsenic exposure for evaluating effects of exposure to cacodylic acid has not been established and cannot be inferred, the literature on inorganic arsenic is not considered in this report. Methylation of inorganic arsenic to DMA was long thought to be a detoxification pathway. More recently, however, the trivalent methylated forms of arsenic, DMA^{III} and MMA^{III}, have been shown to be toxic—following acute exposure MMA^{III} is about 4 times more toxic than inorganic arsenic and DMA^{III} has similar toxicity to that of $arsenic^{III}$ (NRC, 2001). Urinary excretion of DMA appears to be species-dependent; rapid excretion occurs in many animals. Rats, however, accumulate DMA in red cells and tissues.

Few animal studies are available on the noncancer health effects of cacodylic acid, but previous reports indicate that high, maternally toxic doses are fetotoxic and teratogenic in rats and mice. With respect to carcinogenicity, there is evidence that cacodylic acid can promote skin tumorigenesis in animals that are initiated chemically or with UVB radiation. Evidence of cacodylic acid's pulmonary and bladder carcinogenic activity has also been seen in mice and rats, respectively. In other studies, however, cacodylic acid did not promote kidney tumors or lung tumors in nitrosamine-initiated rats.

A primary mechanism of the acute toxicity of arsenic is interference of cellular respiration, but the mechanisms underlying the effects of cacodylic acid are not well understood. Some data indicate that cacodylic acid acts through induction of oxidative damage or damage to DNA, and it has been shown to affect microtubule networks at particular points in mitosis. A recent study demonstrated that cacodylic acid causes necrosis of the epithelium of the urinary bladder followed by regenerative hyperplasia, and other studies have found that cacodylic acid is a potent inducer of apoptosis (or programed cell death).

Picloram

Few studies have been conducted on the toxicity of picloram, but those done indicate that it is relatively nontoxic. Two of three carcinogenicity studies reviewed in *VAO* indicate that pilcoram is not carcinogenic; a third was positive for liver tumors, but on review of the data, an Environmental Protection Agency committee concluded that the tumors were due to contamination with hexachlorobenzene (HCB). The *VAO* committee did note, however, that because the study was carried out with technical picloram, the compound used in Vietnam most likely contained similar amounts of HCB. Although the data on reproductive effects are not extensive, no effects considered to be treatment-related have been seen. Notably, a study of the male-mediated reproductive toxicity of Tordon 75D® (a commercial mixture of 2,4-D and picloram) found no effects on fetal survival or malformations. Another commercial mixture of 2,4-D and picloram, Tordon 202C®, had immunotoxic effects, reducing antibody production in mice in response to sheep red-cell inoculation at concentrations only marginally above those expected to be encountered after recommended application of the herbicide. Once again, however, the relevance of the few effects seen to human health outcomes is not known; taken together, the data indicate that picloram is relatively nontoxic.

TCDD

In contrast with the effects of the herbicides themselves, the effects of TCDD, a contaminant of 2,4,5-T, have been studied extensively. TCDD is hydrophobic and therefore is absorbed well across membranes, distributes to all compartments of the body, and partitions with lipids. Data also indicate that TCDD is transferred across the placenta to the fetus and is transferred to neonates through lactation. The enzyme cytochrome P450 1A2 (CYP1A2) plays an important role in the distribution of TCDD. Studies of TCDD in Ranch Hand Vietnam veterans indicate that it has a mean half-life of 7.6 years. Recent studies in two people exposed to very high amounts of TCDD, however, showed an elimination half-life of 1.5 and 2.9 years in the more and less exposed people, respectively, indicating that the half-life depends on body burden. Recent data from Seveso also indicate that the half-life is shorter in the first 3 months after exposure than from 3 to 16 years after exposure. Those data on half-life are consistent with a two-compartment toxicokinetic model for TCDD. Olestra somewhat increased the excretion of TCDD in the two heavily exposed patients, and this is consistent with earlier studies that indicate that the diet can affect the toxicokinetics of TCDD. A recent study in rats demonstrated that dietary seaweed can increase TCDD excretion, but whether the increase would occur in humans is not known. Evidence also suggests that the half-life is correlated with body weight. TCDD concentrations are often measured in blood, and previous and recent autopsy

studies indicate that blood concentrations correlate with tissue concentrations. Studies have also been conducted to validate physiologically based pharmacokinetic models to estimate the distribution and tissue concentrations of TCDD. Such models appear to be useful for toxicokinetic predictions.

Many effects have been observed in animals after exposure to TCDD, and TCDD is considered more toxic than the active ingredients of the herbicides used in Vietnam. Sensitivity to the lethal effects of TCDD varies among species and strains, but most species studied develop a wasting syndrome after acutely toxic doses that is characterized by a loss of body weight and fatty tissue. One target of TCDD is the liver, where lethal doses of TCDD cause necrosis, but the effect depends on the species exposed. Effects on the structure and function of the liver are also seen at lower doses. A recent study demonstrated that TCDD inhibits the ability of the liver to accumulate vitamin A.

TCDD may affect, directly or indirectly, many organs of the endocrine system in a species-specific manner; for example, thyroid-hormone concentrations have been shown to be affected. But some of the results of studies of thyroid hormones are contradictory, and this makes their interpretation difficult.

The adult nervous system has been shown to be sensitive to TCDD only at high doses. After in utero exposure, however, even the effects at high doses are not straightforward: in utero TCDD exposure decreases performance in some learning and memory tasks but improves performance in other tasks.

In animals, one of the most sensitive systems to TCDD is the immune system. Recent studies have demonstrated that TCDD can alter the numbers of immune cells, the measured activity of the cells, and the ability of animals to fight off infection. Effects on the immune system, however, appear to depend on the species, strain, and developmental stage of the animal studied.

Recent studies have further investigated whether TCDD is involved in endometriosis. TCDD did not affect surgically induced endometrial lesions in rats, although earlier studies demonstrated that prenatal and postnatal exposure of mice to TCDD increased sensitivity to endometrial-lesion growth.

Reproductive and developmental effects have been seen in animals exposed to TCDD, such as effects of developmental exposure to TCDD on sperm counts, sperm production, and seminal vesicle weights in male offspring and effects on the reproductive system in female offspring. In some recent studies, however, the effects on the reproductive system were not accompanied by effects on reproductive outcomes. Effects on the developing cardiovascular system have also been seen after TCDD exposure. The developing nervous system is potentially very sensitive to the effects of TCDD.

TCDD is carcinogenic and an extremely potent promoter of neoplasia in laboratory rats. Liver cancers have been seen consistently after TCDD treatment, and increases in skin cancer, lung cancer, and cancers of the thyroid and adrenal glands have been seen in some studies. A decrease in cancers of the uterus, the pancreas, and the pituitary, mammary, and adrenal glands has also been seen, but

most of those tumors showed decreases only at the high dose and were associated with the decrease in body weight gain, and the decrease in mammary tumors was only seen in one study. In a recent study, there was an increase in hepatic foci at TCDD doses as low as 0.01 ng/kg body weight per day—the lowest dose of TCDD known to promote tumors. Recent data also suggest that promotion of liver tumors by TCDD in female rats depends on continuous exposure.

Data published since *Update 2000* are consistent with the hypothesis that TCDD produces most, if not all, of its effects by binding to a protein that regulates gene expression, the aryl hydrocarbon receptor (AhR). The binding of TCDD to the AhR and interaction of the complex with other proteins is followed by its binding to DNA, which triggers a number of cellular events, including induction of numerous proteins. Research in animals that have been engineered not to express the AhR and in animals with slightly different forms of it provides evidence that the AhR is necessary for the toxicity of TCDD. Modulation of genes by the AhR appears to have developmental-stage-, species-, and cell-specific patterns, which suggest that the molecular and cellular pathways that lead to any particular toxic event are complex.

Additional research has demonstrated that the outcomes of TCDD exposure can be modulated by numerous other proteins with which the AhR interacts. It is plausible, therefore, that the AhR could divert proteins and transcription factors from other signaling pathways; the disruption of the other pathways could have serious consequences for cellular and tissue processes.

Despite the large amount of research conducted on the cellular effects of TCDD, details of the mechanism(s) underlying its effects are not yet determined. Possible mechanisms underlying different effects have been discussed in this chapter and include effects on protein kinase expression, effects on vitamin stores, effects on cellular differentiation and the cell cycle, and oxidative stress. Although the mechanism underlying the carcinogenic effects of TCDD remains unknown, available data indicate that TCDD does not act directly on the genetic material; most genotoxic assays have negative results. Effects on enzymes or hormones could be involved in the carcinogenicity of TCDD.

RELEVANCE TO HUMAN HEALTH

As indicated above, exposure to TCDD has been associated with both cancer and noncancer end points in animals, and most 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. Animal research indicates that TCDD can cause cancers and benign tumors, and it can increase the incidence of some cancers or tumors in the presence of known carcinogens. However, experimental animals differ greatly in their susceptibility to TCDD-induced effects, and the sites at which tumors are

induced vary from species to species. Noncancer health effects also vary according to dose, time, and the animal exposed. Whether the effects of TCDD and other exposures are threshold-dependent—that is, whether some exposures may be too low to induce any effect—is still controversial. The relationship between mechanism and the shape of the dose-response curve, whether it be linear or nonlinear, is complex, not well understood, and may be different for different end points.

Little information is available on the biologic plausibility of causation of health effects by Agent Orange through chemicals other than TCDD. Although concerns have been raised about nondioxin contaminants of herbicides, far too little is known about their distribution and concentration in the formulations used in Vietnam to permit conclusions concerning their impact.

Considerable uncertainty remains about how to apply mechanistic information from nonhuman studies to an evaluation of the potential health effects of herbicide or dioxin exposure in Vietnam veterans. While the data specific to humans is inadequate to demonstrate strong relationships between exposure and disease conditions or pathologies, the growing and abundant evidence from experimental animals and wildlife strongly suggests that similar adverse effects are likely in human populations, the issues of sensitivity and dose-response perhaps being paramount in species differences. It is hoped that as the cellular mechanisms of these compounds are discovered, future *VAO* updates will have better information on which to base conclusions, including better information on the relevance of experimental data to effects in humans.

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4

Overview of Epidemiologic Studies

In seeking evidence of associations between health outcomes and exposure to herbicides and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), many kinds of epidemiologic studies must be considered. Each study type has different strengths and weaknesses (see Chapter 2), and each contributes evidence of an association with the health outcomes considered in Chapters 6–9 of this report. This chapter provides an overview of the design of new epidemiologic studies and reports reviewed by the committee. They include studies published since *Veterans and Agent Orange: Update 2000* (hereafter, *Update 2000*) (IOM, 2001), studies that had been published but were not reviewed by the committees that wrote the prior reports, and studies that have been updated since publication of *Update 2000*. Tables 4-1, 4-2, and 4-3 provide a brief overview of the study design of epidemiologic studies reviewed in both the prior reports and this document. The summaries include the study method used and, if it is known, how the study subjects were selected; how the data were collected; and the inclusion criteria. The tables also list the numbers of subjects in the study and comparison populations and provide brief descriptions of the studies. The purpose of this chapter is to provide a methodologic framework for the health-outcome chapters that follow; no studies are evaluated and results are not discussed in this chapter. The committee's evaluations are presented in the health-outcome chapters. Qualitative critique—of study design, population size, methods of data collection, case and control ascertainment, and exposure assessment—has been reserved for the individual health-outcome chapters.

The text and tables in this chapter are organized into three basic sections—occupational studies, environmental studies, and studies of Vietnam veterans.

Detailed descriptions of many of the study populations are in Chapter 2 of *Veterans and Agent Orange* (hereafter referred to as *VAO*) (IOM, 1994), and the criteria for inclusion in the review are discussed in Appendix A of that report. The studies reviewed addressed exposures to 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant TCDD, cacodylic acid, and picloram. The committee also examined some studies that addressed 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 the specific herbicides to which study participants were exposed or the magnitude of exposure; these complicating factors were considered when the committee weighed the relevance of a study to its findings. If they were available, details of exposure assessment and use of exposure in the analysis are discussed in Chapter 5.

The occupational section covers studies of production workers, agricultural and forestry workers (including herbicide and pesticide appliers), and paper and pulp workers. The environmental section covers studies of populations accidentally exposed to unusual concentrations of herbicides or dioxins as a result of where they live, such as Seveso, Italy; Times Beach, Missouri; and the southern portion of Vietnam. The section on Vietnam veterans covers studies conducted in the United States by the Air Force, the Centers for Disease Control and Prevention (CDC), the Department of Veterans Affairs (VA), the American Legion, and the state of Michigan; it also notes studies of Australian Vietnam veterans.

Many cohorts potentially exposed to dioxins and the herbicides used in Vietnam are monitored periodically, typically every 3–5 years (such as the cohorts of the National Institute for Occupational Safety and Health (NIOSH), the International Agency for Research on Cancer (IARC), the National Cancer Institute (NCI), Seveso, and Ranch Hand). For the sake of thoroughness, the discussions of specific health outcomes in Chapters 6–9 include references to studies discussed in previous Agent Orange reports and new studies, but in making its conclusions, the committee focuses on the most recent update when multiple reports on the same cohorts and end points are available.

Individual researchers who are a part of research consortia evaluating cohorts in large multicenter studies (such as the IARC and NCI cohort studies) sometimes publish reports based solely on the subset of subjects they themselves are monitoring. All the studies are discussed in this report, but when making its conclusions, the committee focuses on the studies of 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 to TCDD. Occupational

groups that have been studied include workers in chemical production plants; agricultural and forestry workers, including farmers and herbicide sprayers; and workers in paper and pulp manufacturing. In addition, some studies use job titles as broad surrogates of exposure or rely on disease-registry data. Exposure characterization varies widely among studies in the exposure metric used, extent of detail, confounding by other exposures, and whether individual or surrogate or group (ecologic) measures are used.

Production Workers

National Institute for Occupational Safety and Health

Starting in 1978, NIOSH began a study to identify all US workers potentially exposed to TCDD in 1942–1984 (Fingerhut et al., 1991). In a total of 12 chemical companies, 5,132 workers were identified from personnel and payroll records as having been involved in production or maintenance processes associated with TCDD contamination. Their possible exposure resulted from working with chemicals in which TCDD was a contaminant, including 2,4,5-trichlorophenol (TCP), 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, so many of the subjects were potentially exposed to many other chemicals, some of which could be carcinogenic.

Before the publication of the first study of the main cohort, 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 two of the plants in the full cohort. The cross-sectional study included comparison of workers at one plant in Newark, New Jersey, in 1951–1969 and at one plant in Verona, Missouri, in 1968–1969 and 1970–1972 with neighborhood controls (Sweeney et al., 1989, 1993; Calvert et al., 1991, 1992; Alderfer et al., 1992). The New Jersey plant manufactured TCP and 2,4,5-T, and the Missouri plant manufactured TCP, 2,4,5-T, and hexachlorophene.

A number of later studies looked at specific health outcomes among the cohort, including pulmonary function (Calvert et al., 1991), liver and gastrointestinal function (Calvert et al., 1992), mood (Alderfer et al., 1992), effects on the peripheral nervous system (Sweeney et al., 1993), porphyria cutanea tarda (Calvert et al., 1994), and effects on reproductive hormones (Egeland et al., 1994). Sweeney et al. (1996, 1997/1998) evaluated noncancer end points, including liver function, gastrointestinal disorders, chloracne, serum glucose concentration, hormone and lipid concentrations, and diabetes in a subgroup of the original Calvert et al. (1991) cohort. Recent studies of the main cohort looked at cardiovascular effects (Calvert et al., 1998); diabetes mellitus, thyroid function, and

endocrine function (Calvert et al., 1999); and immune characteristics (Halperin et al., 1998). Cross-sectional medical surveys reported serum TCDD concentrations and surrogates of cytochrome P450 induction (Halperin et al., 1995) in that cohort. In addition, a follow-up study by Steenland et al. (1999) examined the association between TCDD exposure and cause of death; it looked at specific health outcomes, including cancer (all and site-specific), respiratory disease, cardiovascular disease, and diabetes. *VAO*, *Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996*), *Update 1998*, and *Update 2000* describe the details of those studies.

Since *Update 2000*, Steenland et al. (2001), acting on a research recommendation in *Update 1998*, published a paper that reanalyzed data from two published studies on TCDD and diabetes mellitus: one in US workers (the NIOSH cohort; Calvert et al., 1999) and one in veterans of operation Ranch Hand in which the herbicides were sprayed from planes in Vietnam (Henriksen et al., 1997). Female subjects and nonexposed subjects with serum TCDD greater than 10 ppt were excluded from the NIOSH cohort to make its study design closer to that of the Ranch Hand study. For the analysis by TCDD concentration, NIOSH exposed subjects whose TCDD concentrations were unknown were excluded ($N = 8$). Exposure-response analysis by back-extrapolated TCDD was conducted for exposed subjects (those with TCDD over 10 ppt). Those with TCDD of 10 ppt or lower were considered part of the nonexposed group. The combined study population consisted of 2,759 men: 494 from the NIOSH cohort (267 exposed and 227 nonexposed) and 2,265 from the Ranch Hands (990 exposed and 1,275 nonexposed). A subject was considered to have diabetes on the basis of having been diagnosed by a physician, an oral glucose tolerance test of over 200 mg/dL (as was used in the Ranch Hands study), or a fasting glucose of 126 mg/dL or more (as was used in the NIOSH study). Covariates considered included smoking history, alcohol history, family history of diabetes, body-mass index, year of birth, race, current use of medications that might contribute to diabetes or increased serum glucose, and education. Data were analyzed for prevalence of diabetes, fasting glucose, and time from last exposure to diagnosis of diabetes. A separate analysis of data from each cohort and an analysis of the combined data were conducted for each outcome.

A reanalysis of an earlier published chemical plant study conducted by the National Institute for Occupational Safety and Health (NIOSH) suggested that although cancer incidence may increase at high dioxin exposures, this increase is preceded by a significant reduction in tumor incidence at lower exposures (Kayajanian, 2002).

Monsanto

Included in the NIOSH study cohort (Fingerhut et al., 1991) were a number of people at Monsanto's production facilities on whom studies have been con-

ducted. One set of Monsanto studies was based on an accidental exposure that occurred on March 8, 1949, in the TCP production process at the Nitro, West Virginia, plant (Zack and Suskind, 1980; Moses et al., 1984; Collins et al., 1993). Others focused on exposure of Monsanto workers involved in numerous aspects of 2,4,5-T production (Zack and Gaffey, 1983; Moses et al., 1984; Suskind and Hertzberg, 1984). The Monsanto studies are discussed in more detail in *VAO*. No new studies have been published on these subjects.

Dow Chemical Company

Several studies have been conducted on Dow Chemical Company production workers and are summarized in *VAO*, *Update 1996*, *Update 1998,* and *Update 2000*. The populations in these studies, except for one report by Bond et al. (1988), were included in the NIOSH cohort (Fingerhut et al., 1991). Originally, Dow conducted a study on workers engaged in the production of 2,4,5-T (Ott et al., 1980) and a study on TCP manufacturing workers exhibiting chloracne (Cook et al., 1980). Extension and follow-up studies compared potential exposure to TCDD with medical-examination frequency and morbidity (Bond et al., 1983), and reproductive outcomes after potential paternal TCDD exposure (Townsend et al., 1982). A prospective mortality study of Dow employees diagnosed with chloracne or classified as having chloracne on the basis of clinical description (Bond et al., 1987) was also conducted.

In addition, Dow assembled a large cohort at the Midland, Michigan, plant (Cook et al., 1986, 1987; Bond et al., 1989b). Exposure to TCDD was characterized in this cohort on the basis of chloracne diagnosis (Bond et al., 1989a). Within this large Midland cohort, a cohort study of women (Ott et al., 1987) and a case–control study of soft-tissue sarcoma (STS) (Sobel et al., 1987) were conducted.

Dow has also undertaken a large-scale cohort mortality study of workers exposed to herbicides in several of its plants (Bond et al., 1988; Bloemen et al., 1993; Ramlow et al., 1996).

Since *Update 2000*, an update on mortality in a cohort of 1,517 Dow employees was published (Burns et al., 2001), extending the follow-up to 1994. A job–exposure matrix assessing potential phenoxy herbicide exposure for 1945–1982 had been created by an industrial hygienist with categories for time-weighted average 2,4-D concentrations of greater than 1.0 mg/m^3, 0.1–1.0 mg/m^3, and less than 0.1 mg/m^3. For follow-up from 1983 to 1994, a new category of "very low" was added for jobs that had nondetectable exposure-monitoring concentrations or in which less than 50% of time was spent in a low-exposure area and no time was spent in a high-exposure area. The limit of detection of the monitoring was not specified by the authors. A total of 495 workers worked for any length of time in either high- or moderate-exposure jobs. Standardized mortality ratios were calculated to compare the total male cohort of 1,517 workers with the US population.

To control for the healthy-worker effect, comparisons were made with an internal reference cohort of all other Dow employees at the Midland plant. For the exposed cohort, the total number of person-years of follow-up was 39,799, for an average of 26.2 years.

BASF

In Germany, an accident on November 17, 1953 during the manufacture of TCP at BASF Aktiengesellschaft resulted in the exposure of some workers in the plant predominantly to TCDD. *VAO*, *Update 1996*, and *Update 1998* summarize studies on those workers. The studies include a mortality study of persons initially exposed or later involved in cleanup (Thiess et al., 1982), an update and expansion of that study (Zober et al., 1990), and a morbidity follow-up (Zober et al., 1994). In addition, Ott and Zober (1996) examined cancer incidence and mortality in another cohort of workers exposed to TCDD after the accident during reactor cleanup, maintenance, or demolition. No new studies have been published on those cohorts since *Update 1998*.

International Agency for Research on Cancer

To avoid problems of small studies with insufficient power to detect increased cancer risks, IARC created a multinational registry of workers exposed to phenoxy herbicides, chlorophenols, and their contaminants (Saracci et al., 1991). The IARC registry includes information on mortality and exposure of 18,390 workers—16,863 men and 1,527 women. *Update 1996* describes the individual national cohorts included in the registry.

In a study including individuals from 10 countries, cancer mortality from STS and malignant lymphoma was evaluated (Kogevinas et al., 1992). Two nested case–control studies were also undertaken using the IARC cohort to evaluate the relationship between STS and non-Hodgkin's lymphoma (Kogevinas et al., 1995). In an update and expansion, Kogevinas et al. (1997) assembled national studies from 12 countries that used the same protocol (jointly developed by study participants and coordinated by IARC) and studied cancer mortality. Vena et al. (1998) studied nonneoplastic mortality in the IARC cohorts. A cohort study of cancer incidence and mortality was conducted among 701 women from seven countries who were occupationally exposed to chlorophenoxy herbicides, chlorophenols, and dioxins (Kogevinas et al., 1993). *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* highlight those studies.

In addition, a number of the smaller cohorts that comprise the IARC cohort have been evaluated apart from the IARC-coordinated efforts. They include Danish production workers studied by Lynge (1985, 1993); British production workers studied by Coggon et al. (1986, 1991); Dutch production workers studied by Bueno de Mesquita et al. (1993); German production workers studied by Manz et

al. (1991), Becher et al. (1996), Flesch-Janys et al. (1995), and Flesch-Janys (1997); factory workers from the Netherlands studied by Hooiveld et al. (1998); and Austrian production workers studied by Neuberger et al. (1998, 1999) and Jäger et al. (1998). *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* discuss those studies in more detail. No new studies have been published on the IARC cohort or the smaller cohorts that comprise the IARC cohort.

Other Chemical Plants

Studies have reviewed health outcomes among chemical workers in the UK exposed to TCDD as a result of an industrial accident in 1968 (May, 1982, 1983; Jennings et al., 1988), production workers in the former Soviet Union involved in the production of 2,4-D (Bashirov, 1969), factory workers in Prague who exhibited symptoms of TCDD toxicity 10 years after occupational exposure to 2,4,5-T (Pazderova-Vejlupkova et al., 1981), 2,4-D and 2,4,5-T production workers in the United States (Poland et al., 1971), white men employed at a US chemical plant manufacturing flavors and fragrances (Thomas, 1987), and US chemical workers engaged in the production of PCP, lower-chlorinated phenols, and esters of chlorophenoxy acids (Hryhorczuk et al. 1998); 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 (Tonn et al., 1996); and immune effects in a cohort of workers formerly employed at a German pesticide-producing plant (Jung et al. 1998). *VAO*, *Update 1998*, and *Update 2000* detail those studies. No studies at other chemical plants have been published since *Update 2000*.

Agricultural and Forestry Workers

Cohort Studies

Agricultural Workers *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* detail a number of cohort studies examining health effects in people involved in agricultural activity. They include studies of proportionate mortality among Iowa farmers (Burmeister, 1981) and among male and female farmers in 23 states (Blair et al., 1993), cancer mortality among Danish and Italian farmers (Ronco et al., 1992) and among a cohort of rice growers in the Novara Province of northern Italy (Gambini et al., 1997), cancer incidence among farmers licensed to spray pesticides in the southern Piedmont area of Italy (Corrao et al., 1989) and among female Danish gardeners (Hansen et al., 1992), sperm abnormalities among Argentine farmers (Lerda and Rizzi, 1991), cancer birth defects among the offspring of Norwegian farmers (Kristensen et al., 1997), the incidence of spontaneous abortion in couples living on family farms in Ontario, Canada (Arbuckle et al. 1999), and immunologic changes in 10 farmers who mixed and applied commer-

cial formulations containing the chlorophenoxy herbicides (Faustini et al., 1996). In addition, a set of Canadian studies, called the Mortality Study of Canadian Male Farm Operators, evaluated the risk to farmers of death and specific health outcomes, including non-Hodgkin's lymphoma (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). On the basis of data from the Swedish Cancer Environment Register (which links population census data, including occupation, with the Swedish Cancer Registry), cohort studies evaluated cancer mortality and farm work (Wiklund, 1983), STS and malignant lymphoma among agricultural and forestry workers (Wiklund and Holm, 1986; Wiklund et al., 1988a), and the risk of NHL, Hodgkin's disease (HD), and multiple myeloma in relation to numerous occupational activities (Eriksson et al., 1992). Brain, lymphatic, and hematopoietic cancers in Irish agricultural workers have also been studied (Dean, 1994).

Since *Update 2000*, Masley et al. (2000) have conducted a cross-sectional survey of households in an agriculture-based rural area of Saskatchewan, Canada, to understand the short-term effects of environmental pesticide exposure on immune, neurobehavioral, and lung function. Some 3,327 people living in 1,185 private households in three towns or three rural municipalities were targeted in the survey; 875 households (74%) accepted the canvassing package, and 549 (46%) returned a completed questionnaire. Designed to be answered by an adult household representative, the questionnaire included sections about physical environmental factors (occupational and home or garden pesticide or fertilizer use, and so on), demographic characteristics of people living in the household, and general health problems, health concerns, or respiratory symptoms of each person in the household. The proportion of households using pesticide and fertilizer was determined by dividing the total number of "yes" responses by the number of "yes" and "no" responses (missing responses were not included).

In addition, a number of studies have been published on the basis of data from the Ontario Family Farm Health Study, a Canadian research effort investigating male pesticide exposures and pregnancy outcomes. *Update 2000* discusses a study in which Arbuckle et al. (1999) examined the incidence of spontaneous abortion in couples selected from the 1986 Canadian Census of Agriculture and living on family farms in Ontario. Farming families were contacted by telephone and were considered eligible if they were married or "living as married," if they lived year-round on the farm, and if the wife was not older than 44 years. Eligible families were sent three questionnaires. One questionnaire, addressed to the farm operator, collected data on current and historical pesticide use. A second, addressed to the husband, collected demographic, socioeconomic, and lifestyle information; medical history; and information on his activities on the farm, date of moving to the farm, and pesticide exposure both at home and on the farm. A third, addressed to the wife, collected information similar to that on the husbands but

also collected a complete reproductive history. Pesticide use was recorded for specific pesticides by month and year. Pregnancy-outcome data were merged with pesticide use at the corresponding times. Potential confounders were recorded (such as parental age, smoking, and alcohol consumption), as was the period during which they were present. Telephone screening identified 2,946 eligible couples (36.5% of all operating farms), among whom 1,898 (64%) completed all three questionnaires. Pregnancies were excluded if there was missing information (such as outcome, delivery date, or gestational age at delivery), if they occurred when the woman was not living on the farm, if the study husband might not have been the father, or in the case of multiple gestations, ectopic pregnancies, or hydatidiform-mole pregnancies. The 2,110 women enrolled in the study had a total of 5,853 pregnancies.

Since *Update 2000*, three other studies have been identified that report reproductive outcomes from that questionnaire and that cohort. Savitz et al. (1997) investigated the effects of male pesticide exposure on a number of pregnancy outcomes, including number of live births, number of preterm births, number of small-for-gestational-age births, number of miscarriages, and sex ratios. Of the 5,853 pregnancies of the 2,110 women enrolled in the study, 3,984 were included in the analysis.

Curtis et al. (1999) examined the effect of pesticide exposure on time to pregnancy in the same cohort. For all "planned" pregnancies (pregnancies that occurred when the women reported not using any form of birth control) they recorded any method of birth control that the couple had discontinued to try to conceive and the number of months or cycles it took to conceive. They analyzed 1,048 couples and 2,012 planned pregnancies.

Arbuckle et al. (2001) conducted further analyses of the data from that cohort to explore the critical windows of exposure (the point during gestation at which the fetus is sensitive to pesticide exposure), the target sites of interactions among the pesticides, and other risk factors for spontaneous abortion. Spontaneous-abortion data were correlated with exposure information to determine pesticide-exposure opportunities in the months leading up to conception, during the first trimester, and in early term (less than 12 weeks) or late term (12–19 weeks). In this study, 2,010 women participated and provided information on 3,936 pregnancies.

Forestry Workers Studies have been conducted among forestry workers potentially exposed to the types of herbicides used in Vietnam. The 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 that investigated the prevalence of acne and liver dysfunction (van Houdt et al., 1983). *VAO* describes these studies in greater detail.

Since *Update 1998*, Thörn et al. (2000) have reported on mortality and cancer incidence in a cohort of Swedish lumberjacks. The cohort analyzed con-

sisted of men and women who were Swedish residents and were employed by one Swedish forestry company at some time in the period 1954–1967. The approximate volume and concentration of phenoxy acids used daily in a particular work task or job category were obtained from former employees. Pay slips were used to determine the time spent in particular work tasks, and exposure to phenoxy acids was estimated from the time spent in particular job categories. Employees who were exposed to phenoxy acids for more than 5 working days were considered to have been exposed; employees not exposed to any types of pesticides were used as the nonexposed or control group; people who were exposed to other pesticides (including DDT) were excluded from the study. Mortality was determined from the National Register of Causes of Death, new cancer cases were determined from the Swedish Cancer Register, and death certificates with cause of death were provided by Statistics Sweden. Data were available on 261 exposed and 243 unexposed members of the cohort. Standardized mortality ratios and cancer incidence (all and site-specific) ratios were calculated for each group by using ratios expected from the death and cancer registries.

Herbicide and Pesticide Sprayers 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), reproductive outcomes among male chemical appliers in New Zealand (Smith et al., 1981, 1982), and doctor visits resulting from pesticide exposure in Iowa and North Carolina (Alavanja et al., 1998). Other studies examined the risk of cancer—including STS, HD, NHL, and prostate cancer—among pesticide and herbicide appliers in Sweden (Dich and Wiklund, 1998; 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 Minnesota pesticide appliers (Garry et al., 1994, 1996a,b), lung-cancer morbidity in male agricultural plant-protection workers in the former German Democratic Republic (Barthel, 1981), British Columbia sawmill workers potentially exposed to chlorophenate wood preservatives (Dimich-Ward et al., 1996; Hertzman et al., 1997; Heacock et al. 1998), and cancer risk among pesticide users in Iceland (Zhong and Rafnsson, 1996). Some of those studies included agricultural- and forestry-worker cohorts; details of the study design and results are included in *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*.

Since *Update 2000*, Hoppin et al. (2002) have conducted a study that examined chemical predictors of wheeze among pesticide appliers in Iowa and North Carolina in the Agricultural Health Study. Of 52,000 farmers certified for pesticide application, 20,468 farmer applicators completed both an enrollment and a

secondary questionnaire and were included in the study. Of the respondents, 3,838 recorded episodes of wheeze in the previous year when responding to this question: "How many episodes of wheezing in your chest have you had in the past 12 months. No wheezing or whistling, 1–2 episodes, 3–6 episodes, 7–12 episodes, more than 12 episodes." Pesticide exposures and related activities of study participants were assessed in relation to episodes of wheeze. A base logistic-regression model controlling for age, state, smoking history (current, past, or never), and asthma-atopy status was evaluated for the exposures.

Case–Control Studies

In 1977, case-series reports in Sweden (Hardell, 1977, 1979) of a potential connection between soft-tissue sarcoma and exposure to phenoxyacetic acids prompted several case–control studies in Sweden to investigate the possible association (Hardell and Sandstrom, 1979; Eriksson et al., 1979, 1981, 1990; Hardell and Eriksson, 1988; Wingren et al., 1990). After the initial reports on STS (Hardell, 1977, 1979), case–control studies of other cancer outcomes were also conducted in Sweden, including studies of HD, NHL, and other lymphomas (Hardell et al., 1980, 1981; Hardell and Bengtsson, 1983); HD and NHL (Persson et al., 1989, 1993); NHL (Olsson and Brandt, 1988; Hardell and Eriksson, 1999); nasal and nasopharyngeal carcinomas (Hardell et al., 1982); gastric cancer (Ekström et al., 1999); and primary or unspecified liver cancer (Hardell et al., 1984). To address criticism regarding potential observer bias in some of the case–control series, Hardell (1981) conducted another case–control study on colon cancer. Hardell et al. (1994) also examined the relationship between occupational exposure to phenoxyacetic acids and chlorophenols and various characteristics related to NHL—including histopathologic measures, stage, and anatomic location—on the basis of the NHL cases from a previous study (Hardell et al., 1981).

Prompted by the Swedish studies (Hardell, 1977, 1979), Smith and coworkers undertook a set of case–control studies in New Zealand to evaluate the association between phenoxy herbicide 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 on phenoxy herbicide and chlorophenol exposure and the risks of malignant lymphoma, NHL, and multiple myeloma (Pearce et al., 1985, 1986a,b, 1987).

Geographic patterns of increased leukemia mortality in white men in the central part of the United States prompted a study of the leukemia mortality in Nebraska farmers (Blair and Thomas, 1979). Additional case–control studies were later conducted on leukemia in Nebraska (Blair and White, 1985), in Iowa (Burmeister et al., 1982) on the basis of the cohort study of Burmeister (1981), in Iowa and Minnesota (Brown et al., 1990), and on leukemia associated with NHL in eastern Nebraska (Zahm et al., 1990).

Case–control studies have been conducted in various US populations on

other cancers, including NHL (Cantor, 1982; Cantor et al., 1992; Zahm et al., 1993; Tatham et al., 1997); multiple myeloma (Morris et al., 1986; Boffetta et al., 1989; Brown et al., 1993); cancers of the stomach and prostate, NHL, and multiple myeloma (Burmeister et al., 1983); STS, HD, and NHL (Hoar et al., 1986); NHL and HD (Dubrow et al., 1988); and STS and NHL (Woods et al., 1987; Woods and Polissar, 1989).

Other studies outside the United States have looked at cancer end points: 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 in 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); and renal-cell carcinoma in the Denmark Cancer Registry (Mellemgaard et al., 1994). In addition, Nanni et al. (1996) conducted a population-based case–control study, based on the work of Amadori et al. (1995), of occupational and chemical risk factors for lymphocytic leukemia and NHL in northeastern Italy.

Noncancer end points have also been investigated in case–control studies. End points studied were spontaneous abortion (Carmelli et al., 1981); congenital malformations (García et al., 1998); immunosuppression and subsequently decreased host resistance to infection among AIDS patients with Kaposi's sarcoma (Hardell et al., 1987); mortality in US Department of Agriculture extension agents (Alavanja et al., 1988, 1989); spina bifida in offspring associated with paternal occupation (Blatter et al., 1997); mortality from neurodegenerative diseases associated with occupational risk factors (Schulte et al., 1996); Parkinson's disease (PD) associated with occupational and environmental risk factors (Liou et al., 1997); PD associated with various rural factors, including exposure to herbicides and wood preservatives (Seidler et al., 1996); PD associated with occupational risk factors (Semchuk et al., 1993); and birth defects in offspring of agricultural workers (Nurminen et al., 1994). Those studies are discussed in detail in *VAO*, *Update 1996*, or *Update 1998*. No new case–control studies of agricultural and forest workers have been published since *Update 2000*.

Paper and Pulp Workers

Workers in the paper and pulp industry can be exposed to TCDD and other dioxins that are generated during the bleaching process in the production and treatment of some paper and paper products. *VAO* describes studies of workers potentially exposed to TCDD in paper and pulp mills and various health outcomes, including general mortality in workers at five mills in Washington, Oregon, and California (Robinson et al., 1986); cancer incidence among male Finn-

ish paper-mill workers (Jappinen and Pukkala, 1991); respiratory health in a New Hampshire mill (Henneberger et al., 1989); and cause-specific mortality among white men employed in plants identified by the United Paperworkers International Union (Solet et al., 1989). *Update 2000* describes studies of cancer risks among Danish workers in the paper industry (Rix et al., 1998) and oral-cancer risks among occupationally exposed workers in Sweden (Schildt et al., 1999). No new studies of paper and pulp workers have been published since *Update 2000*.

ENVIRONMENTAL STUDIES

The occurrence of accidents and industrial disasters has offered opportunities to evaluate the long-term health effects of exposure to TCDD and other potentially hazardous chemicals.

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. Of the various indicators used to estimate individual exposure, soil contamination with 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 less 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 were conducted on the basis of those exposure categories. The studies are reviewed extensively in *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* and are summarized here.

Caramaschi et al. (1981) presented the distribution of chloracne among Seveso children, and Mocarelli et al. (1986) measured several chemicals in the blood and urine of children who had had chloracne. In a follow-up, dermatologic and laboratory tests were conducted among a group of the children with chloracne and controls (Assennato et al., 1989a).

Other studies looked at specific health effects associated with TCDD exposure among Seveso residents, including chloracne, birth defects, spontaneous abortion, and 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); neurologic disorders (Boeri et al., 1978; Filippini et al., 1981); cancer incidence (Pesatori et al., 1992, 1993; Bertazzi et al., 1993); and the sex ratio of offspring who were born in zone A (Mocarelli et al., 1996). A

2-year prospective controlled study was conducted of workers potentially exposed to TCDD during cleanup of the most highly contaminated areas after the accident (Assennato et al., 1989b).

Seveso residents have had long-term follow-up of their health outcomes, especially cancer. Bertazzi and colleagues conducted 10-year mortality follow-up studies among adults and children who were 1–19 years old at the time of the accident (Bertazzi et al., 1989a,b, 1992), 15-year follow-up studies (Bertazzi et al., 1997, 1998), and a 20-year follow-up study (Bertazzi et al., 2001). Pesatori et al. (1998) also conducted a 15-year follow-up study to update noncancer mortality.

A recent study by Warner et al. (2002) used data from the Seveso Women's Health Study to evaluate the association between individual serum TCDD and breast-cancer risk in women who resided in Seveso at the time of the accident in 1976. The study included 981 women who were infants to 40 years old at the time of the accident, had resided in the most contaminated areas (A and B), and had adequate stored serum collected shortly after the explosion for TCDD measurements. In addition to giving informed consent, study participants donated blood and were interviewed. In some cases medical records were obtained, and gynecologic examinations and transvaginal ultrasonography were conducted. Serum TCDD was measured, and Cox proportional hazards modeling was used for the primary analysis.

Times Beach and Quail Run Cohorts

During early 1971, byproducts 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, including around Times Beach and Quail Run areas. TCDD was a contaminant of the mixtures sprayed, and the contamination was reported by the Environmental Protection Agency. A number of studies were conducted to evaluate health effects of the potential exposure (Evans et al., 1988; Hoffman et al., 1986; Stehr et al., 1986; Stehr-Green et al., 1987; Stockbauer et al., 1988; Webb et al., 1987). *VAO* discusses those studies, and no more recent studies have been published.

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) summarized the unpublished results of those 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 those studies. No studies have been published since *Update 1996*.

Other Environmental Studies

VAO, *Update 1996*, and *Update 1998* report on numerous studies focusing on reproductive outcomes of potential environmental exposure in Oregon (US 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 other studies have focused on different outcomes of environmental exposure. They include examinations of 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); lymphomas and STS in Italy (Vineis et al., 1991); neuropsychologic effects in Germany (Peper et al., 1993); early-onset Parkinson's disease in Oregon and Washington (Butterfield et al., 1993); adverse health effects after an electric transformer fire in Binghamton, New York (Fitzgerald et al., 1989); skin cancer in Alberta, Canada (Gallagher et al., 1996); NHL, HD, and chronic lymphocytic leukemia in a rural Michigan community (Waterhouse et al., 1996); cancer mortality in four northern wheat-producing states (Schreinemachers, 2000); HD, NHL, multiple myeloma, and acute myeloid leukemia in various regions of Italy (Masala et al., 1996); effects of inhalation exposure to TCDD and related compounds in wood preservatives on cell-mediated immunity in German day-care center employees (Wolf and Karmaus, 1995); mortality and cancer incidence in two cohorts of Swedish fishermen whose primary exposure route was assumed to be diet (Svensson et al., 1995); immune effects in hobby fishermen in the Frierfjord in southeastern Norway (Lovik et al., 1996); and immunologic effects of prenatal and postnatal PCB or TCDD exposure in Dutch infants from birth to the age of 18 months (Weisglas-Kuperus et al., 1995).

Since *Update 2000*, Revich et al. (2001) have reported on TCDD exposure and public health in Chapaevsk, Russia, where a chemical plant produced hexachlorocyclohexane and its derivatives from 1967 to 1987. The plant now produces crop-protection materials. TCDD exposure was assessed by sampling the air, drinking water and soil in the town, human milk from 40 women (pooled to seven samples), blood from 14 people, and cow's milk from privately owned cattle. Official statistics, including demographic and medical statistics, were used to assess cancer risk and reproductive health. Congenital and morphogenetic effects were assessed by examining the medical records of 369 children born in Chapaevsk in 1990–1995 to those for the Samara region and for all of Russia. A study by Revazova et al. (2001) investigated cytogenetic effects among the

women residents of Chapaevsk. Three groups of women were examined: 15 women working in the fertilizer plant, 16 women with no known occupational TCDD exposures but living 1–3 km from the plant, and 14 women with no known occupational TCDD exposure who lived 5–8 km from the plant. All women were 20–40 years old. The women's plasma TCDD was measured, chromosomal aberrations were assessed in peripheral blood lymphocytes, and micronuclei and other nuclear anomalies were assessed in buccal epithelium.

VIETNAM-VETERAN 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 and in Australia and Vietnam. Exposures in those studies have been measured on various levels, and health outcomes have been evaluated with various comparison or control groups. This section is organized primarily by research sponsor because it is more conducive to methodologic presentation of the articles. In the studies, exposure measures range from individual exposures of Ranch Hands, as reflected in serum TCDD measurements, to service in Vietnam as a surrogate of TCDD exposure in some state studies.

It should also be noted that a variety of comparison groups have been used for the veteran cohort studies: (a) 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; (b) Vietnam era veterans who were in the military at the time of the conflict but did not serve in Vietnam; (c) non-Vietnam veterans who served in other wars or conflicts such as the Korean War or World War II; and (d) various US male populations (either state or national). This is also discussed in Chapter 5 of this report.

United States

Operation Ranch Hand

The men responsible for most of the aerial spraying of herbicides in Vietnam were Air Force volunteers who participated in Operation Ranch Hand. To determine whether exposure to herbicides, including Agent Orange, had adverse human health effects, the Air Force made a commitment to Congress and the White House in 1979 to conduct an epidemiologic study of Ranch Hands (AFHS, 1982). *VAO*, *Update 1996*, *Update 1998*, *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (hereafter referred to as *Type 2 Diabetes*) (IOM, 2000), and *Update 2000* discuss reports and papers addressing this cohort in more detail.

A retrospective matched-cohort study design was implemented to examine morbidity and mortality; follow-up was scheduled to continue until 2002. Na-

tional Personnel Records Center and US Air Force Human Resources Laboratory records were searched and cross-referenced to identify 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 individually matched on age, type of job (based on Air Force specialty code), and race (white or not white). The rationale for matching on those variables was to control for age-related effects, educational and socioeconomic status, and potential race-related differences in development of chronic disease. Ranch Hands and controls performed similar combat or combat-related jobs, so many potential confounders related to the physical and psychophysiologic effects of combat stress and the Southeast Asia environment were potentially controlled for (AFHS, 1982). Rank was also used as a surrogate of exposure. Alcohol use and smoking were controlled for when they were known risk factors for the end point of interest.

Ten matches formed a control set for each exposed subject. For the mortality study, each subject classified as exposed 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, with 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 examination occurred in 1982; the final examination was scheduled for 2002. Morbidity is ascertained through questionnaire and physical examination, which emphasize dermatologic, neuropsychiatric, hepatic, immunologic, reproductive, and neoplastic conditions. Some 1,208 Ranch Hands and 1,668 comparison subjects were eligible for baseline examination. Initial questionnaire response rates were 97% for the exposed cohort and 93% for the nonexposed; baseline physical examination responses were 87% and 76%, respectively (Wolfe et al., 1990). For the 1987 examination and questionnaire (Wolfe et al., 1990), 84% of Ranch Hands (N = 955) and 75% of comparison subjects (N = 1,299) were fully compliant. Mortality outcome was obtained and reviewed by using US Air Force Military Personnel Center records, the VA's Death Beneficiary Identification and Record Location System (BIRLS), and the Internal Revenue Service database of active social security numbers. Death certificates were obtained from the appropriate health departments (Michalek et al., 1990). For this study, 84% of the 1,148 eligible Ranch Hands (N = 952), 76% of the original comparison group (N = 912), and 65% 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 with those described previously for the 1982 and 1987 examinations.

Ranch Hands were divided into three categories on the basis of their potential exposure:

- *Low potential*. This group included pilots, copilots, and navigators. Exposure was primarily through preflight checks and spraying.
- *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.
- *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); the first (1984), second (1987), third (1992), and fourth (1997) follow-up examinations (AFHS, 1987, 1990, 1995, 2000); and the reproductive-outcomes study (AFHS, 1992; Wolfe et al., 1995; Michalek et al., 1998d). Mortality updates have been published for 1984–1986, 1989, and 1991 (AFHS, 1984b, 1985, 1986, 1989, 1991a). An interim technical report updated the cause-specific mortality among Ranch Hands through 1993 (AFHS, 1996), and Michalek et al. (1998b) reported on a 15-year follow-up of postservice mortality in veterans of Operation Ranch Hand, updating their cause-specific mortality study (1990). Serum TCDD was measured in 1982 (36 Ranch Hands; Pirkle et al., 1989), 1987 (866 Ranch Hands; AFHS, 1991b), and 1992 (455 Ranch Hands; AFHS, 1995). Serum TCDD analysis of the 1987 follow-up examinations was published in 1991 (AFHS, 1991b).

Other Ranch Hand publications have addressed the relationship between serum TCDD and reproductive hormones (Henriksen et al., 1996); sex ratios (Michalek et al., 1998c); TCDD and diabetes mellitus, glucose, and insulin (Henriksen et al., 1997); serum TCDD and diabetes mellitus (Longnecker and Michalek, 2000); insulin, fasting glucose, and sex hormone-binding globulin (Michalek et al., 1999a); immunologic responses (Michalek et al., 1999b); skin disorders (Burton et al., 1998); skin cancer (Ketchum et al., 1999); and TCDD and infant death (Michalek et al., 1998a).

Since *Update 2000*, Michalek et al. (2001a) have studied the relationship between serum TCDD and hepatic abnormalities in the cohort of veterans of Operation Ranch Hand previously described in Wolfe et al. (1990). Study participants were examined and medical records retrieved in 1982, 1985, 1987, and 1992. During the 1992 examination, 1,109 Ranch Hands and 1,493 comparisons subjects volunteered for additional testing for hepatic disease. Subjects whose TCDD measurements were missing, subjects who had detectable TCDD below the limit of quantification, comparison veterans with TCDD greater than 10 ppt, subjects who received no result, and subjects with a history of liver disease before service in Southeast Asia were excluded. According to TCDD concentrations, the Ranch Hands and comparison subjects were categorized as having "background,"

"low," or "high" exposure. After those exclusions, data on 987 Ranch Hands (background, 402; low, 284; high 283) and 1,266 comparison subjects were analyzed. Liver conditions were grouped into hepatomegaly, nonalcoholic chronic liver disease and cirrhosis, and other liver disorders; and nonspecific increases in transaminase or lactic acid dehydrogenase, other nonspecific abnormal serum enzyme values, and nonspecific abnormal results on liver-function studies were also recorded. Liver-function tests included measurement of alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, lactic dehydrogenase, alkaline phosphatase, and total bilirubin. At the time of the examination, participants were asked about their history of liver problems, and liver abnormalities were verified by personal physicians or laboratory analysis completed during the examination. Medical records were used to determine whether identified liver conditions had occurred in the period between service in Southeast Asia and April 1993. All liver conditions were classified according to International Classification of Disease coding. Confounders—including lifetime smoking history, lifetime drinking history, lifetime industrial chemical exposure, race, and birth year—were adjusted for.

Michalek et al. (2001b) studied the relationship between serum TCDD and peripheral neuropathy in the cohort of veterans of Operation Ranch Hand previously described in Wolfe et al. (1990). Study participants underwent TCDD body-burden analysis and neurologic examinations at the 1982, 1985, 1987, 1992, and 1997 physical examinations. TCDD was measured in 2,101 veterans at the 1997 examination. Subjects with TCDD below the limit of detection were assigned a value equal to the limit of detection divided by the square root of 2. Ranch Hand veterans with TCDD not exceeding 10 ppt were assigned to the background category. Those with TCDD greater than 10 ppt and a 1982 TCDD measurement not exceeding the median (94 ppt) were assigned to the low category. Ranch Hands with TCDD concentrations greater than 10 ppt and a 1982 TCDD measurement greater than the median were assigned to the high category. Neurologic status of participants was evaluated through a standardized neurologic examination conducted by a board-certified neurologist. Nerve-conduction velocities were measured during the 1992 examination, and vibrotactile thresholds in 1992 and 1997. Study participants were identified as having possible, probable, or diagnosed peripheral neuropathy if one, two, or all three, respectively, of the following outcomes occurred bilaterally: absence of the Achilles reflex, abnormal vibration at the ankle, and abnormal pin-prick reaction. Veterans who had a history of neurologic disorders before service in Southeast Asia, whose TCDD measurements were missing, who had conditions that would interfere with an assessment of the peripheral nerves, or who had specific neurologic disorders of known causes unrelated to TCDD exposure were excluded. Sample sizes in the 1997 primary analysis after exclusions were 761 Ranch Hands (background, 338; low, 213; high, 210) and 1,086 comparison subjects. Adjustments

were made in the primary analysis for age, height, alcohol consumption, occupation, diabetes, and body-mass index (BMI).

Michalek et al. (2001c) also studied the relationship of serum TCDD to hematologic results in the cohort of Ranch Hand veterans previously described in Wolfe et al. (1990). Hematologic function and TCDD body burden were analyzed for veterans who participated in the 1982, 1985, 1987, and 1992 physical examinations. According to TCDD concentrations, the Ranch Hands and comparison subjects were categorized as having "background," "low," or "high" exposures. Veterans whose TCDD measurements were missing or nonquantifiable, and comparison subjects with TCDD greater than 10 ppt were excluded. Subjects with TCDD below the limit of detection were assigned a value of 0 ppt. Veterans who had a fever at the time of examination or who tested positive for human immunodeficiency virus were also excluded from the analysis. After exclusions, data on 953 Ranch Hands and 1,280 comparison subjects were analyzed. At each examination, red-cell count, hemoglobin, hematocrit, mean corpuscular volume, white-cell count, platelet count, and erythrocyte sedimentation rate were measured. BMI, smoking patterns, and alcohol consumption were estimated for study participants at each examination.

Barrett et al. (2001) studied the relationship between serum TCDD and cognitive function among the cohort of Ranch Hand veterans previously described in Wolfe et al. (1990). At the 1987 physical examination, or the 1992 examination if the testing was not completed at the earlier examination, blood was drawn from each veteran and assayed for TCDD. After exclusion of veterans with no TCDD measurement or a nonquantifiable TCDD result and comparison veterans with TCDD greater than 10 ppt—and one veteran who had epilepsy before the 1982 physical examination—data on 937 Ranch Hands (background, 388; low, 274; high, 275) and 1,052 comparison subjects were analyzed. An alternative analysis was conducted that included all veterans with a TCDD measurement stratified by quintile of the TCDD distribution. Nonquantifiable TCDD results were assigned the value of half the limit of quantitation. Cognitive function was tested at the 1982 examination with the Halstead-Reitan neuropsychologic test battery, the revised Wechsler adult intelligence scale, the Wechsler memory scale Form 1, and the reading subset of the wide-range achievement test. In the primary analysis, adjustments were made for military occupation, age, race, drinking history, marital status, combat-exposure quartile, psychiatric-diagnosis indicators, and psychotropic-medication indicators.

As noted earlier, Steenland et al. (2001) published a paper that reanalyzed data from two previously published studies on TCDD and diabetes mellitus: one in US workers (NIOSH study; Calvert et al., 1999) and one in Ranch Hands (Henriksen et al., 1997). In that paper, the data from the NIOSH cohort are reanalyzed with a cohort selection closer to that used in the Ranch Hand study. The combined data from the NIOSH study and the Ranch Hand study are also

analyzed. Further details on those reanalyses can be found in the "Occupational Studies" section of this chapter.

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 those studies in detail. The first was a case–control interview study of birth defects among offspring of men who served in Vietnam (Erickson et al., 1984a,b).

To examine concerns about Agent Orange more directly, CDC conducted the Agent Orange Validation Study to evaluate TCDD in US Army veterans compared with exposure estimates based on military records and TCDD in veterans who did not serve in Vietnam (CDC, 1989a). Using those exposure estimates, CDC 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: physical health, reproductive outcomes and child health, and psychosocial characteristics (CDC, 1987, 1988a,b,c, 1989b).

Using data from the VES, CDC examined the postservice mortality (through 1983) in a cohort of 9,324 US Army veterans who served in Vietnam compared with 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, CDC designed a study using VES subjects (Decoufle et al., 1992).

Finally, 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 and nasal, nasopharyngeal, and primary liver cancers (CDC, 1990d).

No CDC studies have been published since 1990.

Department of Veterans Affairs

DVA has conducted numerous cohort and case–control studies, which *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* discuss in detail. One of the first was a proportionate-mortality study conducted by Breslin et al. (1988). Study subjects were ground troops who served in the US 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 were reported deceased as

of July 1, 1982, was assembled from DVA's BIRLS. A random sample of 75,617 names was selected from the list. Cause of death was ascertained for 51,421 men, including 24,235 who served in Vietnam. On the basis of the 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 deaths. Later, Bullman et al. (1990) examined whether Army I Corps Vietnam veterans had cancer mortality similar to that of other Army Vietnam-era veterans, using the study design of Breslin et al. (1988). Watanabe et al. (1991) conducted an additional study comparing the Vietnam-veteran mortality experience of Breslin et al. (1988) with three referent groups and with additional follow-up through 1984. A third follow-up proportionate-mortality study using the veterans from Breslin et al. (1988) and Watanabe et al. (1991) was also conducted (Watanabe and Kang, 1996).

DVA also examined the morbidity and mortality experience of a subgroup of Vietnam veterans potentially exposed to high concentrations of herbicides from some US Army Chemical Corps units (Thomas and Kang, 1990). In an extension of Thomas and Kang (1990), Dalager and Kang (1997) compared mortality among veterans of the Chemical Corps specialties, including Vietnam veterans and non-Vietnam veterans. Watanabe and Kang (1995) also examined postservice mortality among Marine Vietnam veterans compared with Vietnam era marines who did not serve in Vietnam. Mortality among female Vietnam veterans was assessed by Thomas et al. (1991) and updated in Dalager et al. (1995a).

DVA has evaluated specific disease and health outcomes—including case–control studies of STS (Kang et al., 1986, 1987), NHL (Dalager et al., 1991), testicular cancer (Bullman et al., 1994), HD (Dalager et al., 1995b), and lung cancer (Mahan et al., 1997)—and has conducted a co-twin study of self-reported physical health in a series of Vietnam-era monozygotic twins (Eisen et al., 1991).

DVA has also examined other outcomes—including posttraumatic stress disorder (PTSD) (True et al., 1988; Bullman et al., 1991), suicide, motor-vehicle accidents (Farberow et al., 1990), and smoking behavior (McKinney et al., 1997)—among Vietnam veterans and has studied cause-specific mortality among veterans with nonlethal (combat and noncombat) wounds sustained during the Vietnam War (Bullman and Kang, 1996). *VAO* and *Update 1998* discuss those studies in detail. In many of the studies, exposure to Agent Orange is not discussed; exposure to "combat" is evaluated as the risk factor of interest.

Since *Update 1998*, DVA has published a study on pregnancy outcomes among US female Vietnam veterans (Kang et al., 2000a). Of 5,230 women, 4,390 whose permanent tour of duty included service in Vietnam were alive as of January 1, 1992. From a pool of 6,657 potential control subjects whose military unit did not include service in Vietnam, 4,390 who were alive as of January 1, 1992, were randomly selected as controls. A questionnaire was administered on demographic background, general health, lifestyle, menstrual history, pregnancy history, pregnancy outcomes, and military experience, including nursing occupa-

tion and combat exposure. Information on pregnancy complications—including smoking, infections, medications, exposure to x-rays, occupational history, and exposure to anesthetic gases, ethylene oxide, herbicides, and pesticides was collected for each pregnancy. The first pregnancy after the beginning of Vietnam service was designated as the index pregnancy for each woman. For the comparison group, the first pregnancy after July 4, 1965, was used as the index pregnancy. Odds ratios were calculated for reproductive history and pregnancy outcomes. The study analyzed data on 3,392 Vietnam and 3,038 non-Vietnam veterans and on 1,665 Vietnam and 1,912 non-Vietnam veteran indexed pregnancies.

DVA has also published a study on gynecologic cancers among US Vietnam veterans (Kang et al., 2000b). Of 5,230 potential study participants, 4,390 women veterans who served in Vietnam in July 1965–March 1973 were located and alive as of January 1, 1992. From a pool of 6,657 potential control female Vietnam-era veterans whose tour of duty did not include service in Vietnam, 4,390 who were alive as of January 1, 1992, were randomly selected as controls. A 45-min health questionnaire was administered that included questions on demographic background, general health, smoking and drinking history, menstrual history, pregnancy history, pregnancy outcomes, and military experience, including nursing occupation and combat exposure. Medical and hospital records were reviewed to document self-reported cancers of the breast, ovary, uterus, and cervix. Additional information was obtained from military records regarding effect modifiers and confounders. Of the total of 8,780 women, 500 (250 Vietnam veterans and 250 controls) were used in a feasibility pilot study and therefore were not included in the final analysis. A total of 6,430 women completed the full telephone interview.

In response to a study recommendation in *VAO*, Kang et al. (2001) conducted a preliminary long-term health study of US Army Chemical Corps Vietnam veterans. A review of morning reports, military personnel records, and class rosters from the Army Chemical School resulted in a pool of 2,872 Vietnam veterans and 2,737 non-Vietnam veterans who were eligible for the study. Army Chemical Corps veterans were considered eligible if they had served in the US Army on active duty for a minimum of 18 months and whose military record reflected service in Vietnam with chemical operations duties in July 1965–March 1973. Non-Vietnam veteran controls had similar military histories with respect to branch, length, and period of service and military occupation but did not have active duty in Vietnam. A random sample of 284 Vietnam and 281 non-Vietnam veterans were selected from the larger pool (Vietnam veterans, 2,872; eligible non-Vietnam veterans, 2,737) for the study. A computer-assisted telephone interview was administered to collect information on veterans' exposures, health problems, and offspring. Military records supplemented and validated self-reported interview data to the extent possible. Blood samples were collected, and

serum TCDD concentrations were collected from 50 Vietnam veterans and controls.

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 were conducted on veterans who had served in Southeast Asia and veterans who served elsewhere (Snow et al., 1988; Stellman et al., 1988a,b,c). No new studies have been published on this cohort.

State Studies

Several states have conducted studies of Vietnam veterans. Most of the 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; Clapp, 1997), Michigan (Visintainer et al., 1995), New Jersey (Kahn et al., 1988; Fiedler and Gochfeld, 1992, Kahn et al., 1992a,b,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).

Other US Vietnam Veteran Studies

Additional studies have been conducted to examine a number of health outcomes, including spontaneous abortion (Aschengrau and Monson, 1989) and late adverse pregnancy outcomes in spouses of Vietnam veterans (Aschengrau and Monson, 1990) and PTSD among monozygotic twins who served during the Vietnam era (Goldberg et al., 1990). After a published study indicated 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 those studies, and no new studies have been published.

Australia

The Australian government has commissioned studies to investigate health risks to Australian veterans. Studies of birth anomalies (Donovan et al., 1983, 1984; Evatt, 1985), mortality (Commonwealth Institute of Health, 1984a,b,c; Evatt, 1985; Fett et al., 1987a,b; Forcier et al., 1987; Crane et al., 1997a,b), deaths

from all causes (Fett et al., 1987b), cause-specific mortality (Fett et al., 1987a), and morbidity (AIHW, 1999, 2000; CDVA 1998a,b) have been conducted. A revised morbidity study has also been published (AIHW, 2001). An independent study in Tasmania evaluated numerous reproductive and childhood health problems for association with paternal Vietnam service (Field and Kerr, 1988). In addition, O'Toole et al. (1996a,b,c) described self-reported health status in a random sample of Australian Army Vietnam veterans. *VAO*, *Update 1998*, and *Update 2000* describe the studies. No new studies or data have been published since the acute myelogenous leukemia report (IOM, 2001).

Other Vietnam-Veteran Studies

A team of Vietnamese scientists examined Vietnamese veterans who served in a "dioxin-sprayed zone," looking at antinuclear and sperm autoantibodies (Chinh et al., 1996). Available details of this study are presented in *Update 1998*. No other studies in similar cohorts have been published.

TABLE 4-1 Epidemiologic Studies—Occupational Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
PRODUCTION WORKERS				
New NIOSH Studies				
Steenland et al., 2001	Cohort	A study to reexamine and compare diabetes data from the NIOSH cohort and the United States Air Force Ranch Hands in order to reconcile differences between the two study methods and protocols	267 NIOSH workers 990 Ranch Hands	227 NIOSH comparisons 1,275 Ranch Hand comparisons
***NIOSH Studies Reviewed in* Update 2000**				
Calvert et al., 1999	Cohort	Continuing follow-up of workers employed more than 15 years ago at two plants that manufactured substances contaminated with TCDD to evaluate associations between serum TCDD and serum glucose (diabetes), TSH, total T_4, and T_3	281	260
Steenland et al., 1999	Cohort	Mortality study of workers at 12 industrial plants that produced chemicals contaminated with TCDD, using a job-exposure matrix to estimate TCDD exposure categories. End points reported are all cancers, ischemic heart disease, and diabetes	5,132 (3,538 with exposure data divided into septiles of cumulative exposure; 608 who had chloracne)	—

Calvert et al., 1998	Cohort	Continuing follow-up of workers employed more than 15 years ago at two plants that manufactured substances contaminated with TCDD to evaluate the association between TCDD exposure and cardiovascular outcomes	281	260
Halperin et al., 1998	Cohort	Continuing study of a cohort of TCDD-exposed workers at two plants that manufactured substances contaminated with TCDD to assess the association between serum TCDD and immunological outcome variables for eligible workers and matched neighborhood controls	259	243
***NIOSH Studies Reviewed in* Update 1998**				
Sweeney et al., 1996, 1997/1998	Cross-sectional	Study of numerous noncancer end points 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 P450 induction in same group as Calvert et al. (1991)	281	260

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***NIOSH 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
***NIOSH 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 at least 15 years prior to assessment of chronic bronchitis, COPD, ventilatory function, thorax, and lung abnormalities, compared to matched neighborhood controls	281	260

Fingerhut et al., 1991	Cohort	Cancer mortality in male workers from 12 plants producing TCDD contaminated chemicals (1942–1984), compared to US population	5,172	—
***Monsanto Studies Reviewed in* VAO**				
Collins et al., 1993	Cohort	Mortality of workers (through 1987) exposed and not exposed 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 nonexposed workers at same Monsanto plant	204	163

continues

TABLE 4-1 *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 rates of standardized US population	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 US male population standard	121	—
New Dow Studies				
Burns et al., 2001	Cohort	Study comparing mortality in a cohort of chemical workers who manufactured or formulated 2,4-D between 1945 and 1994	1,567 workers	40,600 nonexposed chemical workers; US population
***Dow Studies Reviewed in* Update 1998**				
Ramlow et al., 1996	Cohort	Study of mortality in a cohort of workers exposed to pentachlorophenol (PCP)	770	36,804 nonexposed workers; US population
***Dow Studies Reviewed in* Update 1996**				
Bloemen et al., 1993	Cohort	Additional years of follow-up of Bond et al. (1988) study cohort through 1986	878	36,804 nonexposed workers; US population

***Dow 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 US white males and all other male employees not exposed	878	36,804 employees not exposed; US white male population
Bond et al., 1987	Cohort	Extension of Cook et al. (1980) study, mortality through 1982	322	2,026 employees without chloracne; US white male population
Cook et al., 1987; Ott 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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Cook et al., 1986	Cohort	Mortality experience (1940–1979) of men manufacturing chlorinated phenols compared to US white men	2,189	—
Bond et al., 1983	Cross-sectional	Study of differences in workers potentially exposed and not exposed 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 US white men	61	—

Ott et al., 1980	Cohort	Mortality experience among workers exposed to 2,4,5-T in manufacturing (1950–1971) compared to mortality experience of US white men	204	—
***BASF Studies Reviewed in* Update 2000**				
Zober et al., 1997	Cohort (1953 accident) Cross-sectional (1988 cohort)	Review and summary of previous BASF studies of morbidity and mortality in workers exposed to TCDD after BASF accidents in 1953 and 1988	154 surviving (as of 1989) members of 1953 accident cohort 42 exposed (1988) extruder personnel	No comparison group
***BASF Studies Reviewed in* Update 1998**				
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	—
***BASF Studies Reviewed in* Update 1996**				
Zober et al., 1994	Cohort	Morbidity experience in the same group as Zober et al. (1990)	158	161
***BASF 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 (FRG)	247	—

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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	180,000 (town); 1.8 million (district); 60.5 million (FRG); two groups of 74 each from other cohort studies
***IARC Studies Reviewed in* Update 2000**				
Neuberger et al., 1999	Austrian chloracne cohort	Morbidity up to 1993 of exposed chemical workers assessed by health insurance data and health examination, laboratory measures, and interviews with participating survivors and controls	159, including 50 who participated in examination	Two control groups comparable to the 50 participants—numbers not given
Hooiveld et al., 1998	Cohort	Mortality (through 1991), using SMRs, of workers at one Dutch factory assessed in relation to work and exposure history; SMR and relative risk analyses	562 (serum samples for 50); 140 males at accident	567
Jäger et al., 1998	Cohort	Preliminary data from Neuberger et al. (1999; English abstract only)	159 in original cohort; 56 screened; 49 full data	Matched nonexposed controls
Neuberger et al., 1998	Cohort of exposed cases	Preliminary data from Neuberger et al. (1999)	50	Age- and sex-matched controls; number not given

Vena et al., 1998	Cohort	International study (36 cohorts from 12 countries) of workers producing or spraying phenoxy acid herbicides and chlorophenols, categorized into one of three TCDD or higher chlorinated dioxin categories. Noncancer mortality (1939–1992) was analyzed by standardized mortality rate comparisons and by Poisson multiple regression	21,863	No comparison group
Flesch-Janys, 1997	Cohort	Mortality (1952–1984) study of German workers exposed to TCDD and other contaminants in the production of herbicides and insecticides. SMRs and Cox regression models were calculated	1,189	—
***IARC Studies Reviewed in* Update 1998**				
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)	—

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
***IARC 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	—

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 nonexposed employees
***IARC 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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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	—
***Studies from Other Chemical Plants Reviewed in* Update 2000**				
Hryhorczuk et al., 1998	Cohort	Morbidity study of workers involved in pentachlorophenol production at one factory between 1938 and 1978 and nonexposed workers at the same factory. Assesses chloracne, prophyria, and general health status	366	303

Jung et al., 1998	Cohort	Self-selected group of former workers at pesticide-producing factory participated in physical examination, laboratory measures, and questionnaires. Associations between serum PCDD/F, infectious disease, and immunologic measures were assessed	192	
		Lymphocyte proliferation and chromate resistance tests were compared between a subgroup of the mostly highly exposed workers at the study factory and an nonexposed group of workers in another industry	29 (highly exposed subgroup)	28 (external nonexposed group)
***Studies from Other Chemical Plants Reviewed in* Update 1998**				
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 from Other Chemical Plants 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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 US white men and for cancers compared to local men	1,412	—
May, 1982, 1983	Cohort	Health outcomes among workers exposed and probably exposed to TCDD following a 1968 accident, compared to nonexposed 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 porphyria cutanea tarda (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 AND FOREST PRODUCTS

New Cohort Studies of Agricultural Workers Studies

Arbuckle et al., 2001	Cohort	Spontaneous abortions in couples living on full-time family-run farms in Ontario, Canada	2,110 women; 3,936 pregnancies	none
Masley et al., 2000	Cross-sectional survey	Targeted survey of households in an agricultural-based rural area of Saskatchewan, Canada	548 households; 1,407 individuals	none
Curtis et al., 1999	Cohort	Time to pregnancy in couples living on full-time family-run farms in Ontario, Canada	2,012 pregnancies	none
Savitz et al., 1997	Cohort	Male pesticide exposure and pregnancy outcome among full-time family-run farms in Ontario, Canada	1,898 couples; 3,984 pregnancies	none

***Cohort Studies of Agricultural Workers Reviewed in* Update 2000**

Arbuckle et al., 1999	Cohort	Spontaneous abortions in couples living on full-time family-run farms in Ontario, Canada	2,110 women (3,936 pregnancies)	none

***Cohort Studies of Agricultural Workers Reviewed in* Update 1998**

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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Faustini et al., 1996	Cohort	Study of immune system components and function among farmers who mixed and applied commercial formulations containing the chlorophenoxy herbicides 2,4-D and MCPA	10	Internal comparison
***Cohort Studies of Agricultural Workers Reviewed in* Update 1996**				
Dean, 1994	Cohort	Study of mortality from brain and hematopoietic cancers of agricultural workers compared to nonagricultural 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
***Cohort Studies of Agricultural Workers 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 or older (1971–1987) compared to Canadian prairie province rates	155,547	—

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Ronco et al., 1992	Cohort	Study of cancer incidence (1970–1980) among male and female Danish farm workers 15 to 74 years old, compared to expected numbers of cancers among persons economically active, and study of cancer mortality (November 1981–April 1982) among male and female Italian farmers 18 to 74 years old compared to persons in other occupational groups	No *N* given	No *N* given
Lerda and Rizzi, 1991	Cohort	Study of farmers exposed to 2,4-D, as measured in urine, compared to unexposed men 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 NHL 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
New Cohort Studies of Forestry Workers				
Thörn et al., 2000	Cohort	Study of mortality and cancer incidence in a cohort of Swedish lumberjacks exposed to phenoxy herbicides	261	243
***Cohort Studies of Forestry Workers Reviewed in* VAO**				
Green, 1991	Cohort	Mortality experience of male forestry workers (1950–1982) in Ontario, compared to expected mortality of the male Ontario population	1,222	—

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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, compared to nonexposed workers	54	54
New Cohort Studies of Herbicide and Pesticide Sprayers				
Hoppin et al., 2002	Cohort	Study predicting wheeze among farmers who applied pesticide in the Agricultural Health Study	3,838 appliers with wheeze	16,630 appliers without wheeze
***Cohort Studies of Herbicide and Pesticide Sprayers Reviewed in* Update 2000**				
Alavanja et al., 1998	Cohort	Analysis of self-reported health care visits having resulted from pesticide use by Iowa and North Carolina pesticide appliers	35,879	None
Dich et al., 1998	Cohort	Study of men licensed for pesticide application in Sweden. Cancer cases ascertained from cancer registry and standardized incidence ratio reported for prostate cancer	20,025	—

Cohort Studies of Herbicide and Pesticide Sprayers Reviewed in **Update 1998**

Heacock et al., 1998	Cohort	Fertility study among British Columbia workers potentially exposed to chlorophenate wood preservatives in 14 sawmills between 1955 and 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 to chlorophenate wood preservatives in 11 sawmills between 1950 and 1985	23,829	2,658
Dimich-Ward et al., 1996	Cohort; Nested case-control	Analysis of birth defects among offspring born between 1952 and 1988 of the Hertzman et al. (1997) cohort	19,675 births among 9,512 fathers	5 nondefect births as 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 of male pesticide appliers in Minnesota born between 1989 and 1992	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 males and 589 females)	—

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***Cohort Studies of Herbicide and Pesticide Sprayers 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, 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
***Cohort Studies of Herbicide and Pesticide Sprayers Reviewed in* VAO**				
Swaen et al., 1992	Cohort	Cancer mortality experience (through 1987) among Dutch male herbicide appliers licensed before 1980, compared to total male Dutch population	1,341	—
Bender et al., 1989	Cohort	Cancer mortality of Minnesota highway maintenance workers compared to expected numbers based on white Minnesota men	4,849	—
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 US and Florida men	3,827	—
Riihimaki et al., 1983	Cohort	Cancer morbidity and mortality in cohort of 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	113 pregnancies (chemicals not 2,4,5-T); 486 pregnancies (2,4,5-T)	401 pregnancies (not exposed)

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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

***Case-Control Studies Reviewed in* Update 2000**

Ekström et al., 1999	Case-control	All new cases of histologically confirmed gastric adenocarcinoma in two geographic areas in Sweden; age- and gender-matched control group randomly selected using computerized population register	565	1,164
Hardell and Eriksson, 1999	Case-control	Male cases 25 or older with histopathologically confirmed NHL during 1987–1990 in northern and mid-Sweden; age matched controls from National Population Registry	404	741
Garcia et al., 1998	Case-control	Matched-paired study of congenital malformations or defects in an agricultural region of Spain	261	261

***Case-Control Studies Reviewed in* Update 1998**

Blatter et al., 1997	Case-control	Multicenter Dutch study of paternal occupation and risk of spina bifida in offspring (1980–1992)	222	764
Liou et al., 1997	Case-control	Study of occupational and environmental risk factors and Parkinson's disease (PD) in Taiwan (1993–1995)	120	240

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Tatham et al., 1997	Nested case-control	Population-based study of occupational risk factors for subgroups of NHL patients based on the CDC's Selected Cancers Study (CDC, 1990a,b,c,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 (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	Based on 130,420 death certificates	
Seidler et al., 1996	Case-control	Study of PD and various rural factors, including exposure to herbicides and wood preservatives in Germany	380	379 neighborhood controls; 376 regional controls
***Case-Control 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 defects in 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	NHL: 93 HD: 31	204
Semchuk et al., 1993	Case-control	Study of cases of PD (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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
***Case-Control 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 controls
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 (MM), 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 United States Department of Agriculture (USDA) forest or soil conservationists (1970–1979) evaluated for specific cancer excess; case-control study of specific cancers identified from PMR analysis	1,411	—

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Boffetta et al., 1989	Nested case-control	National study of MM 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 to 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) cohort for association with phenoxy herbicides in farm workers	576	694
Alavanja et al., 1988	PMR analysis with nested case-control	Mortality experience of USDA extension agents (1970–1979) evaluated for specific cancer excess; case-control study of specific cancers identified from PMR analysis	1,495	—

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 and 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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Hardell et al., 1987	Case-control	Study of Kaposi's sarcoma in AIDS patients (23–53 years old) 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-9 200-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, and 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 MM, including farm use of herbicides	698	1,683
Pearce et al., 1986a	Case-control	Study of male MM cases diagnosed 1971–1981 in New Zealand, compared to controls for other cancers for potential association with phenoxy herbicides and chlorophenols	76	315
Pearce et al., 1986b	Case-control	Study of NHL cases (ICD-9 202) 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 update (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

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Blair and White, 1985	Case-control	Study of leukemia cases by cell type in Nebraska (1957–1974) compared to nonleukemia deaths for association with agricultural practices	1,084	2,168
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 old 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 MM 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 years old, 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

continues

TABLE 4-1 *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 old 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 old 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 paternal exposure to 2,4-D	134	311
Eriksson et al., 1979, 1981	Case-control	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	(1) Cases of STS (Hardell and Sandstrom, 1979) and malignant lymphomas (Hardell et al., 1981) compared to colon cancer cases (2) Colon-cancer cases 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	Cases of malignant lymphomas (HD, NHL, unknown) diagnosed in men 25–85 years old, between 1974 and 1978 in northern Sweden, compared to population-based controls for association with occupational exposure to phenoxyacetic acids and chlorophenols	60 HD 109 NHL	338

continues

TABLE 4-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Blair and Thomas, 1979	Case-control	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	Cases of STS (26–80 years old) diagnosed between 1970 and 1977 in northern Sweden, compared to population-based controls for association with occupational exposure to phenoxyacetic acids and chlorophenols	52	206
PAPER AND PULP WORKERS				
***Paper and Pulp Worker Studies Reviewed in* Update 2000**				
Schildt et al., 1999	Case-control	Matched study of histopathologically verified oral cancer cases. Mailed exposure questionnaire on lifetime occupational history, oral cancer risk factors, pesticide use, smoking, SES, and place of residence	410	410
Rix et al., 1998	Cohort	Cancer incidence rates of blue-collar workers at three Danish paper mills were compared to population rates from national population and mortality registers	14,788 (14,362 were identified for follow-up)	—

***Paper and Pulp Worker 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, New Hampshire, paper and pulp industry, compared to expected mortality in US 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 US men	201	—
Robinson et al., 1986	Cohort	Mortality experience through March 1977 of white male workers employed in five paper or pulp mills compared to expected number of deaths among U.S. population	3,572	—

[a]The dash (—) indicates the comparison group is based on a population (e.g., US white males, country rates), and details are given in the text for specifics of the actual population.

ABBREVIATIONS: CDC, Centers for Disease Control and Prevention; COPD, chronic obstructive pulmonary disease; HD, Hodgkin's disease; IARC, International Agency for Research on Cancer; ICD, International Classification of Diseases; MM, multiple myeloma; NIOSH, National Institute for Occupational Safety and Health; NHL, non-Hodgkin's lymphoma; PMR, proportionate mortality ratio; SEER, surveillance, epidemiology, and end results; STS, soft-tissue sarcoma; *Update 2000*, *Veterans and Agent Orange: Update 2000* (IOM, 2001); *Update 1998, Veterans and Agent Orange*: *Update 1998* (IOM, 1999); *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).

TABLE 4-2 Epidemiologic Studies—Environmental Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
New Studies from Seveso				
Warner et al., 2002	Cohort	Study to evaluate the association between individual serum TCDD levels and breast cancer risk in women who participated in the Seveso Women's Health Study	15	981
***Studies from Seveso Reviewed in* Update 2000**				
Bertazzi et al., 2001	Cohort	Mortality (through 1996) study of residents in industrial accident exposure-related geographic regions	804 zone A 5,941 zone B 38,624 zone R	232,745
Bertazzi et al., 1998; Pesatori et al., 1998	Cohort	Mortality (through 1991) study of residents in industrial accident exposure-related geographic regions	805 zone A 51,943 zone B 38,625 zone R	232,747
***Studies from Seveso Reviewed in* Update 1998**				
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 zone A 5,943 zone B 38,625 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 to 1984 and (2) 1985 to 1994	(1) 74 births (28 male, 48 female) (2) 124 births (60 male, 48 female)	

***Studies from Seveso 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 nonexposed 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 postaccident decade compared to age-matched residents of neighboring nonexposed areas	Approximately 20,000	167,391
***Studies from Seveso 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
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 mortality experience of nonexposed residents in neighboring towns	556 zone A 3,920 zone B 26,227 zone R	167,391

continues

TABLE 4-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Barbieri et al., 1988	Cohort	Comparison of prevalence of peripheral nervous system involvement among Seveso residents with chloracne, compared to residents of 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 birth mothers who were 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 noncontaminated 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 in 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 nonexposed areas	308	305
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 nonexposed 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 nonexposed mothers	402 births	804 births
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 exposure)	36 (low exposure)

continues

TABLE 4-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 exposure)	36 (low exposure)
***Studies of Vietnamese 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 of Vietnamese 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

Constable and Hatch, 1985	Review	Summaries of reproductive outcomes among Vietnamese populations, includes nine unpublished studies		
Other New Environmental Studies				
Revazova et al., 2001	Cohort	Cytogenetic effects in women exposed to different levels of dioxin while living in Chapaevsk, Russia	15 possibly exposed workers	30 nonexposed but living close to plant
Revich et al., 2001	Cohort	Study of dioxin exposures in Chapaevsk, Russia which looked at various health outcomes in children and adults	Children and adults in Chapaevsk, Russia	Samara region and all of Russia
Other Environmental Studies Reviewed in **Update 2000**				
Schreinemachers, 2000	Cross-sectional	Study of cancer mortality rates in four northern wheat-producing states using wheat acreage per county as surrogate for exposure	—	—
Other Environmental Studies Reviewed in **Update 1998**				
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, MM, and AML in Italy by region	HD: 421 NHL: 1,822 MM: 325 AML: 263	Internal comparison by region

continues

TABLE 4-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 prenatal andpostnatal 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 inhalation exposure to TCDD and related compounds in wood preservatives on cell-mediated immunity in German day care center employees	221	189
***Other Environmental 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
Other Environmental 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 design	Presentation of rates (1985–1988) of NHL, HD, and STS in men and women 15–74 years old 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 nonexposed controls
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	—
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 studies	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 or herbicides	437	724
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 or lip in Iowa and Michigan, compared to other live births	187	985

continues

TABLE 4-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 period	9,614 births	15,000 births
Nelson et al., 1979	Ecological design	Study of prevalence of oral cleft palates in high, medium, and low 2,4,5-T sprayed areas in Arkansas (1948–1974)	—	—
US 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	1,666 births—unsprayed area; 4,120 births—urban area

[a]The dash (—) indicates the comparison group is based on a population (e.g., US white males, country rates), with details given in the text for specifics of the actual population.

ABBREVIATIONS: AML, acute myelogenous leukemia; HD, Hodgkin's disease; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; STS, soft-tissue sarcoma; *Update 2000, Veterans and Agent Orange: Update 2000* (IOM, 2001); *Update 1998, Veterans and Agent Orange: Update 1998* (IOM, 1999); *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).

TABLE 4-3 Epidemiologic Studies—Veterans' Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
UNITED STATES STUDIES				
New Ranch Hand Studies				
Michalek et al., 2001a	Cohort	Based on physical examination through 1992 and medical records reviewed through March of 1993; analyzed association between serum dioxin levels and hepatic abnormalities	931	1,199
Michalek et al., 2001b	Cohort	Based on physical examination in 1982, 1985, 1987, 1992, and 1997 and medical records through 1997; analyzed association between serum dioxin levels and peripheral neuropathy	926 918 872 834 761	1,067 1,144 1,131 1,110 1,086
Michalek et al., 2001c	Cohort	Based on physical examination in 1982, 1985, 1987, and 1992 and medical record through 1997; analyzed association between serum dioxin levels and hematological function	1,046 1,017 996 953	1,223 1,292 1,298 1,280
Barrett et al., 2001	Cohort	Based on tests of cognitive function in 1982, and dioxin levels measured in 1987 and 1992; analyzed association between serum dioxin levels and cognitive function	937	1,052

continues

TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Steenland et al., 2001	Cohort	A study to reexamine and compare diabetes data from the NIOSH cohort and the United States Air Force Ranchs Hands in order to reconcile differences between the two study methods andprotocols	267 NIOSH workers 990 Ranch Hands	227 NIOSH comparisons 1,275 Ranch Hand comparisons
***Ranch Hand Studies Reviewed in* Update 2000**				
AFHS, 2000	Cohort	Evaluation of 266 health-related end points, including assessments of 10 clinical areas: general health, neoplasia, neurological, psychological, gastrointestinal, cardiovascular, hematologic, endocrine, immunologic, and pulmonary	995	1,299
Longnecker and Michalek, 2000	Cohort	Based on physical examination and medical record review through 1992, analyzed association between serum dioxin concentrations and diabetes mellitus among the comparison group (no Ranch Hands)	—	1,281 1,197
Ketchum et al., 1999	Cohort	Based on physical examination and medical record review through 1992, analyzed association between serum dioxin levels and cancer, skin cancer, and other than skin cancer	1,109 980 922 980	1,493 1,275 1,202 1,275

Michalek et al., 1999a	Cohort	To further elucidate the relationship between dioxin and diabetes mellitus, this analysis studies the effect of dioxin body burden on the relationship between sex hormone-binding globulin and insulin and fasting glucose	952 871	1,281 1,121
Michalek et al., 1999b	Cohort	Based on physical examinations in 1982, 1985, 1987, and 1992, examination of immunologic response and exposure to dioxin among Ranch Hand and comparison cohorts	952 914 372 358	1,281 1,186 491 456
Burton et al., 1998	Cohort	Based on physical examination and medical record review through 1992, analyzed association between serum dioxin levels and occurrence and timing (relative to Southeast Asia service) of chloracne and acne	952 930 476	1,281 1,200 598
Michalek et al., 1998b	Cohort	Updates all-cause and cause-specific postservice mortality (through 1993) among veterans of Operation Ranch Hand, using standardized mortality ratios	1,261	19,080
Michalek et al., 1998c	Cohort	Prospective study of exposure and long-term health, survival, or reproductive outcome	1,208 veterans 903 offspring	1,549 veterans 1,254 offspring
Michalek et al., 1998d	Cohort	Third report in a series investigating dioxin body burden and preterm birth, intrauterine growth retardation, and infant death among offspring of Ranch Hand veterans	995 932 859	1,299 1,202 1,223

continues

TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***Ranch Hand Studies Reviewed in* Update 1998**				
Michalek et al., 1998a	Cohort	Paternal serum dioxin concentrations and infant death among 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 levels, 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)
***Ranch Hand Studies Reviewed in* Update 1996**				
AFHS, 1995	Cohort	Mortality updates of Ranch Hands 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	1,202
***Ranch Hand Studies Reviewed in* VAO**				
AFHS, 1992	Cohort	Reproductive outcomes of participants in the Air Force Health Study (AFHS)	791	942
AFHS, 1984a, 1987, 1990, 1991b, 1995	Cohort	Baseline morbidity and follow-up exam results of the AFHS	1,208 (baseline)	1,668 (baseline)
AFHS, 1983, 1984b, 1985, 1986, 1989, 1991a	Cohort	Mortality updates of Ranch Hands 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)
Michalek et al., 1990	Cohort	Mortality of Ranch Hands compared with Air Force C-130 air and ground crew veterans in Southeast Asia	1,261	19,101
Wolfe et al., 1990	Cohort	Health status of Ranch Hands at second follow-up, compared with Air Force C-130 air and ground crew veterans in Southeast Asia	995	1,299

continues

TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***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 (VES)	7,924	7,364
O'Brien et al., 1991	Cohort	Interview report and mortality for NHL based on VES	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
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 sarcoma	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 US Army enlisted men 1965–1971	2,490	1,972
CDC, 1988a	Cohort	VES—random sample of US Army enlisted men 1965–1971: psychosocial outcomes	2,490	1,972
CDC, 1988b	Cohort	VES: physical health outcomes	2,490	1,972
CDC, 1988c	Cohort	VES: reproductive outcomes	12,788 children	11,910 children
CDC, 1987; Boyle et al., 1987	Cohort	VES: mortality	9,324	8,989
Erickson et al., 1984 a,b	Case-control	CDC birth defects study of children born in the Atlanta area between 1968 and 1980, comparing fathers' Vietnam experience and potential Agent Orange exposure between birth defects cases and normal controls	7,133	4,246
New Department of Veterans Affairs (VA) Studies				
Kang et al., 2000a	Cohort	Self-report pregnancy outcomes for female Vietnam veterans compared to contemporary female veterans not deployed to Vietnam. Odds ratios were calculated for reproductive history and various birth defects	3,392 women; 1,665 women with an indexed pregnancy	3,038 women; 1,912 women with an indexed pregnancy

continues

TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Kang et al., 2000b	Cohort	Study of gynecologic cancers among female Vietnam veterans compared to femae veteran controls	484	5,946
Kang et al., 2001	Cohort	Study evaluating the health of Army Chemical Corps Vietnam veterans compared to Army Chemical Corps veterans who did not serve in Vietnam	2,872	2,737
***Department of Veterans Affairs (VA) Studies Reviewed in* Update 1998**				
Dalager and Kang,1997	Cohort	Morbidity and mortality experience (1968–1987) of Army Chemical Corps Vietnam veterans compared to US 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 men hospitalized without cancer; 111 patients with colon cancer
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	—

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	Postservice mortality among Marine Vietnam veterans	10,716	9,346
***VA Studies Reviewed in* Update 1996**				
Dalager et al., 1995a	Cohort	Update of Thomas et al. (1991) through December 31, 1995	4,586	5,325
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
***VA 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

continues

TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 US 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 US male population	24,145 Army, 5,501 Marines	(1) 27,145 Army, 4,505 Marines (2) 32,422 combined Vietnam era (3) US 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 US men	894	—
True et al., 1988	Cross-sectional	PTSD and Vietnam combat experience evaluated among Vietnam-era veterans	775	1,012

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

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TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***State Studies Reviewed in* Update 1998**				
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
***State 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
***State Studies Reviewed in* VAO**				
Fiedler and Gochfeld, 1992; Kahn et al., 1992a,b,c	Cohort	New Jersey study of outcomes in select group of herbicide-exposed Army, Marine, and Navy Vietnam veterans, compared to veterans self-reported as unexposed	10 Pointman I 55 Pointman II	17 Pointman I 15 Pointman II
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

Levy, 1988	Cross-sectional	Study of PTSD in chloracne as indicator of TCDD-exposed 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
Anderson et al., 1986b	Cohort	Mortality experience of Wisconsin Vietnam-era veterans and Vietnam veterans compared to US 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, and Vietnam-era veterans compared to nonveterans; Vietnam veterans compared to Vietnam-era veterans	615 Vietnam veterans 610 Vietnam-era veterans	—

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TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Pollei et al., 1986	Cohort	Study of chest radiographs of New Mexico Agent Orange Registry Vietnam veterans compared to radiographs of control Air Force servicemen 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 of 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 US Veteran 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
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 or by 27 weeks compared to women delivering at 37 weeks	201	1,119
AUSTRALIAN STUDIES				
***Australian Studies Reviewed in* Update 2000**				
AIHW, 1999	Cohort	Validation of the male veterans study (CDVA, 1998a) using medical documents, doctors' certification and records on a disease or death registry	6,842	—

continues

TABLE 4-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
CDVA, 1998a	Cohort	Self-reported data on male members of the Australian Defence Force and the Citizen Military Force who landed in Vietnam or entered Vietnamese water. Questions on physical (including reproductive history) and mental health, and that of their partner(s) and children	49,944 mailed; 39,955 responded	—
CDVA, 1998b	Cohort	Self-reported data on female members of the Australian Defence Force and the Citizen Military Force who landed in Vietnam or entered Vietnamese water. Questions on physical (including reproductive history) and mental health, and that of their partner(s) and children	278 mailed 225 responded	—
Australian Studies Reviewed in* Update *1998				
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,b,c	Cross-sectional	Survey of self-reported health status (1989–1990) of Australian Army Vietnam veterans	641	—

***Australian 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 Reviewed in* Update 1998**				
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 age-matched controls; 36 additional male controls

[a] The dash (—) indicates the comparison group is based on a population (e.g., US white males, country rates), with details given in the text for specifics of the actual population.

ABBREVIATIONS: HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; PTSD, posttraumatic stress disorder; STS, soft-tissue sarcoma; CDVA, Commonwealth Department of Veterans' Affairs; *Update 2000, Veterans and Agent Orange: Update 2000* (IOM, 2001); *Update 1998, Veterans and Agent Orange: Update 1998* (IOM, 1999); *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).

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5

Exposure Assessment

Assessment of human exposure to herbicides and the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a key element in determining whether specific health outcomes are linked to them. This chapter reviews information on occupational and environmental exposures to herbicides and TCDD, including exposure of Vietnam veterans. The purpose of this chapter is to discuss exposure assessment and the exposure assessment that has been conducted in some of the epidemiologic studies as background for the health-outcome chapters that follow; no studies are evaluated and results are not discussed in this chapter. The committee's evaluations are presented in the health-outcome chapters. A more complete discussion of the exposures and a detailed review of the US military's wartime use of herbicides in Vietnam can be found in Chapters 3 and 6 of *Veterans and Agent Orange* (IOM, 1994) and in Chapter 5 of *Veterans and Agent Orange: Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), and *Update 2000* (IOM, 2001). Reviews of the most recent studies of the absorption, distribution, metabolism, and excretion of herbicides and TCDD can be found in the discussion of toxicokinetics in Chapter 3 of this report.

EXPOSURE ASSESSMENT FOR EPIDEMIOLOGY

Exposure to chemical contaminants can be defined as the amount of the contaminant that contacts a body barrier and is available for absorption over a defined period. Ideally, exposure assessment would quantify the amount of chemical at the site of toxic action in the tissue of an organism. In studies of human populations, however, it is not usually possible to measure those concentrations.

Instead, exposure assessments are based on chemical measurements in either environmental media or biological specimens. In either case, exposure serves as a surrogate for dose. Exposure assessments based on measurements of chemical contaminants in the environment attempt to quantify the amount of the contaminant that contacts a body barrier over a defined time period. Exposure can occur via three routes: inhalation, skin contact, and ingestion. Exposure can also be assessed by measuring chemicals or their metabolites in human tissues. Such biomarkers of exposure integrate absorption from all routes. The evaluation of biomarkers can be complex, since most markers are not stable for long periods of time. Knowledge of pharmacokinetics is essential to the linkage of measurements at the time of sampling with past exposures. Similarly, biomarkers that have the possibility of being biomarkers of effect, such as DNA adducts, show promise, but do not necessarily provide accurate measures of past exposures; that is, there is no evidence that currently measured DNA adducts have any relationship to occupational or environmental exposures experienced years before.

Quantitative assessments based on environmental or biologic samples are rarely available for epidemiologic studies; instead, investigators must rely on a mixture of qualitative and quantitative information to produce exposure estimates. One can usefully distinguish a few basic approaches to exposure assessment for epidemiology (Checkoway et al., 1989; Armstrong et al., 1994). The simplest approach compares the members of a group presumably exposed to a toxic agent with the general population or with a nonexposed group. The advantages of that approach are its simplicity and the ease of interpretation of results. If, however, only a small fraction of the group is exposed to the agent, any increased risk posed by exposure of this subgroup may not be detectable when the risk of the entire group is assessed.

A more refined method of exposure assessment assigns each study subject to an exposure category, such as high, medium, low, and no exposure. Disease risk in each group can then be calculated separately and compared with a reference or nonexposed group. This method, in contrast with the simple exposed–nonexposed comparison above, can evaluate the presence or absence of a dose–response trend. In some cases, more detailed information is available, and quantitative exposure estimates can be developed. Such estimates are sometimes referred to as exposure metrics. Exposure metrics integrate quantitative estimates of exposure intensity (such as air concentration or extent of skin contact) with exposure duration to produce an estimate of cumulative exposure. Ideally, such refined estimates reduce errors associated with misclassification and thereby increase the power of statistical analysis to identify true associations between exposure and disease.

Occupational-exposure studies tend to rely on work histories, job titles, and workplace measurements of contaminant concentration, which can be combined to create a job–exposure matrix, in which a quantitative exposure estimate is

assigned to each job or work task, and time spent on each job or task is calculated. Such metrics are able to incorporate exposure mitigation factors, such as process changes, engineering controls, or the use of protective clothing. The production-worker cohort analysis conducted by the US National Institute for Occupational Safety and Health (NIOSH) is a good example of a study that has used those methods.

Many environmental-exposure studies use proximity to a contaminant source as the primary means of exposure classification. If, for example, an industrial facility emits a chemical contaminant, investigators may create geographic zones around the facility and assign exposure categories to individuals on the basis of location of residence. That approach was taken in the case of a serious industrial accident in Seveso, Italy, that contaminated nearby areas with TCDD. Assessments of this kind are often refined to include knowledge of exposure pathways (how chemicals move from the source through the environment) and personal behavior, and sometimes include measurements of chemicals in environmental samples such as soil.

Biomarkers of exposure can provide crucial information for both occupational and environmental studies, in that a quantitative exposure estimate can be assigned to each individual in the study. The most important biomarker in the context of Vietnam veterans' exposure to Agent Orange is the measurement of TCDD in serum. Studies of the absorption, distribution, and metabolism of TCDD have been conducted over the last 20 years. In the late 1980s, the Centers for Disease Control (CDC) developed a highly sensitive assay to detect TCDD in serum and demonstrated a high correlation between serum TCDD and TCDD in adipose tissue (Patterson et al., 1986, 1987). The serum TCDD assay is now used extensively to evaluate TCDD exposure in Vietnam veterans and other populations.

OCCUPATIONAL EXPOSURE TO HERBICIDES AND TCDD

The committee reviewed many epidemiologic studies of occupationally exposed groups for evidence of an association between health risks and exposure to TCDD and the herbicides used in Vietnam; primarily the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and chlorophenols. In reviewing the studies, the committee explicitly considered two types of exposure: exposure to TCDD itself and exposure to the various herbicides, particularly 2,4-D and 2,4,5-T. Separate consideration was necessary because of the possibility that, for example, some health effects may be associated with exposure to 2,4-D in agriculture and forestry. TCDD is an unwanted byproduct of 2,4,5-T production, but not of 2,4-D, although small quantities of other dioxins can be found in 2,4-D.

Studies of occupational exposure to dioxins focus primarily on chemical-plant workers who produce phenoxy herbicides or chlorophenols. Other occupationally exposed groups include workers in agriculture and forestry who spray herbicides, sawmill workers exposed to chlorinated dioxins from contaminated wood preservatives, and pulp and paper workers exposed to dioxins through the pulp-bleaching process.

Production Work

US National Institute for Occupational Safety and Health Cohort Study

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. More than 5,000 workers in 12 companies were identified from personnel and payroll records as TCDD-exposed. Exposure status was determined initially through a review of process operating conditions, employee job duties, and analytic records of TCDD in industrial-hygiene samples, process streams, products, and waste (Fingerhut et al., 1991). Occupational exposure to TCDD-contaminated processes was confirmed by measuring serum TCDD in 253 cohort members. Duration of exposure was defined as number of years worked in processes contaminated with TCDD and was used as the primary exposure metric in the study. The use of duration of exposure as a surrogate for cumulative exposure was based on the high correlation (Pearson correlation coefficient = 0.72) between log-transformed serum TCDD and years worked in TCDD-contaminated processes. Duration of exposure for individual workers was calculated from work records, and exposure-duration categories were created, such as, <1 year, 1 to <5 years, 5 to <15 years, and 15+ years. In some cases, information was not available to determine duration of exposure, so a separate metric called duration of employment was defined as the total time that each worker was employed at the study plant.

The NIOSH cohort study was updated recently (Steenland et al., 1999), and a more refined exposure assessment was conducted. Workers whose records lacked adequate information to determine duration of exposure were excluded. The final analysis was restricted to eight plants because four plants (with 591 workers) lacked records on the degree of TCDD contamination of their work processes or lacked the detailed work histories required to estimate TCDD exposure by job. Another 38 workers at the remaining eight plants were eliminated because they worked in a process in which TCDD contamination could not be estimated. Finally, another 727 workers with exposure to both pentachlorophenol and TCDD were eliminated to avoid possible confounding of any TCDD effects by pentachlorophenol. Those restrictions led to a subcohort of 3,538 workers (69% of the overall cohort).

The exposure assessment for the subcohort was based on a job–exposure matrix (Piacitelli and Marlow, 1997). The matrix assigned each worker a quantitative exposure score for each year of work. The score was based on three factors: concentration of TCDD in micrograms per gram of process materials, fraction of the day when the worker worked in the specific process, and a qualitative contact value (0.01–1.5) based on the estimated TCDD contamination reaching exposed skin or the potential for inhalation of TCDD-contaminated dust. The scores for each year of work were combined to yield a cumulative exposure score for each worker. The new exposure analysis presumably reduced misclassification (exclusion of nonexposed workers) and uncertainty (exclusion of workers with incomplete information) and improved accuracy (more detailed information on daily exposure).

Most recently, Steenland et al. (2001) conducted a detailed exposure–response analysis from data on workers at one of the original 12 companies in the cohort study. A group of 170 workers were identified with serum TCDD greater than 10 ppt, as measured in 1988. The investigators conducted a regression by using the following information: the work history of each worker, the exposure scores for each job held by each worker over time, a simple pharmacokinetic model for the storage and excretion of TCDD, and an estimated TCDD half-life of 8.7 years. That pharmacokinetic model allowed calculation of the estimated serum TCDD concentration at the time of last exposure of each worker. Results of the analysis were used to estimate serum TCDD over time due to occupational exposure for all 3,538 workers in the subcohort defined in 1999.

International Agency for Research on Cancer Cohort

A multisite study by the International Agency for Research on Cancer involved 18,390 production workers and herbicide sprayers in 10 countries (Saracci et al., 1991). The full cohort was established by using the International Register of Workers Exposed to Phenoxy Herbicides and Their Contaminants. Twenty cohorts were combined for this analysis: one each from Canada, Finland, and Sweden; two each from Australia, Denmark, Italy, the Netherlands, and New Zealand; and seven from the United Kingdom. There were 12,492 production workers and 5,898 sprayers in the full cohort.

Questionnaires were constructed for factories producing chlorophenoxy herbicides or chlorinated phenols and for spraying cohorts. These were completed with the assistance of industrial hygienists, workers, and factory personnel. Industry and production records were also used. Job histories were examined when available. Workers were classified as exposed, probably exposed, exposure unknown, or nonexposed. Exposed workers (N = 13,482) comprised all known to have sprayed chlorophenoxy herbicides and all who worked in particular aspects of chemical production. Two cohorts (N = 416) had no job titles available but

were deemed probably exposed on the basis of professional judgment. Workers with no exposure information ($N = 541$) were classified as "exposure unknown." Nonexposed workers ($N = 3,951$) were those never employed in parts of factories that produced chlorophenoxy herbicides or chlorinated phenols and those who never sprayed chlorophenoxy herbicides. Review of the later analysis indicated that the lack of detailed exposure information on workers prevented meaningful classification beyond exposed and nonexposed.

An expanded and updated version of this cohort study was published in 1997 (Kogevinas et al., 1997). The expanded cohort added herbicide production workers in 12 plants in the United States (the NIOSH cohort) and four plants in Germany. Exposure was reconstructed by using individual job records, company exposure questionnaires developed specifically for the study, and, in some cohorts, measurements of TCDD and other dioxin and furan congeners in serum and adipose tissue and in the workplace. The 21,863 workers exposed to phenoxy herbicides or chlorophenols were classified in three categories: those exposed to TCDD or higher-chlorinated dioxins ($N = 13,831$), those not exposed to TCDD or higher-chlorinated dioxins ($N = 7,553$), and those with unknown exposure to TCDD or higher-chlorinated dioxins ($N = 479$). Several exposure metrics were constructed for the cohort—years since first exposure, duration of exposure (in years), year of first exposure, and job title—but detailed methods were not described. No new studies of the full cohort have been reported since *Update 2000*.

Researchers have also conducted studies of various subgroups of the IARC cohort. Flesch-Janys et al. (1995) did an update of the 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 concentrations for the German population, the authors estimated PCDD/F exposure of the 190 workers with serum or adipose measurements of PCDD/F. The authors then regressed the estimated PCDD/F exposure of these workers at the end of their exposure against the length of time they worked in each production department in the plant. The authors also estimated the contribution of the time worked in each production department to the PCDD/F exposure. The working-time "weights" were then used with the work histories of the remainder of the cohort to estimate the PCDD/F exposure of each cohort member at the end of the person's exposure. The epidemiologic analysis used the 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 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 em-

ployment at the plant; it could have included some periods without exposure. Analysis was based on time since first exposure.

Hooiveld et al. (1998) reported on an update of a mortality study of workers at two chemical factories in the Netherlands. The study included analysis by estimated maximal serum TCDD concentration. That was estimated for each member of the cohort by measuring serum TCDD of 144 subjects, including production workers known to be exposed to dioxins, workers in herbicide production, nonexposed production workers, and workers known to be exposed as a result of an accident that occurred in 1963. Assuming first-order TCDD elimination with an estimated half-life of 7.1 years, $TCDD_{max}$ was extrapolated for a group of 47 workers; then a regression model was constructed to estimate the effect of exposure as a result of the accident, of duration of employment in the main production department, and of time of first exposure before (or after) 1970 on the estimated $TCDD_{max}$ for each cohort member.

Dow Cohorts

Workers at Dow Chemical Co. facilities who manufactured, formulated, or packaged 2,4-D have been the subject of a cohort analysis since the 1980s (Bond et al., 1988). Industrial hygienists developed a job–exposure matrix that ranked employee exposures as low, moderate, or high on the basis of available air-monitoring data and professional judgment. The job–exposure matrix was merged with employee work histories to assign an exposure magnitude to each employee job assignment. A cumulative dose was then developed for each of the 878 employees by multiplying the representative 8-h time-weighted average (TWA) exposure value for each job assignment by the number of years the job was held and then summing the products across all jobs. A 2,4-D TWA of 0.05 mg/m^3 was used for low, 0.5 mg/m^3 for moderate, and 5 mg/m^3 for high exposure. The role of dermal exposure in these facilities does not appear to have been factored into the exposure estimates. It is not clear to what extent the use of air measurements alone provides accurate classification of workers into low-, moderate-, and high-exposure groups. Biologic monitoring of 2,4-D in a subset of workers could provide a straightforward evaluation of the validity of the job–exposure matrix but was apparently not undertaken in this study. Follow-up reports were published in 1993 (Bloemen et al., 1993) and most recently in 2001 (Burns et al., 2001); neither of these studies modified the exposure-assessment procedures of the original study.

A cohort study of manufacturing workers exposed to pentachlorophenol was also conducted by Dow (Ramlow et al., 1996). Exposure assessment was based on consideration of the available industrial-hygiene and process data, including process and job-description information obtained from veteran employees, process and engineering controls change information, industrial-hygiene surface-

wipe sample data, area exposure monitoring, and personal breathing-zone data. Jobs with higher estimated potential exposure involved primarily dermal exposure to airborne pentachlorophenol (PCP) in the flaking–prilling–packaging area; the industrial-hygiene data suggest about a 3-fold difference between the potential highest- and lowest-exposure areas. All jobs were therefore assigned an estimated exposure intensity score on a scale of 1–3 (from lowest to highest potential exposure intensity). Reliable information concerning use of personal protective equipment was not available for modification of estimated exposure intensity.

Cumulative PCP and TCDD exposure indexes were calculated for each subject by multiplying the duration of each exposed job by its estimated exposure intensity and then summing across all exposed jobs.

Other Production-Worker Studies

Several other occupational studies of workers involved in chemical production plants have relied on job titles as recorded on individual work histories and company personnel records to classify exposure (Ott et al., 1980; Zack and Gaffey, 1983; Coggon et al., 1986, 1991; Cook et al., 1986; Zober et al., 1990). Similarly, exposure of chemical-plant workers has been characterized by worker involvement in various production processes, such as synthesis, packaging, waste removal, shipping, and plant supervision (Manz et al., 1991; Bueno de Mesquita et al., 1993).

Agricultural, Forestry, and Other Outdoor Work

Occupational studies of agricultural workers have estimated exposure to herbicides or TCDD with various methods. In the simplest method, data on a person's occupation were derived from death certificates, cancer registries, or hospital records (Burmeister, 1981). Although such information is relatively easy to obtain, it is not possible to estimate duration or intensity of exposure from it or to determine the specific type of herbicide or chemical to which a worker was exposed. Some studies of agricultural workers attempted to investigate differences in occupational practices, allowing identification of subsets of workers who were likely to have had higher herbicide exposure (Hansen et al., 1992; Musicco et al., 1988; Ronco et al., 1992; Vineis et al., 1986; Wiklund and Holm, 1986; Wilklund et al., 1988a). Other studies 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 (Blair and White, 1985; Cantor, 1982; Gordon and Shy, 1981). Still other studies attempted to refine exposure estimates by categorizing exposure on the basis of the number of years employed in a specific occupation as a surrogate for exposure duration, using supplier records of amounts of herbicides purchased to estimate exposure or

estimating the acreage sprayed to determine the amount used (Morrison et al., 1992; Wigle et al., 1990). 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 by using written records, signed statements, or telephone contacts with co-workers or former employers (Carmelli et al., 1981; Woods and Polissar, 1989).

Forestry workers and other outdoor workers, such as highway-maintenance workers, are likely to have been exposed to herbicides and other chemicals to various degrees (see Table 4-1 for summary of studies). Exposure has been classified in a manner similar to that in other studies, for example, by number of years employed, job category, and occupational title.

The Ontario Farm Family Health Study has produced several studies relevant to phenoxyacetic acid herbicide exposures, including 2,4-D. A study of male pesticide exposure and pregnancy outcome (Savitz et al., 1997) developed an exposure metric based on self-reported involvement in five activities associated with pesticide exposure: mixing or applying crop herbicides, crop insecticides and fungicides, livestock chemicals, yard herbicides, and building pesticides. Subjects were asked whether they participated in those activities during each month. A man's exposure classification was based on his activities in 3-month windows. The exposure classification was refined with questions regarding use of protective equipment and specificity of pesticide use.

A related study included analysis of 2,4-D residues in semen as a biomarker of exposure (Arbuckle et al., 1999a). The study began with 773 potential participants, but only 215 eventually consented to the study. Of the 215, 97 provided semen and urine samples for 2,4-D analysis.

The Ontario Farm Family Health Study also examined the effect of pesticide exposure, including 2,4-D, on time to pregnancy (Curtis et al., 1999) and the risk of spontaneous abortion (Arbuckle et al., 1999b; 2001). About 2,000 farm couples participated in the study. Exposure information was pooled from interviews with husbands and wives to construct a history of monthly agricultural and residential pesticide use. Exposure classification was based on a yes–no response for each month. Data on such variables as acreage sprayed and use of protective equipment were collected but were not available for all cases. More recent studies have used herbicide biomonitoring in a subset of the population to evaluate the validity of self-reported predictors of exposure (Arbuckle et al., 2002). Assuming that the presence of 2,4-D in urine was an accurate measure of exposure and that the results of the questionnaire indicating 2,4-D use were more likely to be subject to exposure classification error (that is, the questionnaire results were less accurate than the urine analysis), the questionnaire's prediction of exposure, when compared to the urine 2,4-D concentrations, had a sensitivity of 57% and a specificity

of 86%. In multivariate models, the variables pesticide formulation, protective clothing and gear, application equipment, handling practice, and personal-hygiene practice were significant as predictors of urinary herbicide concentrations in the first 24-h after application was initiated.

Herbicide and Pesticide Spraying

Studies of herbicide sprayers are relevant because it can be presumed that appliers had more sustained exposure to herbicides; however, they were also likely to be exposed to a multiplicity of chemicals, and this would complicate the assessment of any individual or group exposure to specific phenoxy herbicides or TCDD. Some studies have attempted to measure exposure of appliers on the basis of information from work records on acreage sprayed or number of days of spraying. Employment records can also be used to extract information on the chemicals sprayed.

One surrogate indicator of herbicide exposure is receipt of a license to spray. Several studies have specifically identified licensed or registered pesticide and herbicide appliers (Blair et al., 1983; Smith et al., 1981, 1982; Swaen et al., 1992; Wiklund et al., 1988b, 1989). Individual estimates of the intensity and frequency of exposure were rarely quantified in the studies that the committee examined, however, and many appliers were known to have applied many kinds of herbicides, pesticides, and other chemicals. In addition, herbicide spraying is generally a seasonal occupation, and information may not be available on possible exposure-related activities during the rest of the year.

One study provided information on serum TCDD concentrations in herbicide sprayers. Smith et al. (1992) analyzed blood from nine professional spray appliers in New Zealand who first sprayed before 1960 and were also spraying in 1984. The duration of actual spray work varied from 80 to 370 months. Serum TCDD was 3–131 ppt on a lipid basis (mean = 53 ppt). The corresponding values for age-matched controls were 2–11 ppt (mean = 6 ppt). Serum TCDD was positively correlated with the number of months of professional spray application.

Several studies have evaluated various herbicide exposures during spraying in terms of type of exposure, routes of entry, and routes of excretion: (Ferry et al., 1982; Frank et al., 1985; Lavy et al., 1980a,b; Libich et al., 1984; Kolmodin-Hedman and Erne, 1980; and Kolmodin-Hedman et al., 1983). On the basis of those studies, it appears that the major route of exposure is dermal absorption, with 2–4% of the chemical that contacts the skin being absorbed into the body during a normal workday. Air concentrations of the herbicides were usually less than 0.2 mg/m^3. Absorbed phenoxy acid herbicides are virtually cleared within 1 day, primarily through urinary excretion. Typical measured excretion by ground crews was 0.1–5 mg/day, and that by air crews was less.

A recent study of Canadian farmers examined pesticide exposures of men (McDuffie et al., 2001). Data on pesticide exposure were collected by questionnaires, including information on specific chemicals (including 2,4-D), frequency of application, and duration of exposure. A small validation study ($N = 27$) was performed to test the self-reported pesticide-use data against records of purchase. Investigators reported an "excellent concordance" between the two sources, but did not provide a statistical analysis. A study of 98 professional turf sprayers in Canada developed new models to predict 2,4-D dose (Harris et al., 2001). Exposure information was gathered with self-administered questionnaires. Urine samples were collected throughout the spraying season (24-h samples on 2 consecutive days). Estimated 2,4-D doses were developed from the data and used to evaluate the effect of protective clothing and other exposure variables.

A number of other studies regarding agricultural use of pesticides published recently do not provide specific information on exposure to 2,4-D, TCDD, or other compounds relevant to Vietnam veterans' exposure (Bell et al., 2001a,b; Duell et al., 2001).

Paper and Pulp Mill Work

Another occupational group likely to be exposed to TCDD and chlorinated phenols consists of paper and pulp mill workers. They are likely to have received various degrees of exposure as part of the bleaching process in the production of paper products. Pulp and paper production workers are also likely to be exposed to other chemicals in the workplace according to, for example, the type of paper mill or pulping operation and the product manufactured (Henneberger et al., 1989; Jappinen and Pukkala, 1991; Robinson et al., 1986; Solet et al., 1989). In a study of a cohort of Danish paper mill workers (Rix et al., 1998), there were no direct measures of exposure of the workers, and a qualitative assessment of chemicals used in paper manufacture by department does not include chlorinated organic compounds, although chlorine, chlorine dioxide, and hypochlorite were used. No new studies of those populations have been reported since *Update 2000*.

Sawmill Work

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. Wood is dipped in those chemicals and then cut and planed in the mills. Most exposure is dermal, although some exposure can occur by inhalation (Hertzmann et al., 1997; Teschke et al., 1994). No new studies in those populations have been reported since *Update 2000*.

ENVIRONMENTAL EXPOSURE TO HERBICIDES AND TCDD

The committee reviewed several new studies of TCDD-exposed populations associated with industrial facilities, including recent investigations at Seveso, Italy. The committee also reviewed exposure studies related to Agent Orange use in Vietnam.

Industrial Exposure

Seveso, Italy

One of the largest industrial accidents involving environmental exposure to TCDD occurred in Seveso in July 1976 as a result of an uncontrolled reaction during trichlorophenol production. Various indicators were used to estimate individual exposure. TCDD measurements in soil have been used extensively as the indicator of individual exposure. Three areas were defined surrounding the release point on the basis of soil sampling for TCDD (Bertazzi et al., 1989). Zone A was the most heavily contaminated, and all residents of it were evacuated within 20 days. Zone B was less contaminated, and children and pregnant women in their first trimester were urged to avoid it during daytime. Zone R had some contamination, and consumption of crops grown there was prohibited.

Data on serum TCDD concentrations in Zone A residents have been presented by Mocarelli et al. (1990, 1991) and earlier by CDC (1988a). In those with severe chloracne (N = 10), serum TCDD was 828–56,000 ppt of lipid weight. Those without chloracne (N = 10) had serum TCDD concentrations of 1,770–10,400 ppt. TCDD was non-detectable in all controls but one. The highest of those concentrations exceeded any that had been estimated at the time for TCDD-exposed workers on the basis of backward extrapolation and a half-life of 7 years. Data on nearby soil concentrations, number of days that a person stayed in Zone A, and whether local food was consumed were considered in evaluating TCDD. That none of those data correlated with serum TCDD suggested strongly that the exposure of importance was from fallout on the day of the accident. The presence and degree of chloracne did correlate with TCDD. It appears that adults are much less likely than children to develop chloracne after an acute exposure, but surveillance bias may have played some role in this finding. Recent updates (Bertazzi et al., 1998; 2001) have not changed the exposure-assessment approach.

The validity of exposure classification by zone was tested recently as a part of the Seveso Women's Health Study (Eskenazi et al., 2001). Investigators measured serum TCDD in samples collected in 1976–1980 from 601 residents (97 from Zone A and 504 from Zone B). The women completed a questionnaire in 1996–1998 regarding age, chloracne history, animal mortality, consumption of homegrown food, and location at the time of the explosion. Participants did not know their TCDD concentrations at the time of the interview, although most

knew their zone of residence. Interviewers and TCDD analysts were blinded to participants' zone of residence. Zone of residence explained 24% of the variability in serum TCDD. Addition of the questionnaire data improved the regression model, explaining 42% of the variance. Those findings demonstrate a significant association between zone of residence and serum TCDD, but much of the variability in TCDD concentrations is still unexplained by these models.

Times Beach, Missouri

A number of reports have provided information on exposure to TCDD from environmental contamination in the Times Beach area of Missouri (Andrews et al., 1989; Patterson et al., 1986). In 1971, TCDD-contaminated sludge from a hexachlorophene production facility was mixed with waste oil and sprayed in various community areas for dust control. Soil contamination in some samples exceeded 100 ppb. One of the Missouri sites with the highest TCDD soil concentrations was the Quail Run mobile-home park. Residents were considered exposed if they had lived in the park for at least 6 months during the time when the contamination occurred (Hoffman et al., 1986). Other investigations of Times Beach have estimated exposure risk on the basis of residents' reported occupational and recreational activities in the sprayed area. Exposure has been estimated from duration of residence and TCDD soil concentrations.

Andrews et al. (1989) provided the most extensive data on human adipose tissue TCDD in 51 exposed persons—persons who had ridden or cared for horses at arenas sprayed with TCDD-contaminated oil, who lived in areas where such oil had been sprayed, who were involved in trichlorophenol (TCP) production, or who were involved in TCP nonproduction activities, such as laboratory and maintenance workers—and 128 nonexposed controls. Persons were considered exposed if they lived near, worked with, or had other contact for 2 years or more with soil contaminated with TCDD at 20–100 ppb or for 6 months or more with soil contaminated with TCDD at or over 100 ppb. Of the exposed population samples, 87% had adipose tissue TCDD concentrations less than 200 ppt; however, TCDD concentrations in seven of the 51 exposed individuals were 250–750 ppt. In nonexposed persons, adipose tissue TCDD ranged from nondetectable to 20 ppt, with a median of 6 ppt. On the basis of a 7-year half-life, it is calculated that two of the study participants would have had adipose tissue TCDD near 3,000 ppt at the time of the last date of exposure. No new studies have been published since *Update 2000*.

Doubs, France

Viel et al. (2000) reported on an investigation of apparent clusters of cases of soft-tissue sarcoma and non-Hodgkin's lymphoma in the vicinity of a municipal

solid-waste incinerator in Doubs, France. The presumptive source of TCDD in the region is a municipal solid-waste incinerator in the Besançon electoral ward in western Doubs. A measurement of dioxin emissions from the incinerator, measured in international toxicity equivalent (I-TEQ) units, showed a level of 16.3 ng I-TEQ per cubic meter, far in excess of the EU standard of 0.1 ng I-TEQ per cubic meter. In addition, measurements of TCDD in cow's milk from three farms near the incinerator suggested that it was highest at the farm closest to the incinerator, but the measurements were all well below the EU guideline of 6 ng I-TEQ/kg of fat.

Chapaevsk, Russia

Researchers in the Samara region of Russia have identified a chemical plant in Chapaevsk as a major source of TCDD pollution (Revich et al., 2001). In 1967–1987 the plant produced hexachlorocyclohexane (lindane) and its derivatives. Since then, the plant has produced various crop-protection chemicals. Dioxins have been detected in air, in soil, in the town's drinking water, and in cow's milk. However, the researchers do not include a description of air, soil, or water sampling methods in their report. The number of samples analyzed were small for some media (two drinking-water samples, seven breast-milk samples pooled from 40 women, and 14 blood samples) and unreported for others (air, soil, and vegetables). Results from the samples suggested increased dioxin around the center of Chapaevsk compared with outlying areas. This conclusion was based primarily on dioxin concentrations measured in soil: 141 ng TEQ/kg soil less than 2 km from the plant, compared to 37 ng TEQ/kg soil 2-7 km from the plant, and 4 ng TEQ/kg soil 7-10 km from the plant. Concentrations outside the city (10-15 km from the plant) were approximately 1 ng TEQ/kg soil. The authors also compared levels measured in Chapaevsk with those measured in other Russian cities with industrial facilities. The data presented do not allow direct comparison of dioxin soil concentrations as a function of distance from the industrial facilities. However, the highest TCDD concentrations in the Chapaevsk study (those nearest the plant) were higher than maximum concentrations reported in four other studies referenced in the article. Residence in the city of Chapaevsk was used as a surrogate for exposure in the epidemiologic analyses presented in the report. No attempt was made to create exposure categories based on residential location within the city, nor with occupational or lifestyle factors that might have influenced TCDD exposure.

Other Studies

Two other epidemiologic studies have been conducted in association with industrial-facility emissions. Chemical-combustion records in the Zeeburg area of Amsterdam in the Netherlands were used as a surrogate for exposure to dioxins

in a study of orofacial clefts (ten Tusscher et al., 2000). Location downwind or upwind of an incineration source was used to define exposure and reference groups for the study. A study of soft-tissue sarcomas in the general population was conducted in northern Italy around the city of Mantua (Costani et al., 2000). Several industrial facilities are in Mantua, and residential proximity to them was presumed to result in increased TCDD exposure, but TCDD was not measured in the environment or in human tissues.

Studies in Vietnam

Several studies have investigated exposure to herbicides among the residents of southern Vietnam (Constable and Hatch, 1985), comparing unexposed residents of the South with residents of the North. Other studies have attempted to identify wives of veterans of North Vietnam who served in South Vietnam. Records of herbicide sprays have been used to refine exposure measurements, comparing individuals who lived in sprayed villages in the South with those living in unsprayed villages. In some studies, residents of villages were considered exposed if a recorded herbicide mission passed within 10 km of the village center (Dai et al., 1990). Other criteria for classifying exposure included length of residence in a sprayed area and number of times the area had reportedly been sprayed.

A small number of studies provide information on TCDD concentrations in Vietnamese civilians exposed during the war. Schecter et al. (1986) detected TCDD in 12 of 15 samples of adipose tissue taken at surgery or autopsy in South Vietnam during 1984. The concentrations in the positive samples were 3–103 ppt. TCDD was not detected in nine samples from residents of North Vietnam who had never been to South Vietnam; detection sensitivity was 2–3 ppt. Analysis of three breast-milk samples collected in 1973 from Vietnamese women thought to have been exposed to Agent Orange yielded concentrations of 77–230 ppt on a lipid basis.

Most recently, 43 residents of Bien Hoa City provided blood samples for TCDD analysis (Schecter et al., 2002). Bien Hoa City is in the southern part of South Vietnam, and the surrounding area was treated heavily with Agent Orange. The median lipid-normalized TCDD concentration was 67 ppt in these residents, compared with an average of 2 ppt in residents of Hanoi. The study also indicated that TCDD exposure of this population was continuing, presumably through consumption of fish and other foods.

MILITARY USE OF HERBICIDES IN VIETNAM

Military use of herbicides in Vietnam began in 1962, was expanded during 1965 and 1966, and reached a peak from 1967 to 1969. The herbicides were used by the US Air Force's Operation Ranch Hand to defoliate inland hardwood

forests, coastal mangrove forests, and to a lesser extent cultivated land by spraying from 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 about 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; that 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 river banks.

Four compounds were used in the herbicide formulations—2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, and cacodylic acid. The chlorinated phenoxy acids (2,4-D and 2,4,5-T) persist in soil only a few weeks (Buckingham, 1982). Picloram is more mobile than 2,4-D and 2,4,5-T and is extremely persistent in soils. Cacodylic acid contains an organic form of arsenic.

The type of herbicide was identified by a code name referring to the color of the band around the 55-gal drum that contained it. The code names included Agents Orange, White, Blue, Purple, Pink, and Green (Table 5-1). From 1962 to 1965, Agents Purple, Blue, Pink, and Green were used; from 1965 to 1970, Agents Orange, White, and Blue were used; and from 1970 to 1971, Agents White and Blue were used (Young and Reggiani, 1988). Further details on the herbicides used are presented in the earlier reports (IOM, 1994, 1996, 1998, 2000).

In addition to the 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, minefields, ammunition storage areas, and other specialized sites requiring control of grasses and woody

TABLE 5-1 Major Herbicides Used in Operation Ranch Hand, 1962–1971

Code Name	Formulation	Purpose	Amount Sprayed, gal.	Period of Use
Purple	2,4-D and 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	2,4-D and 2,4,5-T	General defoliation	11,261,429	1965–1970
White (Tordon 101™)	2,4-D and picloram	Forest defoliation, long-term control	5,246,502	1965–1971

Data from MRI (1967), NAS (1974), and Young and Reggiani (1988).

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 numbers of military personnel potentially exposed to those chemicals are not available.

Ground Spraying of Herbicides

The number of US military personnel exposed to herbicides is impossible to determine precisely, but most of those assigned to Operation Ranch Hand can be presumed to have been exposed to Agent Orange and other herbicides. In addition, the US Army Chemical Corps, using hand equipment and helicopters, conducted smaller spray operations, such as defoliation around special forces camps; clearance of perimeters of airfields, depots, and other bases; and small-scale crop destruction (Thomas and Kang, 1990; Warren, 1968).

Units and individuals other than 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, and engineering personnel required the use of herbicides for removal of underbrush and dense growth in constructing fire-support bases.

Because the herbicides were not considered to present a health hazard, few precautions were taken to prevent troop exposure to the chemicals. The precautions prescribed were consistent with those applied in the domestic use of herbicides that existed before the Vietnam conflict (US GAO, 1979).

New information looking into the assessment of wartime exposure to herbicides in Vietnam is being gathered through a research project being overseen by an Institute of Medicine (IOM) committee. This work is being conducted in response to a request for proposals on this topic (IOM, 1997).

TCDD in Herbicides Used in Vietnam

TCDD is a contaminant of 2,4,5-T. Small quantities of other dioxins are present in 2,4-D. The concentration of TCDD in any given lot of 2,4,5-T depends on the manufacturing process (Young et al., 1976), and different manufacturers produced 2,4,5-T with different concentrations of TCDD.

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 they 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 ppm to almost 50 ppm and averaged 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 be present at less than 0.05 ppm (NAS, 1974).

Agents Green, Pink, and Purple—used early in the program (before 1965)—contained 16 times the mean TCDD content of formulations used during 1965–1970 (Young et al., 1978). Analysis of archive samples of Agent Purple reported TCDD as high as 45 ppm (Young, 1992). The mean concentration of TCDD in Agent Purple was estimated to be 32.8 ppm; that in Agents Pink and Green, 65.6 ppm (Young et al., 1978). It has been estimated that about 368 lbs of TCDD was sprayed in Vietnam over a 6-year period (Gough, 1986).

EXPOSURE ASSESSMENT IN STUDIES OF VIETNAM VETERANS

Different approaches have been used to estimate the exposure of Vietnam veterans, including self-reported exposures, record-based exposure estimates, and biomarkers of TCDD exposure. Each approach is limited in its ability to determine individual exposure. Some studies rely on such gross markers 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 that type include the CDC Vietnam Experience Study and Selected Cancers Study, Department of Veterans Affairs mortality studies, and most studies of veterans conducted by states. This approach almost surely dilutes the health effects of herbicides because many members of the cohort presumed to be exposed to herbicides may, in reality, not have been.

Ranch Hand Studies

Job title while in the military has been shown to be a valid exposure classification for Air Force Ranch Hand personnel, who were responsible for aerial spraying of herbicides. Biomarker studies of the Ranch Hand personnel 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 was detected for jobs that involved more frequent handling of herbicides (AFHS, 1991).

The exposure index initially proposed in the Air Force Ranch Hand study relied on military records of TCDD-containing herbicides (Agents Orange, Purple, Pink, and Green) sprayed as reported in the HERBS tapes for the period starting July 1965 and on military procurement records and dissemination information for the period before July 1965. In 1991, the exposure index was compared with the results of the Ranch Hand serum TCDD analysis. The exposure index and the TCDD body burden correlated weakly.

Michalek et al. (1995) developed several indexes of herbicide exposure for members of the Ranch Hand cohort and tried to relate them to the measurements of serum TCDD from 1987 to 1992. Self-administered questionnaires completed by veterans of Operation Ranch Hand were used to develop three indexes for herbicide or TCDD exposure: number of days of skin exposure, percentage of skin area exposed, and the product of number of days of skin exposure, percentage of skin exposed, and a factor for the concentration of TCDD in the herbicide. A fourth index used no information gathered from individual subjects. It was calculated by multiplying the volume of herbicide sprayed during a specific individual's tour of duty by the concentration of TCDD in herbicides sprayed in that period and dividing the product by the number of crew members in each job specialty at that time.

Each of the four models tested was significantly related to serum TCDD, although each explained only 19–27% 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 indexes, explained 60% of the variability in serum TCDD. When the questionnaire-derived indexes were applied within each job classification, days of skin exposure added statistically significantly, but not substantially, to the variability explained by job alone.

Recent studies of the same population have used serum TCDD as the primary exposure index to examine possible associations with hepatic abnormalities, peripheral neuropathies, hematologic disorders, and cognitive functioning (Barrett et al., 2001; Michalek et al., 2001a,b,c).

Army Chemical Corps Studies

Members of the US Army Chemical Corps performed ground and helicopter chemical operations and were thereby involved in the direct handling and distribution of herbicides in Vietnam. This population has only recently been identified for detailed study of health effects related to herbicide exposure (Thomas and Kang, 1990). Results of an initial feasibility study were reported recently (Kang et al., 2001). It recruited 565 veterans: 284 Vietnam veterans and 281 non-Vietnam veteran controls. Blood samples were collected from 50 Vietnam veterans and 50 control veterans, and 95 of the samples met quality-assurance–quality-control standards set by the CDC laboratory. Comparison of the entire Vietnam cohort with the entire non-Vietnam cohort showed that the geometric mean TCDD concentrations did not differ significantly ($p = 0.6$). Analysis of questionnaire responses indicated that the Vietnam veterans who reported spraying herbicides had higher TCDD concentrations than those who reported no spraying activities. The authors concluded that Agent Orange exposure was a likely contributor to

TCDD concentrations in Vietnam veterans with a history of spraying herbicides. The main study of 5,000 Vietnam veterans, including analysis of an additional 900 blood specimens, continues.

Other Vietnam Veterans

Surveys of Vietnam veterans who were not part of the Ranch Hand or Army Chemical Corps groups indicate that 25–55% believe that they were exposed to herbicides (CDC, 1989; Erickson et al., 1984a,b; Stellman and Stellman, 1986). A few attempts have been made to estimate exposure of the Vietnam veterans who were not part of the Ranch Hand or Army Chemical Corps groups. In 1983, CDC was assigned by the US government to conduct a study of the possible long-term health effects of Vietnam veterans' exposures to Agent Orange. The CDC Agent Orange study (CDC, 1985) attempted to classify veterans' exposure to herbicides that occurred during military service. That involved determining the proximity of troops to Agent Orange spraying by using military records to track troop movement and the HERBS tapes to locate herbicide-spraying patterns. The CDC Birth Defects Study developed an exposure opportunity index to score Agent Orange exposure (Erickson et al., 1984a,b).

In 1987, CDC conducted the Agent Orange Validation Study to test the validity of the various indirect methods used to estimate exposure of ground troops to Agent Orange in Vietnam. The study measured serum TCDD in a nonrandom sample of Vietnam veterans and Vietnam-era veterans who did not serve in Vietnam (CDC, 1988b). Vietnam veterans were selected for further study on the basis of their estimated number of Agent Orange hits, derived from the number of days on 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 157 veterans, and the "high" exposure group 191 veterans. Blood samples were obtained from 66% of Vietnam veterans ($N = 646$) and 49% of the eligible comparison group of veterans ($N = 97$). More than 94% of those whose serum was obtained had served in one of five battalions.

The median serum TCDD in Vietnam veterans was 4 ppt, with a range of less than 1 to 45 ppt, and two veterans had concentrations above 20 ppt; the distributions of these measurements were nearly identical with those in the control group of 97 non-Vietnam veterans. In other words, the CDC validation study found that study subjects could not be distinguished from controls on the basis of serum TCDD. In addition, none of the record-derived estimates of exposure and neither type of self-reported exposure to herbicides identified Vietnam veterans who were likely to have currently high serum TCDD (CDC, 1988b). The study concluded that it is unlikely that military records alone can be used to identify a large number of US Army veterans who might have been heavily exposed to TCDD in Vietnam.

In addition, the serum TCDD measurements in Vietnam veterans suggest that the exposure to TCDD in Vietnam was substantially less, *on the average*, than that of persons exposed as a result of the industrial explosion in Seveso, or of the heavily exposed occupational workers that are the focus of many of the studies evaluated by the committee. This estimation of *average* exposure does not preclude the existence of a heavily exposed subgroup of Vietnam veterans.

In 1997, a committee convened by IOM developed a request for proposals (RFP) seeking individuals and organizations capable of conducting research to develop one or more historical exposure reconstruction approaches suitable for epidemiologic studies of herbicide exposure among US veterans during the Vietnam War (IOM, 1997). The RFP resulted in a project called "Characterizing Exposure of Veterans to Agent Orange and Other Herbicides in Vietnam" (Stellman, 2002). The project, initiated in 1998, has created a geographic information system (GIS) for Vietnam with a grid resolution of 0.01 degree latitude and 0.01 degree longitude. Herbicide-spray records from US military agencies have been integrated into the GIS and used to produce an exposure-opportunity index. The data will be linked with data on military-unit locations to permit estimation of exposure-opportunity scores for individuals. The results of the project will be published in the peer-reviewed literature in the next two years.

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6

Cancer

Cancer is the second leading cause of death in the United States. Among men aged 45–64, the group that includes most Vietnam veterans, the risk of dying from cancer nearly equals the risk of dying from heart disease, the overall leading cause of death in the United States (US Census, 1999). In 2002, about 555,500 Americans are expected to die from cancer—more than 1,500 people per day. In the United States, one of every four deaths is from cancer (ACS, 2002).

In this chapter, the committee summarizes and reaches conclusions about the strength of the evidence from 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. The categories of association and the committee's approach to categorizing the health outcomes are discussed in Chapters 1 and 2.

In assessing a possible relation between herbicide exposure and risk of cancer, one key issue is the magnitude of exposure of those included in a study. As noted in Chapter 5, the detail and accuracy of exposure assessment vary widely among the studies reviewed by the committee. A small number of studies use a biomarker of exposure, for example, the presence of TCDD 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.

In this chapter, background information about each cancer, including data on its incidence in the general US population, is followed by a brief summary of the findings described in the previous Agent Orange reports (*Veterans and Agent Orange*, hereafter referred to as *VAO*, IOM, 1994; *Veterans and Agent Orange: Update 1996*, hereafter, *Update 1996*, IOM, 1996; *Veterans and Agent Orange: Update 1998*, hereafter, *Update 1998*, IOM, 1999; and *Veterans and Agent Orange: Update 2000*, hereafter, *Update 2000*, IOM, 2001), a discussion of the most recent scientific literature, and a synthesis of the material reviewed. Where appropriate, the literature is discussed by exposure type (occupational, environmental, and Vietnam veteran). Each section ends with the committee's conclusion regarding the strength of the evidence from epidemiologic studies, biologic plausibility, and evidence regarding Vietnam veterans.

As mentioned above, data on cancer incidence in the general US population are included in the background sections. Those data provide context for the consideration of cancer risks in Vietnam veterans. Incidences are reported for people 45–59 years old 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 can have a profound effect on risk. Prostatic cancer incidence, for example, is nearly 11 times higher in men 55–59 years old than in men 45–49 years old and more than twice as high in blacks 45–59 years old as in whites in this age group (NCI, 2000). The figures presented for each cancer are estimates for the entire US population, not precise predictions for the Vietnam-veteran cohort. It should be remembered that numerous factors may influence the incidences reported here—including personal behavior (such as smoking and diet), genetic predisposition, and medical history. Those factors may make a particular person more or less likely than the average person to contract a given cancer. Incidence data are reported for all races and also separately for blacks and whites. The data reported are for 1995–1999, the most recent data available at the time this report was written.

Great uncertainties remain about the magnitude of potential risk posed by exposure to herbicides and TCDD in the occupational, environmental, and veteran studies reviewed by the committee. Many of those studies have inadequate controls for important confounders, and the information needed to extrapolate from the exposure in the studies to that of individual Vietnam veterans is lacking. The committee therefore cannot measure the risk likely to have been experienced by Vietnam veterans due to exposure to herbicides in Vietnam; it offers qualitative observations where data permit.

GASTROINTESTINAL TRACT TUMORS

Gastrointestinal tract tumors include some of the most common cancers. The committee reviewed the data on colon cancer (ICD-9 153.0–153.9), rectal cancer

(ICD-9 154.0–154.1), stomach cancer (ICD-9 151.0–151.9), and pancreatic cancer (ICD-9 157.0–157.9). According to American Cancer Society (ACS) estimates, about 200,200 people will be diagnosed with those cancers in the United States in 2002 and some 98,700 will die from them (ACS, 2002). Colon cancer accounts for about half those diagnoses and deaths. Collectively, gastrointestinal tract tumors are expected to account for 15% of new diagnoses and 18% of cancer deaths in 2002. The average annual incidences for gastrointestinal cancers are shown in Table 6-1.

The incidences of stomach, colon, rectal, and pancreatic cancers increase with age in people 45–59 years old. In general, incidence is higher in men than in women, and is higher in blacks than in whites. Besides age and race, risk factors for those cancers vary but always include family history of the same form of cancer, some 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*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was limited or suggestive evidence of *no* association between exposure to the chemicals of interest (2,4-dichlorophenoxyacetic acid, 2,4-D; 2,4,5-trichlorophenoxyacetic acid, 2,4,5-

TABLE 6-1 Average Annual Incidence (per 100,000) of Selected Gastrointestinal Cancers in United States[a]

	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	5.9	4.6	11.7	10.0	9.2	16.9	18.1	15.7	24.3
Females	2.7	2.1	4.8	4.7	3.6	10.6	7.3	6.1	11.3
Colon									
Males	15.3	14.6	21.3	34.2	32.0	56.8	62.5	61.0	85.0
Females	15.9	14.1	25.8	27.5	24.5	46.3	47.9	45.6	73.5
Rectal									
Males	8.6	7.9	10.3	16.5	15.1	21.6	26.7	27.0	26.2
Females	6.0	5.6	6.0	9.6	9.0	12.0	15.2	14.6	16.6
Pancreatic									
Males	6.1	5.8	10.3	12.8	12.0	25.7	21.6	19.8	42.5
Females	3.5	3.3	6.0	7.9	7.4	11.7	14.6	13.8	25.3

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

T, or its contaminant TCDD; picloram; or cacodylic acid) and gastrointestinal tumors. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Tables 6-2, 6-3, 6-4, and 6-5 for a summary of the studies).

Update of the Scientific Literature

Occupational Studies

In an occupational study, Burns et al. (2001) updated the mortality in chemical workers exposed in the production of 2,4-D. Members of that cohort are male employees of Dow Chemical Company who manufactured or formulated 2,4-D in 1945–1994. Their mortality experience is compared with national rates and with that in more than 40,000 other company employees who worked at the same location. There were 330 deaths in the 1,517 male employees who have an average follow-up of 26.2 years. Fewer deaths than expected from all malignant neoplasms and specifically cancers of the digestive organs and peritoneum (International Classification of Diseases, Eighth Revision 150–59) were found. There were 16 observed deaths compared with 21.5 expected, for a standardized mortality ratio (SMR) of 0.7 (0.4–1.2, 95% confidence interval [CI]).

Environmental Studies

An environmental study of residents of Chapaevsk, a Russian industrial community on the Volga River with documented contamination of the food and water supply by dioxins and other chemicals, demonstrated a higher incidence of colon cancer in males (22.7 per 100,000 per year) than Russia as a whole (17.9) or the Samara region of Russia (21.7), which includes Chapaevsk. Female residents of Chapaevsk did not have a higher incidence (13.3) than Russia as a whole (14.1) or Samara (15.4) (Revich et al., 2001). However, female residents of Chapaevsk did have a higher incidence of stomach cancer (33.9) than Russia (20.7) or Samara (17.6). Male residents of Chapaevsk had a lower incidence of stomach cancer (45.3) than Russia (48.1) but a higher incidence than Samara (44.0). Both male and female residents of Chapaevsk had a lower incidence of rectal cancer (15.3 and 7.0, respectively) than Russia (16.6 and 10.3) or Samara (17.1 and 11.2). Because of the lack of adjustment for confounding, the likelihood of multiple exposures, the absence of information on the completeness and accuracy of cancer diagnoses, and the ecologic study design, this study provides little evidence for associations with gastrointestinal cancers.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000*.

Synthesis

With only rare exceptions, studies on gastrointestinal cancers and exposure to herbicides in production, from agricultural use, from environmental sources, and among veteran populations found estimated relative risks close to 1.0, providing no evidence of any increase in risk. The updated analysis of mortality among US chemical workers at a Dow plant (Burns et al., 2001) did not report site-specific gastrointestinal cancers, and there was a nonsignificant increase in the SMR for all gastrointestinal cancers in the highest-exposed subgroups.

Conclusions

Strength of Evidence from Epidemiologic Studies

VAO and the previous updates concluded that there is limited or suggestive evidence of *no* association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) 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. On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the present committee finds that there is still limited or suggestive evidence of *no* association between exposure to the chemicals of interest and gastrointestinal cancers.

Biologic Plausibility

No animal studies have found an increased incidence of gastrointestinal cancer after exposures to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The available data on Vietnam veterans do not suggest an association between TCDD or herbicide exposure and any gastrointestinal cancers.

TABLE 6-2 Selected Epidemiologic Studies—Stomach Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers—cancer of the digestive organs	16	SMR 0.7 (0.4–1.2)
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	13	1.0 (0.6–1.8)
Hooiveld et al., 1998	Dutch chemical production workers	3	1.0 (0.2–2.9)
Rix et al., 1998	Danish paper mill workers		
	Male	48	1.1 (0.8–1.4)
	Female	7	1.0 (0.4–2.1)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	39	0.9 (0.7–1.3)
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)
Ott and Zober, 1996	BASF cleanup workers	3	1.0 (0.2–2.9)
	TCDD <0.1 µg/kg of body wt	0	
	TCDD 0.1–0.99 µg/kg of body wt	1	1.3 (0.0–7.0)
	TCDD >1 µg/kg of 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	US 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	2	0.7 (01.–2.7)
Collins et al., 1993	Monsanto 2,4-D production workers	0	0 (0.0–1.1)
Kogevinas et al., 1993	IARC cohort—females		NS
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farm workers	286	0.9 (*)
Swaen et al., 1992	Dutch herbicide appliers	1	0.5[b] (0.0–2.7)
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)

continues

TABLE 6-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Zober et al., 1990	BASF production workers—basic cohort	3	3.0 (0.8–11.8)
Alavanja et al., 1989	USDA forest or 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	6	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)
Lynge, 1985	Danish male production workers	12	1.3 (*)
Blair et al., 1983	Florida pesticide appliers	4	1.2 (*)
Burmeister et al., 1983	Iowa residents—farming exposures	1,812	1.3 ($p < 0.05$)
Wiklund, 1983	Swedish agricultural workers	2,599	1.1 (1.0–1.2)[c]
Burmeister, 1981	Farmers in Iowa	338	1.1 ($p < 0.01$)
Axelson et al., 1980	Swedish railroad workers—total exposure	3	2.2 (*)
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of stomach cancer in males		45.3 in Chapaevsk; 44.0 in Samara Region[d]
	Age-adjusted incidence (100,000) of stomach cancer in females		33.9 in Chapaevsk; 17.6 in Samara Region[d]
	Mortality standardized to Samara Region		
	Males	59	1.7 (1.3–2.2)
	Females	45	0.7 (0.5–0.9)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	1	0.5 (0.1–3.2)
	Zone A females	2	1.4 (0.3–5.5)
	Zone B males	15	1.0 (0.6–1.6)
	Zone B females	9	1.0 (0.5–1.9)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	1	0.9 (0.1–6.7)
	Zone B males	10	0.8 (0.4–1.5)
	Zone B females	7	1.0 (0.5–2.2)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone A females	1	0.9 (0.0–5.3)
	Zone B males	10	0.8 (0.4–1.5)

TABLE 6-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Zone B females	7	1.0 (0.4–2.1)
	Zone R males	76	0.9 (0.7–1.1)
	Zone R females	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 residents—10-year follow-up—morbidity		
	Zone B males	7	1.0 (0.5–2.1)
	Zone B females	2	0.6 (0.2–2.5)
	Zone R males	45	0.9 (0.7–1.2)
	Zone R females	25	1.0 (0.6–1.5)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	7	0.9 (0.4–1.8)
	Zones A, B females	3	0.8 (0.3–2.5)
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zones A, B, R males	40	0.8 (0.6–1.2)
	Zones A, B, R females	22	1.0 (0.6–1.5)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B males	7	1.2 (0.6–2.6)
VIETNAM VETERANS			
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	32	1.1 (0.7–1.5)
Crane et al., 1997b	Australian national service Vietnam 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] Risk estimate is for stomach and small intestine.
[c] 99% CI.
[d] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; SMR, standardized mortality ratio; USDA, US Department of Agriculture.

TABLE 6-3 Selected Epidemiologic Studies—Colon Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	34	1.2 (0.8–1.6)
Hooiveld et al., 1998	Dutch chemical production workers	3	1.4 (0.3–4.0)
Rix et al., 1998	Danish paper mill workers		
	Males	58	1.0 (0.7–1.2)
	Females	23	1.1 (0.7–1.7)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	27	1.1 (0.7–1.6)
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD 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	
Ott and Zober, 1996[b]	BASF cleanup workers	5	1.0 (0.3–2.3)
	TCDD <0.1 μg/kg of body wt	2	1.1 (0.1–3.9)
	TCDD 0.1–0.99 μg/kg of body wt	2	1.4 (0.2–5.1)
	TCDD >1 μg/kg of body wt	1	0.5 (0.0–3.0)
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	US farmers in 23 states—white males	2,291	1.0 (0.9–1.0)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers	3	1.8 (0.4–5.4)
Collins et al., 1993	Monsanto 2,4-D production workers	3	0.5 (0.1–1.3)
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farm workers	277	0.7 ($p < 0.05$)
Swaen et al., 1992	Dutch herbicide appliers	4	2.6 (0.7–6.5)
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[b]	BASF production workers—basic cohort	2	2.5 (0.4–14.1)
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)

TABLE 6-3 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Thomas, 1987	Flavor and fragrance chemical production workers	4	0.6 (*)
Coggon et al., 1986	British MCPA production workers	19	1.0 (0.6–1.6)
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 et al., 1983	Florida pesticide appliers	5	0.8 (*)
Wiklund, 1983	Swedish agricultural workers	1,332	0.8 (0.7–0.8)[c]
Thiess et al., 1982	BASF production workers	1	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			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of colon cancer in males		22.7 in Chapaevsk; 21.7 in Samara region[d]
	Age-adjusted incidence (100,000) of colon cancer in females		13.3 in Chapaevsk; 15.4 in Samara region[d]
	Mortality standardized to Samara region		
	Males	17	1.3 (0.8–2.2)
	Females	24	1.0 (0.7–1.5)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A females	2	1.8 (0.4–7.0)
	Zone B males	10	1.2 (0.6–2.2)
	Zone B females	3	0.4 (0.1–1.3)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	2	2.6 (0.6–10.5)
	Zone B males	5	0.8 (0.3–2.0)
	Zone B females	3	0.6 (0.2–1.9)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone A females	2	2.6 (0.3–9.4)
	Zone B males	5	0.8 (0.3–2.0)
	Zone B females	3	0.6 (0.1–1.8)
	Zone R males	34	0.8 (0.6–1.1)
	Zone R females	33	0.8 (0.6–1.1)

continues

TABLE 6-3 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relatve Risk (95% CI)[a]
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 residents—10-year follow-up—morbidity		
	Zone B males	2	0.5 (0.1–2.0)
	Zone B females	2	0.6 (0.1–2.3)
	Zone R males	32	1.1 (0.8–1.6)
	Zone R females	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)
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	3	0.6 (0.2–1.9)
	Zones A, B females	3	0.7 (0.2–2.2)
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zones A, B, R males	20	1.0 (0.6–1.5)
	Zones A, B, R females	12	0.7 (0.4–2.2)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000[b]	Air Force Ranch Hand veterans	7	1.5 (0.4–5.5)
AIHW, 1999[b]	Australian Vietnam veterans—male	188	221 expected (191–251)
CDVA, 1998a	Australian Vietnam veterans—male	405[e]	117 expected (96–138)
CDVA, 1998b	Australian Vietnam veterans—female	1[e]	1 expected (0–5)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	78	1.2 (1.0–1.5)
Crane et al., 1997b	Australian national service Vietnam veterans	6	0.6 (0.2–1.5)
Studies Reviewed in *Update 1996*			
Dalager et al., 1995	Women Vietnam veterans	4	0.4 (0.1–1.2)
	Nurses	4	0.5 (0.2–1.7)
Studies Reviewed in *VAO*			
Breslin et al., 1988[f]	Army Vietnam veterans	209	1.0 (0.7–1.3)
	Marine Vietnam veterans	33	1.3 (0.7–2.2)
Anderson et al., 1986a	Wisconsin Vietnam veterans	4	—
Anderson et al., 1986b	Wisconsin Vietnam veterans	6	1.0 (0.4–2.2)

TABLE 6-3 *Continued*

[a] Given when available.
[b] Colon and rectal cancer results are combined in this study.
[c] 99% CI.
[d] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[e] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have cancer of the colon?"
[f] Intestinal and other gastrointestinal cancer results are combined in this study.
* information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

TABLE 6-4 Selected Epidemiologic Studies—Rectal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	6	0.9 (0.3–1.9)
Hooiveld et al., 1998	Dutch chemical production workers	1	1.0 (0.0–5.6)
Rix et al., 1998	Danish paper mill workers	43	0.9 (0.6–1.2)
Studies Reviewed in *Update 1998*			
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	US farmers in 23 states—white males	367	1.0 (0.9–1.1)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers	0	0 (0.0–4.3)
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farmers	309	0.8 ($p < 0.05$)
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 or soil conservationists	9	1.0 (0.5–1.9)
Henneberger et al., 1989	Paper and pulp workers	1	0.4 (0.0–2.1)
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	6	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 et al., 1983	Florida pesticide appliers	2	1.0 (*)
Wiklund, 1983	Swedish agricultural workers	1,083	0.9 (0.9–1.0)[b]

TABLE 6-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of rectal cancer in males		15.3 in Chapaevsk; 17.1 in Samara region[c]
	Age-adjusted incidence (100,000) of rectal cancer in females		7.0 in Chapaevsk; 11.2 in Samara region[c]
	Mortality standardized to Samara region		
	Males	21	1.5 (1.0–2.4)
	Females	24	0.9 (0.6–1.4)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	1	2.2 (0.3–15.6)
	Zone B males	10	1.2 (0.6–2.2)
	Zone B females	3	1.3 (0.4–4.1)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	7	2.9 (1.3–6.2)
	Zone B females	2	1.3 (0.3–5.1)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	7	2.9 (1.2–5.9)
	Zone B females	2	1.3 (0.1–4.5)
	Zone R males	19	1.1 (0.7–1.8)
	Zone R females	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 residents—10-year follow-up—morbidity		
	Zone B males	3	1.4 (0.4–4.4)
	Zone B females	2	1.3 (0.3–5.4)
	Zone R males	17	1.1 (0.7–1.9)
	Zone R females	7	0.6 (0.3–1.3)

continues

TABLE 6-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	3	1.2 (0.4–3.8)
	Zones A, B females	2	1.2 (0.3–4.7)
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zones A, B, R males	10	1.0 (0.5–2.0)
	Zones A, B, R females	7	1.2 (0.5–2.7)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B males	2	1.7 (0.4–7.0)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000[d]	Air Force Ranch Hand veterans	7	1.5 (0.4–5.5)
AIHW, 1999[d]	Australian Vietnam veterans—male	188	221 expected (191–251)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	16	0.6 (0.4–1.0)
Crane et al., 1997b	Australian national service Vietnam 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.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[d] Colon and rectal cancer results are combined in this study.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

TABLE 6-5 Selected Epidemiologic Studies—Pancreatic Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Ojajärvi et al., 2000	Meta-analysis of 161 populations		MRR = 1.0 (0.8–1.3)
Steenland et al., 1999	US chemical production workers	16	1.0 (0.6–1.6)
Hooiveld et al., 1998	Dutch chemical production workers	4	2.5 (0.7–6.3)
Rix et al., 1998	Danish paper mill workers		
	Males	30	1.1 (0.8–1.7)
	Females	2	0.3 (0.0–1.1)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	7	0.9 (0.4–1.9)
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)
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	US farmers in 23 states—white males	1,133	1.1 (1.1–1.2)
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers	3	2.2 (0.5–6.3)
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish self-employed male farm workers	137	0.6 ($p < 0.05$)
Swaen et al., 1992	Dutch herbicide appliers	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	6	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 (*)

continues

TABLE 6-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Blair et al., 1983	Florida pesticide appliers	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			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	1	1.3 (0.2–9.5)
	Zone B males	3	0.6 (0.2–1.9)
	Zone B females	1	0.3 (0.0–2.4)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A males	1	1.9 (0.3–13.5)
	Zone B males	2	0.6 (0.1–2.2)
	Zone B females	1	0.5 (0.1–3.9)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone A males	1	1.9 (0.0–10.5)
	Zone B males	2	0.6 (0.1–2.0)
	Zone B females	1	0.5 (0.0–3.1)
	Zone R males	20	0.8 (0.5–1.2)
	Zone R females	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 residents		
	Zones A, B males	2	1.0 (0.3–4.2)
	Zones A, B females	1	1.6 (0.2–12.0)
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zones A, B, R males	9	0.6 (0.3–1.2)
	Zones A, B, R females	4	1.0 (0.3–2.7)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B males	2	1.1 (0.3–4.5)
VIETNAM VETERANS			
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	38	1.4 (1.0–1.9)
Crane et al., 1997b	Australian national service Vietnam veterans	6	1.5 (*)
Studies Reviewed in *Update 1996*			
Dalager et al., 1995	Women Vietnam Veterans	7	2.8 (0.8–10.2)
	Nurses	7	5.7 (1.2–27.0)

TABLE 6-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Visintainer et al., 1995	Michigan Vietnam veterans	14	1.0 (0.6–1.7)
Studies Reviewed in *VAO*			
Thomas et al., 1991	US Vietnam veterans—females	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.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: IARC; International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

HEPATOBILIARY CANCERS

Hepatobiliary cancers include cancers of the liver (ICD-9 155.0,155.2) and the intrahepatic bile duct (ICD-9 155.1). According to ACS estimates, 11,000 men and 5,600 women will be diagnosed with liver or intrahepatic bile duct cancer in the United States in 2002 and 8,900 men and 5,200 women will die from them (ACS, 2002).

In the United States, liver cancers account for about 1% of new cancer cases and 2.5% of cancer deaths. Misclassification of metastatic cancers as primary liver cancer can lead to overestimating 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 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; at the ages of 45–59 years, it is greater in men than in women and greater in blacks than in whites. The average annual incidence of hepatobiliary cancers is shown in Table 6-6.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD,

picloram, or cacodylic acid) and hepatobiliary cancers. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-7 for a summary of the studies).

Update of the Scientific Literature

An environmental study of residents of Chapaevsk, a Russian industrial community on the Volga River with documented contamination of the food and water supply by dioxins and other chemicals, demonstrated a higher incidence of liver cancer in males and females (13.5 and 5.9 per 100,000 per year, respectively) than in Russia as a whole (7.5 and 5.8) or the Samara region of Russia (6.6 and 2.7), in which the community is located. Because of the lack of adjustment for confounding by socioeconomic, lifestyle, comorbidity and other factors; and because of the likelihood of multiple exposures as well as the ecologic study design, this study cannot be taken as strong evidence for an association.

Vietnam-Veteran Studies

No relevant occupational or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

The evidence from epidemiologic studies is inadequate to link herbicide exposure to hepatobiliary cancer; no new published information was found to change this opinion.

TABLE 6-6 Average Annual Incidence (per 100,000) of Liver and Intrahepatic Bile Duct Cancers in United States[a]

	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	8.0	5.9	15.5	10.0	7.0	20.6	15.9	11.5	30.4
Females	1.9	1.4	3.2	3.0	2.3	4.3	4.2	3.2	7.2

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

TABLE 6-7 Selected Epidemiologic Studies—Hepatobiliary Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	7	0.9 (0.4–1.6)
Rix et al., 1998	Danish paper-mill workers		
	Males	10	1.1 (0.5–2.0)
	Females	1	0.6 (0.0–3.2)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	7	1.3 (0.5–2.6)
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)
Becher et al., 1996	German chemical production workers	1	1.2 (0.0–6.9)
Ott and Zober, 1996	BASF cleanup workers	2	2.1 (0.3–8.0)
	TCDD <0.1 μg/kg of body wt	1	2.8 (0.1–15.5)
	TCDD 0.1–0.99 μg/kg of body wt	0	
	TCDD >1 μg/kg of body wt	1	2.8 (0.1–15.5)
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 appliers	2	0.6 (0.1–2.2)
Blair et al., 1993	US 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)
	Subcohort with ≥ 20-year 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	—

continues

TABLE 6-7 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of liver cancer in males		13.5 in Chapaevsk; 6.6 in Samara region[c]
	Age-adjusted incidence (100,000) of liver cancer in females		5.9 in Chapaevsk; 2.7 in Samara region[c]
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	6	0.5 (0.2–1.2)
	Zone B females	7	1.2 (0.6–2.6)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	4	0.6 (0.2–1.5)
	Zone B females	4	1.1 (0.4–3.1)
	Zone R males	35	0.7 (0.5–1.0)
	Zone R females	25	0.8 (0.6–1.3)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	4	0.6 (0.2–1.4)
	Zone B females	4	1.1 (0.3–2.9)
	Zone R males	35	0.7 (0.5–1.0)
	Zone R females	25	0.8 (0.5–1.3)
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 residents—10-year follow-up—morbidity		
	Zone B males	5	1.8 (0.7–4.4)
	Zone B females	5	3.3 (1.3–8.1)
	Zone R males	11	0.5 (0.3–1.0)
	Zone R females	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 residents		
	Zones A, B males	4	1.5 (0.5–4.0)
	Zones A, B females	1	1.2 (0.2–9.1)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B males	3	1.2 (0.4–3.8)
	Zone R males	7	0.4 (0.2–0.8)

TABLE 6-7 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Hoffman et al., 1986	Residents of Quail Run Mobile Home Park	0	—
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	2	1.6 (0.2–11.4)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	8	0.6 (0.3–1.2)
Crane et al., 1997b	Australian national service Vietnam veterans	1	—
Studies Reviewed in *VAO*			
CDC, 1990	US men born in 1921–1953	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.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; CDC, Centers for Disease Control and Prevention; IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is still inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and hepatobiliary cancer. The evidence regarding association is drawn from previous occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components. Although several of those studies involve sizable cohorts, hepatobiliary cancers are rare; as a result, the number of expected cases is fairly small.

Biologic Plausibility

Rats and mice given TCDD orally for 2 years were evaluated for development of cancer (NTP, 1982). Neoplastic nodules in the livers of female rats were

significantly increased in the high-TCDD-dose group, and a significant increase in hepatocellular carcinomas was noted in high-dose-treated male and female mice. The high dose of TCDD that increased the incidence of neoplasia also increased the incidence of toxic hepatitis in rats and mice of both sexes. Cacodylic acid administered to laboratory animals has induced hepatic neoplasms. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

There are insufficient data to determine whether Vietnam veterans are at increased risk for liver cancer.

NASOPHARYNGEAL CANCER

There are many types of nasal (ICD-9 160.0–160.9) and nasopharyngeal (ICD-9 147.0–147.9) cancers; undifferentiated carcinoma, squamous-cell carcinoma, and lymphomas account for the vast majority of malignancies.

ACS estimates that about 3,600 men and 1,300 women will be diagnosed with nasal, pleural, tracheal, and other respiratory system cancers (this excludes cancers of the larynx, lung, and bronchus) in the United States in 2002 and that some 2,000 men and 800 women will die from them (ACS, 2002). Roughly speaking, nasopharyngeal cancers account for one-third to one-half of those totals. ACS (2002) estimates suggest that about 6,500 men and 2,100 women will be diagnosed with cancers of the pharynx (including nasopharynx, tonsil, oropharynx, hypopharynx, and buccal cavity) and 1,500 men and 600 women will die from them. Nasopharyngeal cancers make up about one-fifth of those cancers. The average annual incidences reported in Table 6-8 show that men are at a greater risk than women for these diseases and that the incidences increase 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, blacks, or other groups; this suggests 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).

TABLE 6-8 Average Annual Incidence (per 100,000) of Nasal and Nasopharyngeal Cancers in United States[a]

	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 Middle Ear									
Males	0.8	0.7	1.4	1.1	1.0	1.4	2.2	2.0	1.9
Females	0.2	0.3	0.2	0.7	0.7	1.4	0.9	0.9	1.1
Nasopharynx									
Males	1.6	0.6	2.1	2.0	1.0	2.0	2.8	2.0	1.4
Females	0.8	0.3	0.6	0.6	0.3	0.3	0.6	0.3	0.8

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and nasopharyngeal cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-9 for a summary of the studies).

Update of the Scientific Literature

An environmental study of female residents of Chapaevsk, a Russian industrial community on the Volga River with documented contamination of the food and water supply by dioxins and other chemicals, demonstrated a higher incidence of pharyngeal cancer (2.1 per 100,000 per year) than in Russia as a whole (0.7) and the Samara region of Russia (0.6), which includes Chapaevsk. Male residents of Chapaevsk did not demonstrate an excess risk (2.2) compared with Russia as a whole (5.9) and the Samara region (2.3) (Revich et al., 2001). The usefulness of these data is restricted due to the lack of adjustment for confounding (e.g. smoking), the likelihood of multiple exposures, the ecologic study design, and the absence of information on the completeness and accuracy of cancer incidence data.

No relevant occupational or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

TABLE 6-9 Selected Epidemiologic Studies—Nasal and Nasopharyngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Caplan et al., 2000	Men selected from population-based cancer registries who have nasal cancer	70	2.2 (1.2–3.7)
Studies Reviewed in *Update 1998*			
Kogevinas et al., 1997	IARC cohort		
	Oral-cavity and pharynx cancer (ICD-9 140–149)	26	1.1 (0.7–1.6)
	Nose and nasal-sinus cancer (ICD-9 160)	3	1.6 (0.3–4.7)
Studies Reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide appliers	1	0.5 (0.0–2.9)
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish and Italian farm workers	11	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			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of pharyngeal cancer in males		2.2 in Chapaevsk; 2.3 in Samara region[b]
	Age-adjusted incidence (100,000) of pharyngeal cancer in females		2.1 in Chapaevsk; 0.6 in Samara region[b]
Studies Reviewed in *VAO*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone R females	2	2.6 (0.5–13.3)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	9	1.0 (0.4–2.8)

TABLE 6-9 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam 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 Vietnam veterans		
	Nasal cancer	0	0 (0.0–>10)
	Nasopharyngeal cancer	1	1.3 (0.0–>10)
Studies Reviewed in *VAO*			
CDC, 1990	US men born in 1921–1953		
	Vietnam veterans	2	0.7 (0.1–3.0)

[a] Given when available.
[b] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: AFHS, Air Force Health Study; CDC, Centers for Disease Control and Prevention; IARC, International Agency for Research on Cancer; ICD-9, *International Classification of Diseases*, Ninth Edition; MCPA, methyl-4-chlorophenoxyacetic acid; NS, not significant.

Synthesis

Nasopharyngeal cancers are relatively rare in the United States. Newly available data do not change the committee's belief that scientific evidence of an association between herbicide exposure and nasopharyngeal cancer is too sparse to draw conclusions.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is still inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and nasopharyngeal cancer.

Biologic Plausibility

No animal studies have found an increased incidence of nasopharyngeal cancer. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides used in Vietnam is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The available data on Vietnam veterans do not suggest an association between TCDD or herbicide exposure and nasopharyngeal cancer.

LARYNGEAL CANCER

According to ACS estimates, 6,900 men and 2,000 women will be diagnosed with cancer of the larynx (ICD-9 161.0–161.9) in the United States in 2002, and 2,900 men and 800 women will die from it (ACS, 2002). Those numbers represent about 1% of new cancer diagnoses and deaths. Cancer of the larynx is more common in men than in women, with an overall ratio in the United States of about 5:1. Incidence increases with age in the group 45–59 years old. The average annual incidence for laryngeal cancer is shown in Table 6-10.

Risk factors for laryngeal cancer 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 some chemicals and dusts may also increase incidence (ACS, 1998).

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was limited or suggestive evidence of an association between exposure to at least one of the chemicals

TABLE 6-10 Average Annual Incidence (per 100,000) of Laryngeal Cancer in United States[a]

	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	4.7	4.3	11.2	10.5	9.8	22.0	17.6	17.1	36.9
Females	1.4	1.1	4.0	2.7	2.4	6.3	4.2	4.1	7.5

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and laryngeal cancers. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Table 6-11 provides summaries of the results of the studies underlying that finding.

Update of the Scientific Literature

Occupational Studies

A small study of 261 Swedish lumberjacks exposed to phenoxyacetic herbicides demonstrated no cases of laryngeal cancer, although one was seen among 241 nonexposed lumberjacks followed as a control group (Thörn et al., 2000). This study is much too small to derive stable estimates of risk.

Environmental Studies

Both male and female residents of Chapaevsk, a Russian industrial community on the Volga River with documented contamination of the food and water supply by dioxins and other chemicals, demonstrated higher incidences of laryngeal cancer (18.0 and 1.1 per 100,000 per year, respectively) than in Russia as a whole (11.3 and 0.4) (Revich et al., 2001). Because of confounding by multiple exposures, the lack of data on alcohol consumption and smoking, and because of the ecologic study design, results of this study cannot be taken as strong evidence of an association with exposure to herbicides, but they are consistent with an increased risk.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

No studies published since *Update 2000* provide strong evidence of the presence or absence of an association between the exposures of interest and laryngeal cancer. Therefore, the conclusion that there is limited or suggestive evidence of an association between laryngeal cancer and the exposures of concern is not challenged by the few data available since *Update 2000*.

TABLE 6-11 Selected Epidemiologic Studies—Laryngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Thörn et al., 2000	Swedish Lumberjacks exposed to phenoxyacetic herbicides	0	(*)
Studies Reviewed in *Update 1998*			
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	US farmer 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-year 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)
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of laryngeal cancer in males		18.0 in Chapaevsk; 11.3 in all of Russia[b]
	Age-adjusted incidence (100,000) of laryngeal cancer in females		1.1 in Chapaevsk; 0.4 in all of Russia[b]
	Mortality standardized to Samara region		
	Males	13	2.3 (1.2–3.8)
	Females	1	0.1 (0.0–0.6)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001[c]	Seveso residents—20-year follow-up		
	Zone B males	55	1.3 (1.0–1.6)
	Zone B females	5	0.8 (0.3–1.9)
Bertazzi et al., 1998[c]	Seveso residents—15-year follow-up		
	Zone B males	40	1.2 (0.9–1.7)
	Zone B females	2	0.5 (0.1–2.0)
	Zone R males	208	0.9 (0.8–1.1)
	Zone R females	35	1.1 (0.8–1.5)
Pesatori et al., 1998[c]	Seveso residents—15-year follow-up		

TABLE 6-11 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Zone A males	5	2.4 (1.0–5.7)
	Zone A females	2	1.3 (0.3–5.3)
	Zone B males	13	0.7 (0.4–1.3)
	Zone B females	8	0.9 (0.4–1.7)
	Zone R males	122	1.0 (0.9–1.3)
	Zone R females	71	0.8 (0.7–1.1)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	4	0.6 (0.2–2.4)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	12	1.3 (0.7–2.3)
Crane et al., 1997b	Australian national service Vietnam veterans	0	0 (0–>10)
Watanabe and Kang, 1996	Army Vietnam veterans compared with US men	50	1.3 (*)
	Marine Vietnam veterans	4	0.7 (*)

[a] Given when available.
[b] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[c] This report did not separate laryngeal from lung and other respiratory cancers.
* Information not provided by study authors.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and laryngeal cancer.

Biologic Plausibility

No animal studies have found an increased incidence of laryngeal cancer associated with exposure to TCDD and the herbicides of concern. A summary of the biologic plausibility of the carcinogencity of TCDD and the herbicides in

general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The conclusion is based on data on other exposed groups. The Air Force Health Study (AFHS) did not find an excess risk of laryngeal cancer among the veterans it studied.

LUNG CANCER

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 ACS estimates, 90,200 men and 79,200 women will be diagnosed with this cancer in the United States in 2002, and about 89,200 men and 65,700 women will die from it (ACS, 2002). Those numbers represent roughly 13% of new cancer diagnoses and 28% of cancer deaths in 2002. The principal types of lung neoplasms are identified collectively as bronchogenic carcinoma (the bronchi are the two main branches of the trachea) and carcinoma of the lung. The lung is also a common site of the development of metastatic cancer.

In men and women, the incidence of lung cancer increases beginning at the age of about 40 years. The incidence in those 50–54 years old is double the incidence in those 45–49 years old, and it doubles again in those 55–59 years old. The rate in black males is consistently higher than that in females or white males. The average annual incidence of lung cancer is shown in Table 6-12.

ACS estimates that more than 90% of lung cancers in males are the result of smoking tobacco (ACS, 1998). Among the other risk factors are occupational exposure to asbestos, chromium, nickel, aromatic hydrocarbons, radioactive ores, and inorganic arsenic. In addition to being synthesized as a herbicide, cacodylic acid, which is dimethylarsinic acid, is a metabolite of inorganic arsenic in hu-

TABLE 6-12 Average Annual Incidence (per 100,000) of Lung and Bronchial Cancer in United States[a]

	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	31.0	27.4	67.7	72.6	66.6	149.8	147.7	129.4	285.8
Females	24.8	24.4	35.0	55.2	55.9	76.2	103.1	107.8	122.9

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

mans. As discussed in Chapter 3, however, the data remain insufficient to conclude that studies of inorganic-arsenic exposure are directly relevant to exposure to cacodylic acid. Therefore, the literature on inorganic arsenic is not considered in this section.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and lung cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Table 6-13 provides summaries of the results of the studies underlying that finding.

Update of the Scientific Literature

Occupational Studies

A cohort of 1,517 male employees of the Dow Chemical Company who were involved in the manufacture or formulation of 2,4-D at some time in 1945–1994 demonstrated no excess mortality from cancer of the lung (SMR = 94) (Burns et al., 2001)

A small study of 261 Swedish lumberjacks exposed to phenoxyacetic herbicides suggested a possible excess of lung cancer by demonstrating single cases in the subgroup of foremen (only 15 persons, with a nominal standardized incidence ratio, SIR, of 417) and female lumberjacks (103 persons, with a nominal SIR of 217), although five such cases were seen among 241 nonexposed lumberjacks followed as a control group (SIR 115, not significant) (Thörn et al., 2000). This study is much too small to derive stable estimates of risk that are generalizable to other populations.

Environmental Studies

Residents of Chapaevsk, a Russian industrial community on the Volga River with documented contamination of the food and water supply by dioxins and other chemicals, demonstrated increased incidences of lung cancer (164.5 per 100,000 per year among men, 19.6 among women) compared with Russia as a whole (89.4 and 9.8, respectively) and the Samara region of Russia (102.4 and 11.1), which includes Chapaevsk (Revich et al., 2001). Data on cigarette-smoking patterns and other exposures were not reported, but arsenic was among the other chemicals to which some residents of Chapaevsk may have been exposed. Because of confounding by multiple exposures and because of the ecologic-study

TABLE 6-13 Selected Epidemiologic Studies—Lung and Bronchial Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4–D production workers	31	SMR = 94 (0.6–1.3)
Thörn et al., 2000	Swedish lumberjack workers exposed to phenoxyacetic herbicides		
	Foremen	1	SIR = 417
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical workers who developed chloracne	30	1.5 (1.0–2.1)
	Two highest cumulative-exposure septiles	19	1.7 (1.2–2.3)
Studies Reviewed in *Update 1998*			
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 appliers	37	1.0 (0.7–1.4)
Blair et al., 1993	US farmers from 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 appliers	12	1.1 (0.6–1.9)
Coggon et al., 1991	Phenoxy herbicide production workers	19	1.3 (0.8–2.1)
	Workers with exposure above "background" levels	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-year 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 NS
Zober et al., 1990	BASF production workers	6	1.6 (*)
	High exposure	4	2.0 (0.6–5.2)
	Chloracne	6	1.8 (0.7–4.0)
Wiklund et al., 1989a	Pesticide appliers in Sweden	38	0.5 (0.4–0.7)

TABLE 6-13 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Bond et al., 1988	Dow 2,4-D production workers (15-year latency)		
	Respiratory cancer	9	1.2 (0.6–2.3)
	Low cumulative exposure	1	0.7 (NS)
	Medium cumulative exposure	2	1.0 (NS)
	High cumulative exposure	5	1.7 (NS)
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)
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 appliers 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			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of lung cancer in males		164.5 in Chapaevsk; 102.4 in Samara region[b]
	Age-adjusted incidence (100,000) of lung cancer in females		19.6 in Chapaevsk; 11.1 in Samara region[b]
	Mortality standardized to Samara region		
	Males	168	3.1 (2.6–3.5)
	Females	40	0.4 (0.3–0.6)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	9	1.5 (0.8–3.0)
	Zone B males	48	1.3 (0.9–1.7)
	Zone B females	4	0.7 (0.3–2.0)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A males	4	1.0 (0.4–2.6)
	Zone B males	34	1.2 (0.9–1.7)
	Zone B females	2	0.6 (0.1–2.3)
	Zone R males	176	0.9 (0.8–1.1)

continues

TABLE 6-13 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Zone R females	29	1.0 (0.7–1.6)
Pesatori et al., 1998	Seveso (respiratory)—15-year follow-up		
	Zone A males	5	2.4 (1.0–5.7)
	Zone A females	2	1.3 (0.3–5.3)
	Zone B males	13	0.7 (0.4–1.3)
	Zone B females	8	0.9 (0.4–1.7)
	Zone R males	122	2.0 (0.9–1.3)
	Zone R females	71	0.8 (0.7–1.1)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone A males	4	1.0 (0.3–2.5)
	Zone B males	34	1.2 (0.9–1.7)
	Zone B females	2	0.6 (0.1–2.1)
	Zone R males	176	0.9 (0.8–1.0)
	Zone R females	29	1.0 (0.7–1.5)
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 et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone A males	2	0.8 (0.2–3.4)
	Zone B males	18	1.1 (0.7–1.8)
	Zone R males	96	0.8 (0.7–1.0)
	Zone R females	16	1.5 (0.8–2.5)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	10	3.7 (0.8–17.1)
AIHW, 1999	Australian Vietnam veterans—male	46	65 expected (49–81)
CDVA, 1998a	Australian Vietnam veterans—male	120[c]	65 expected (49–81)
CDVA, 1998b	Australian Vietnam veterans—female	0[c]	(*)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	212	1.3 (1.1–1.5)
Crane et al., 1997b	Australian national service Vietnam 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 (1.0–1.3)

TABLE 6-13 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Non-Vietnam	77	0.9 (*)
Watanabe and Kang, 1995	Vietnam service Marines vs non-Vietnam	42	1.3 (0.8–2.1)

[a] Given when available.
[b] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[c] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have lung cancer?"
* Information not provided by study authors.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; MCPA, methyl-4-chlorophenoxyacetic acid; PCDD, polychlorinated dibenzodioxin; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

design, results of this study cannot be taken as strong evidence of an association with herbicide or TCDD exposure.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

Evidence remains inconclusive but suggestive regarding an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and lung cancer. Absence of data on smoking and other confounding factors, such as other occupational exposures, limits the usefulness of the results of available studies. The most prominent and convincing evidence came from the National Institute for Occupational Safety and Health (NIOSH) cohort, which experienced some of the highest TCDD exposures of any population studied. Lung was the only individual cancer site that showed an excess among these workers. In addition, numerous lines of mechanistic evidence, discussed below under "Biologic Plausibility," provide further support for the conclusion of limited/suggestive evidence.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and cancer of the lung (carcinomas of the lung and bronchus).

Biologic Plausibility

As noted in *Update 2000*, there is evidence of increased incidence of squamous-cell carcinoma of the lung in rats exposed to high concentrations of TCDD. Cacodylic acid administered to rats has resulted in an increased frequency of carcinoma of the lung. The relevance of those studies to human exposure is not clear. Mechanistic data from in vitro and animal studies, however, also support a role of TCDD as a promoter in the carcinogenic process. Lung tissue has been found to have high concentrations of the aryl hydrocarbon receptor (AhR) that mediates the effects of TCDD, and recent data have shown both CYP1A1 and CYP1A2 to be expressed in lung biopsy specimens from human subjects. Those enzymes are responsible, in part, for the activation of procarcinogens, such as found in tobacco smoke (which also contains AhR ligands), to genotoxic intermediates. Thus, it is biologically plausible that exposure to TCDD may synergize the carcinogenic effects of a variety of other chemicals to which human lung tissue is exposed. A summary of the biologic plausibility of the carcinogencity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Ranch Hand participants show a markedly increased risk of lung cancer, but the extent to which this may be attributable to herbicide or TCDD exposure is not clear.

BONE AND JOINT CANCER

According to the ACS, about 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 2002, and 700 men and 600 women will die from it (ACS, 2002). Primary bone cancers are among the least common malignancies. The bones are, however, frequent sites of secondary tumors of other cancers that have metastasized. Only primary bone

TABLE 6-14 Average Annual Incidence (per 100,000) of Bone and Joint Cancer in United States[a]

	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	0.6	0.6	0.9	1.0	1.1	0.7	1.2	1.3	1.9
Females	0.7	0.8	0.8	0.9	0.9	0.9	0.8	0.9	[b]

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.
[b]Insufficient data to provide meaningful incidence.

cancer is considered here. The average annual incidence of bone and joint cancer is shown in Table 6-14.

Bone cancer is more common in teenagers than in adults. The incidence among people in the age groups of 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 or joint cancer are exposure to ionizing radiation in treatment for other cancers and a history of some noncancer bone diseases.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and bone cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-15 for summaries of the studies).

Update of the Scientific Literature

An environmental study of residents of Chapaevsk, a Russian industrial community on the Volga River with documented contamination of the food and water supply by dioxins and other chemicals, reported seven deaths in male residents due to cancer of the bones or soft tissues (SMR = 2.1 [0.9–4.4]) and seven deaths in female residents (SMR = 1.4 [0.6–3.0]). Because cancers of the bone and soft tissue are combined in this analysis, the results cannot be taken as evidence for an association with bone cancer alone.

TABLE 6-15 Selected Epidemiologic Studies—Bone Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Rix et al., 1998	Danish paper mill workers		
	Males	1	0.5 (0.0–2.7)
	Females	0	—
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	1	46 (0.6–255.2)
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)	3	1.1 (0.2–3.1)
	Workers not exposed to TCDD (or higher-chlorinated dioxins)	2	1.4 (0.2–5.2)
Ramlow et al., 1996	Pentachlorophenol production workers	0	—
Studies Reviewed in *Update 1996*			
Blair et al., 1993	US 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			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Mortality standardized to Samara region (bone, soft tissue cancer)		
	Males	7	2.1 (0.9–4.4)
	Females	7	1.4 (0.6–3.0)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B females	1	2.6 (0.3–19.4)
	Zone R males	2	0.5 (0.1–2.0)
	Zone R females	7	2.4 (1.0–5.7)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B females	1	2.6 (0.0–14.4)
	Zone R males	2	0.5 (0.1–1.7)
	Zone R females	7	2.4 (1.0–4.9)

TABLE 6-15 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
VIETNAM VETERANS			
Studies Reviewed in *Update 1998*			
Clapp, 1997	Massachusetts Vietnam veterans	4	0.9 (0.1–11.3)
AFHS, 1996	Air Force Ranch Hand veterans	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)

[a] Given when available.
[b] 99% CI.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Vietnam-Veteran Studies

No relevant occupational or Vietnam-veteran studies have been published since *Update 2000*.

Synthesis

The committee found no new information to add to the sparse existing dataset. There is no evidence to support a change from the conclusion that 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.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists

between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and bone cancer. The evidence regarding an association is drawn from occupational and environmental studies in which the subjects were exposed to a variety of herbicides and herbicide compounds.

Biologic Plausibility

No animal studies have found an increased incidence of bone cancer after exposure to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

There are no data on which to base a conclusion that Vietnam veterans may or may not be at increased risk for bone cancer because of exposure to herbicides or TCDD.

SOFT-TISSUE SARCOMAS

Soft-tissue sarcoma (STS) (ICD-9 171.0–171.9, 164.1) arises 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. ACS estimates that about 4,400 men and 3,900 women will be diagnosed with STS in the United States in 2002 and that about 2,000 men and 1,900 women will die from it (ACS, 2002). The incidence of STS in the age groups of most Vietnam veterans has no consistent pattern. The average annual incidence of STS is shown in Table 6-16.

TABLE 6-16 Average Annual Incidence (per 100,000) of Soft-Tissue Sarcoma (Including Malignant Neoplasms of Heart) in United States[a]

	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	2.8	4.2	4.0	4.0	5.8	4.3	4.4	3.7
Females	2.3	2.3	3.0	3.0	3.0	3.4	3.7	3.6	4.5

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

Among the risk factors for those cancers are exposure to ionizing radiation from treatment for other cancers and some inherited conditions, including Gardner's syndrome, Li-Fraumeni syndrome, and neurofibromatosis. Several chemical exposures have been identified as possible risk factors (Zahm and Fraumeni, 1997).

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was sufficient information to determine that an association existed between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and STS. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-17 for a summary of the studies).

Update of the Scientific Literature

In an environmental study, Costani et al. (2000) report on an unusually high frequency of STS among the general population living near a chemical plant in the northern Italian city of Mantua, a city of about 50,000 people in a county that is mainly agricultural. A number of industrial activities were developed in the last 20 years, including a paper mill, a petroleum refinery, a petrochemical plant, three thermoelectric plants, three toxic-waste deposits, and a toxic-waste and medical-waste incinerator. A general practitioner's noting five cases of STS over an 8-year period prompted a call to all general practitioners in the area to report cases of STS and to verify the histologic diagnosis. Complete information was obtained on 20 cases diagnosed in 1984–1996. The corresponding number of expected cases based on the provincial cancer registry was 8.9, for an SMR of 2.3 (95% CI 1.3–3.5). The malignancies were classified according to the ICD-10 codes of 8800–9044. The most frequent types were leiomyosarcoma, dermatofibrosarcoma, and fibrosarcoma. The authors hypothesized that the cause of the geographically confined excess was soil contamination, but there were no soil or air samples or exposure data to support the hypothesis.

No relevant occupational or Vietnam-veteran studies have been published since *Update 2000*.

Synthesis

Findings from prior occupational, environmental, and veteran studies show sufficient evidence to link herbicide exposure to STS. An additional environmental-exposure report from Mantua, Italy, adds to this evidence.

TABLE 6-17 Selected Epidemiologic Studies—Soft-Tissue Sarcoma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	0	(*)
Hooiveld et al., 1998	Dutch chemical production workers	0	(*)
Rix et al., 1998	Danish paper mill workers		
	Women in plants 1 and 2	9	2.3 (1.1–4.4)
	Women in plants 1, 2, and 3	11	2.6 (1.3–4.7)
	Women employed in sorting and packing	8	4.0 (1.7–7.8)
	Men employed in sorting and packing	12	1.2 (0.6–2.0)
Studies Reviewed in *Update 1998*			
Hertzman et al., 1997	Canadian sawmill workers	11	1.0 (0.6–1.7)
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher-chlorinated dioxins)	6	2.0 (0.8–4.4)
	Workers not exposed to TCDD (or higher-chlorinated dioxins)	2	1.4 (0.2–4.9)
	Workers exposed to any phenoxy herbicide or chlorophenol	9	2.0 (0.9–3.8)
Ott and Zober, 1996	Workers exposed in 1953 accident	0	0.2 expected
Ramlow et al., 1996	Pentachlorophenol production workers	0	0.2 expected
Studies Reviewed in *Update 1996*			
Kogevinas et al., 1995	IARC cohort	11	(*)
Mack, 1995	US cancer registry data (SEER program) review		
	Male	3,526	(*)
	Female	2,886	(*)
Blair et al., 1993	US farmers in 23 states (white males)	98	0.9 (0.8–1.1)
Lynge, 1993	Danish male production workers	5	2.0 (0.7–4.8)
Kogevinas et al., 1992	IARC cohort (10–19 years after first exposure)	4	6.1 (1.7–15.5)
Studies Reviewed in *VAO*			
Bueno de Mesquita et al., 1993	Phenoxy herbicide workers	0	0 (0.0–23.1)
Hansen et al., 1992	Danish gardeners	3	5.3 (1.1–15.4)
Smith and Christophers, 1992	Male residents of Australia	30	1.0 (0.3–3.1)
Fingerhut et al., 1991	NIOSH cohort	4	3.4 (0.9–8.7)
	Those with 20 years of latency and 1 year of exposure	3	9.2 (1.9–27.0)
Manz et al., 1991	German production workers	0	(*)
Saracci et al., 1991	IARC cohort	4	2.0 (0.6–5.2)
Zober et al., 1990	German production workers	0	(*)
Alavanja et al., 1989	Forest or soil conservationists	2	1.0 (0.1–3.6)
Bond et al., 1988	Dow 2,4-D production workers	0	(*)
Wiklund et al., 1988, 1989b	Swedish agricultural workers	7	0.9 (0.4–1.9)

TABLE 6-17 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Woods et al., 1987	Male residents of Washington State		
	High phenoxy exposure	*	0.9 (0.4–1.9)
	Those with self-reported chloracne	*	3.3 (0.8–14.0)
Coggon et al., 1986	British MCPA chemical workers	1	1.1 (0.03–5.9)
Hoar et al., 1986	Kansas residents		
	All farmers	95	1.0 (0.7–1.6)
	Farm use of herbicides	22	0.9 (0.5–1.6)
Vineis et al., 1986	Italian rice growers	66	
	Among all living women	5	2.4 (0.4–16.1)
Smith et al., 1983, 1984; Smith and Pearce, 1986	New Zealand workers exposed to herbicides	17	1.6 (0.7–3.8)
Lynge, 1985	Danish male production workers	5	2.7 (0.9–6.3)
Balarajan and Acheson, 1984	Agricultural workers in England		
	Overall	42	1.7 (1.0–2.9)
	Those under 75 years old	33	1.4 (0.8–2.6)
Blair et al., 1983	Florida pesticide appliers	0	(*)
Hardell, 1981	Swedish workers		
	Phenoxy herbicide exposure	52	5.5 (2.2–13.8)
Eriksson et al., 1979, 1981	Swedish workers	25	(2.2–10.2) 5.1 matched
ENVIRONMENTAL			
New Studies			
Costani et al., 2000	Residents near a chemical plant in Mantua, Italy	20	SMR = 2.25 (1.3–3.5)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso—20-year follow-up	0	(*)
Viel et al., 2000	Residents near French solid-waste incinerator		
	Spatial cluster	45	1.4 (p = 0.004)
	1994–1995	12	3.4 (p = 0.008)
Bertazzi et al., 1998	Seveso—15-year follow-up	0	(*)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone R males	4	2.1 (0.6–5.4)
Gambini et al., 1997	Rice-growing farmers	1	0.3 expected
Svensson et al., 1995	Swedish fishermen—incidence		
	West coast	3	0.5 (0.1–1.4)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone R males	6	2.8 (1.0–7.3)
	Zone R females	2	1.6 (0.3–7.4)

continues

TABLE 6-17 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *VAO*			
Lampi et al., 1992	Finnish town	6	1.6 (0.7–3.5)
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zone A, B, R males	2	5.4 (0.8–38.6)
	Zone A, B, R females	1	2.0 (0.2–1.9)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone R males	2	6.3 (0.9–45.0)
	Zone B females	1	17.0 (1.8–163.6)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	1	0.8 (0.1–12.8)
AIHW, 1999	Australian Vietnam veterans—male	14	27 expected (17–37)
CDVA, 1998a	Australian Vietnam veterans—male	398[b]	27 expected (17–37)
CDVA, 1998b	Australian Vietnam veterans—female	2[b]	0 expected (0–4)
Studies Reviewed in *Update 1998*			
Clapp, 1997	Massachusetts Vietnam Veterans	18	1.6 (0.5–5.4)
Crane et al., 1997a[c]	Australian military Vietnam veterans		
Crane et al., 1997b	Australian national service Vietnam veterans	4	0.7
	Comparison group	2	—
AFHS, 1996	Ranch Hand veterans	1	—
	Comparisons	1	—
Visintainer et al., 1995	Vietnam veterans	8	1.1 (0.5–2.2)
Watanabe and Kang, 1995	US Marines in Vietnam	0	(*)
Studies Reviewed in *Update 1996*			
Kogan and Clapp, 1988	Vietnam veterans in Massachusetts	9	5.2 (2.4–11.1)
Kang et al., 1986	Vietnam veterans—comparing those who served with those who did not	86	0.8 (0.6–1.1)
Lawrence et al., 1985	Vietnam veterans in New York	2	1.1 (0.2–6.7)
Greenwald et al., 1984	New York State Vietnam veterans	10	0.5 (0.2–1.3)
Studies Reviewed in *VAO*			
Watanabe et al., 1991	Marine Vietnam veterans	8	1.1
Bullman et al., 1990	Army veterans serving in I Corps	10	0.9 (0.4–1.6)
Michalek et al., 1990	Ranch Hand veterans	1	(*)
	Comparisons	1	(*)
Breslin et al., 1988	Army Vietnam veterans	30	1.0
Fett et al., 1987	Australian Vietnam veterans	1	1.3 mortality rate, age-adjusted (0.1–20.0)
Anderson et al., 1986a,b	Wisconsin Vietnam veterans	5	1.5 (0.6–3.5)

TABLE 6-17 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Breslin et al., 1986	Vietnam veterans in Massachusetts	2	3.8 (0.5–13.8)

[a] Given when available.
[b] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have soft-tissue sarcoma?"
[c] Data for different military branches presented separately. Number of exposed cases range from 0–9; all CI's include 1.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; SEER, Surveillance, Epidemiology, and End Results (SEER) Program; SMR, standardized mortality ratio; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is sufficient evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and STS.

Biologic Plausibility

No animal studies have found an increased incidence of STS. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The available data on Vietnam veterans do not permit a conclusion on whether they are at increased risk for STS.

SKIN CANCER—MELANOMA

Skin cancers are generally divided into two broad categories: neoplasms that develop from melanocytes (malignant melanoma) and neoplasms that do not.

TABLE 6-18 Average Annual Cancer Incidence (per 100,000) of Melanoma of the Skin in United States[a]

	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	24.8	28.9	0.9	34.0	38.8	1.7	41.3	46.7	2.3
Females	21.6	25.4	1.8	23.7	27.4	0.9	25.3	29.6	3.0

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

Nonmelanocytic skin cancers (primarily basal-cell and squamous-cell carcinomas) have a far higher incidence than malignant melanoma but are considered less aggressive and therefore more treatable. The average annual incidence of melanoma is shown in Table 6-18. In *VAO* and *Update 1996*, all skin cancers were assessed together. However, beginning with *Update 1998*, the committee chose to address studies assessing malignant melanoma separately from those

TABLE 6-19 Selected Epidemiologic Studies—All (or Unspecified) Skin-Cancer Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative 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			
Studies Reviewed in *Update 1998*			
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)

[a] Given when available.

* Information not provided by study authors.

ABBREVIATIONS: IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; USDA, US Department of Agriculture.

TABLE 6-20 Selected Epidemiologic Studies—All (or Unspecified) Skin-Cancer Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
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 *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	325	1.3 (1.1–1.6)
Ketchum et al., 1999	Ranch Hand (RH) veterans and comparisons through June 1997		
	Comparisons	158	(control group)
	Background-exposure RH veterans	57	1.0 (0.7–1.5)
	Low-exposure RH veterans	44	1.3 (0.8–2.0)
	High-exposure RH veterans	22	0.8 (0.5–1.4)
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)

[a] Given when available.

* Information not provided by study authors.

ABBREVIATIONS: AFHS, Air Force Health Study; CDC, Centers for Disease Control and Prevention.

assessing nonmelanocytic cancers. Because nonmelanocytic cancers are highly treatable, studies of them have been divided further into studies that discuss mortality and studies that discuss incidence. Many studies report results by combining all types of skin cancers or do not specify the type of skin cancers assessed. In the interest of completeness, studies of skin-cancer mortality and morbidity are listed in Tables 6-19 and 6-20.

According to ACS estimates, about 29,000 men and 22,400 women will be diagnosed with cutaneous melanoma (ICD-9 172.0–172.9) in the United States in 2002, and 5,000 men and 2,800 women will die from it (ACS, 2002). About 1,300,000 cases of nonmelanocytic skin cancer (ICD-9 173.0–173.9), primarily basal-cell and squamous-cell carcinomas, are diagnosed in the United States each year (ACS, 2000). Because it is not required to report those cancers to registries, the numbers of cases are not as precise as for other cancers. The ACS estimates that about 1,900 people will die from nonmelanocytic skin cancer in 2002.

Skin cancer is far more likely to occur in fair-skinned people; the risk for whites is roughly 20 times that for dark-skinned blacks. Incidence also increases with age, although more strikingly in males than in females. Other risk factors for melanoma include the presence of some moles on the skin, a suppressed immune system, and excessive exposure to ultraviolet (UV) radiation, typically from the sun. A family history of the disease has been identified 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 most important risk factor for nonmelanocytic skin cancer. Some skin diseases and chemical exposures have also been identified as potential risk factors. Exposure to inorganic arsenic is a risk factor for skin cancer, and cacodylic acid is a metabolite of inorganic arsenic. As discussed in Chapter 3, however, the data remain insufficient to conclude that studies of inorganic-arsenic exposure are directly relevant to exposure to cacodylic acid. Therefore, the literature on inorganic arsenic is not considered in the sections on skin cancer.

SEER incidence data are not available for nonmelanocytic skin cancer.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to herbicides used in Vietnam or the contaminant TCDD and skin cancer. Additional information available to the committee responsible for *Update 1996* did not change that finding. The *Update 1998* committee considered the literature on malignant melanoma separately from that of nonmelanocytic skin cancers. It found that there was inadequate or insufficient information to determine whether an association existed between exposure to herbicides used in Vietnam or the contaminant TCDD and malignant melanoma. The *Update 2000* committee concurred with the findings of the *Update 1998* committee (see Tables 6-21 and 6-22 for summaries of the melanoma mortality and morbidity studies, respectively).

Update of the Scientific Literature

Occupational Studies

Burns et al. (2001) conducted a study of mortality in a cohort of 1,517 male Dow Chemical Company workers involved in the production of 2,4-D in 1945–1994. Information regarding the measurement of exposure and the collection and analysis of data is provided in Chapter 5. No deaths due to melanoma were reported in the study.

Cancer incidence and mortality were analyzed in a cohort of 504 forestry

TABLE 6-21 Selected Epidemiologic Studies—Melanoma Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers		
	Melanoma	0	0.0
Studies Reviewed in *Update 2000*			
Hooiveld et al., 1998	Dutch production workers	1	2.9 (0.1–15.9)
Studies Reviewed in *Update 1998*			
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 fishermen		
	East coast	0	0.0 (0.0–1.7)
	West coast	6	0.7 (0.2–1.5)
Studies Reviewed in *Update 1996*			
Blair et al., 1993	US farmers in 23 states (white male)	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			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A females	1	6.6 (0.9–47.7)
	Zone B males	1	1.7 (0.2–12.5)
	Zone B females	1	1.0 (0.1–7.4)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, and North and South Dakota		
	Males—counties with wheat acreage 23,000–110,999	50	0.8 (0.6–1.1)
	Males—counties with wheat acreage ≥111,000	41	0.8 (0.6–1.1)
	Females—counties with wheat acreage 23,000–110,999	59	1.2 (0.9–1.8)
	Females—counties with wheat acreage ≥111,000	29	0.7 (0.5–1.2)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	1	9.4 (1.3–68.8)
	Zone R males	3	1.1 (0.3–3.7)
	Zone R females	3	0.6 (0.2–2.0)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone R males	3	1.1 (0.2–3.2)
	Zone R females	3	0.6 (0.1–1.8)

continues

TABLE 6-21 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *VAO*			
Bertazzi et al., 1989a	Seveso residents—10-year follow-up Zones A, B, R males	3	3.3 (0.8–13.9)
VIETNAM VETERANS			
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	51	1.3 (1.0–1.8)
Crane et al., 1997b	Australian national service Vietnam 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)

[a] Given when available.
[b] 99% CI.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; IARC, International Agency for Research on Cancer; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

workers in Sweden (Thörn et al., 2000). The cohort included 261 workers exposed to phenoxy herbicides and 243 nonexposed workers. Follow-up data were collected in 1954–1994 on cancer mortality and in 1958–1992 on cancer incidence. The observed frequency of cancer was compared with expected values on the basis of data on the population of Sweden. The only case of melanoma in the cohort was recorded in an exposed female worker (SIR = 95% CI 3.5, 0.1–19.2).

Environmental Studies

An analysis of cancer incidence and mortality was conducted in the city of Chapaevsk in the Samara region of Russia (Revich et al., 2001). Studies of the air, soil, and water in Chapaevsk revealed general dioxin contamination in the environment. Mortality data were not reported for melanoma. The age-adjusted incidence of melanoma in Chapaevsk relative to the Samara region during 1998 was somewhat lower in men (4.2 vs 5.1 per 100,000) but notably higher in women (8.9 vs 3.5). The numbers of cases in Chapaevsk and the Samara region were not given, hypothesis-testing and interval estimation were not performed, and no confounding factors were considered besides age.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000*.

TABLE 6-22 Selected Epidemiologic Studies—Melanoma Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Thörn et al., 2000	Swedish lumberjack workers exposed to phenoxyacetic herbicides		
	Female	1	3.5 (0.1–19.2)
	Male	0	0.0
Studies Reviewed in *Update 1998*			
Hertzman et al., 1997	Sawmill workers	38	1.0 (0.7–1.2)
Svensson et al., 1995	Swedish fishermen		
	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 ($p < 0.05$)
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of melanoma in males		4.2 in Chapaevsk; 5.1 in Samara region[b]
	Age-adjusted incidence (100,000) of melanoma in females		8.9 in Chapaevsk; 3.5 in Samara region[b]
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	16	1.8 (0.8–3.8)
AIHW, 1999	Australian Vietnam veterans—male	483	380 expected (342–418)
Ketchum et al., 1999	Ranch Hand (RH) veterans and comparisons through June 1997		
	Comparisons	9	(control group)
	Background-exposure RH veterans	4	1.1 (0.3–4.5)
	Low-exposure RH veterans	6	2.6 (0.7–9.1)
	High-exposure RH veterans	2	0.9 (0.2–5.6)
CDVA, 1998a	Australian Vietnam veterans—male	2,689[c]	380 expected (342–418)
CDVA, 1998b	Australian Vietnam veterans—female	7[c]	3 expected (1–8)

continues

TABLE 6-22 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *Update 1998*			
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)

[a] Given when available.
[b] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[c] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have melanoma?"
ABBREVIATION: AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs.

Synthesis

The new epidemiologic studies add little information on the association between exposure to herbicides and the incidence of melanoma. Despite the extended period of follow-up (30–40 years), the occupational studies of chemical workers and lumberjacks do not include enough workers to be informative for this outcome. The data on the increased incidence of melanoma in women of Chapaevsk relative to the Samara region and Russia at large are intriguing, but more information is needed about the completeness of surveillance of the incidence of melanoma and the number of cases included in these measures of incidence.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and melanoma. The evidence regarding association is drawn from occupational, environmental, and veteran studies in which subjects were exposed to herbicides and herbicide components.

Biologic Plausibility

Mice were treated topically (on skin surface) for 2 years with TCDD. Under the conditions of the bioassay, fibrosarcomas occurred in the integumentary system of female mice (Huff et al., 1991); this indicates that continuous dermal exposure to TCDD can induce skin tumors (fibrosarcomas, not squamous-cell carcinomas) in laboratory mice. Mechanistic data from in vitro and animal studies also support a role of TCDD as a promoter in the carcinogenic process. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Although previously reviewed studies of US and Australian veterans have reported a higher incidence of melanoma among male, nonblack veterans than among comparison groups, analyses controlling for factors that might influence or be correlated with the incidence of skin cancers do not show a relationship between measures of exposure to the herbicides used in Vietnam and melanoma. The highest melanoma incidence in the AFHS reports was observed in veterans in the low-TCDD category; this would not be expected if there were an association between exposure and melanoma. The strongest evidence to date comes from the medical-validation study of Australian Vietnam veterans. The estimated expected number of cases is considerably lower than the number of reported cases that were validated. Adjustments for potentially important confounders, however, were not carried out. Overall, data on those who served in Vietnam are not adequate to infer an association between malignant melanoma and exposure to herbicides used in Vietnam.

SKIN CANCER—BASAL-CELL AND SQUAMOUS-CELL (NONMELANOMA)

See the preceeding section for background information on skin cancer.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and skin cancer. Additional information available to the committee responsible for *Update 1996* did not change that finding. The *Update 1998* committee considered the literature on nonmelanocytic skin cancers separately from that of malignant melanoma. It found that there was inadequate

TABLE 6-23 Selected Epidemiologic Studies—Other Nonmelanoma (Basal-Cell and Squamous-Cell) Skin Cancer—Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers		
	Non-melanoma skin cancer	0	0.0
Studies Reviewed in *Update 1998*			
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 fishermen		
	East coast	0	0.0 (0.0–15.4)
	West coast	5	3.0 (1.0–7.1)
Studies Reviewed in *Update 1996*			
Blair et al., 1993	US farmers in 23 states (white male)	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]

[a] Given when available.
[b] 99% CI.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and basal-cell or squamous-cell cancers. The *Update 2000* committee concurred with that finding (see Tables 6-23 and 6-24 for summaries of the studies).

Update of the Scientific Literature

Occupational Studies

Burns et al. (2001) conducted a study of mortality from multiple causes in a cohort of 1,517 male workers involved in the production of 2,4-D in 1945–1994. The study is a continuation and extension of previously reported research on

TABLE 6-24 Selected Epidemiologic Studies—Other Nonmelanoma (Basal-Cell and Squamous Cell) Skin Cancer—Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides		
	Foremen	1	16.7 (0.2–92.7)
Studies Reviewed in *Update 1998*			
Zhong and Rafnsson, 1996	Icelandic pesticide users	5	2.8 (0.9–6.6)
Svensson et al., 1995	Swedish fishermen		
	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 ($p < 0.05$)
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of skin cancer in males (non-melanoma)		55.9 in Chapaevsk; 55.7 in Samara region[b]
	Age-adjusted incidence (100,000) of skin cancer in females (non-melanoma)		64.0 in Chapaevsk; 47.6 in Samara region[b]
Studies Reviewed in *Update 1998*			
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)

continues

TABLE 6-24 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone A males	1	2.4 (0.3–17.2)
	Zone B males	2	0.7 (0.2–2.9)
	Zone R males	20	1.0 (0.6–1.6)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	3	1.0 (0.3–3.0)
	Zones A, B females	3	1.5 (0.5–4.9)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans		
	Basal-cell carcinoma	121	1.2 (0.9–1.6)
	Squamous-cell carcinoma	20	1.5 (0.8–2.8)
CDVA, 1998a	Australian Vietnam veterans—male	6,936[c]	(*)
CDVA, 1998b	Australian Vietnam veterans—female	37[c]	(*)
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)

[a] Given when available.
[b] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[c] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have other skin cancers?"
* Information not provided by study authors.
ABBREVIATIONS: AFHS, Air Force Health Study; CDVA, Commonwealth Department of Veterans' Affairs.

workers at the Dow Chemical Company; additional information on study design is presented in Chapter 4. No deaths due to skin cancer (melanoma or other forms) were recorded in the cohort.

The incidence of fatal and non-fatal cancer was assessed in a cohort of 504 forestry workers in Sweden employed in 1954–1967 (Thörn et al., 2000). The cohort included 261 workers exposed to phenoxy herbicides and 243 nonexposed workers. Follow-up data on the occurrence of cancer were collected in 1954–1994 on mortality and in 1958–1992 on incidence. The frequency of cancer in the cohort was compared with that in the population of Sweden at large. Nonmelano-

cytic skin cancer was diagnosed in one exposed foreman in the cohort (SIR = 16.7, 95% CI 0.2–92.7) and three nonexposed workers (SIR = 2.0, 95% CI 0.4–5.8).

Environmental Studies

Revich et al. (2001) assessed cancer incidence and mortality in Chapaevsk, a city in the Samara region of Russia. Studies of the air, water, and soil in Chapaevsk indicated widespread dioxin contamination from a major chemical plant that has produced hexachlorane and chemicals used in herbicides and pesticides. The age-adjusted incidence of skin cancers other than melanoma during 1998 was reported separately for Chapaevsk and for the entire Samara region. The incidence of these cancers was similar in men (55.9 vs 55.7 per 100,000, respectively) and somewhat higher in women (64.0 vs 47.6). No information was provided on the number of cases used to compute those rates, no *p* values or confidence intervals were reported for the comparison of the rates, and no confounding factors besides age were considered.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000*.

Synthesis

There is little new information in the epidemiologic literature on the relationship between herbicide exposure and the occurrence of nonmelanoma skin cancer. Analyses of mortality, such as that of Burns et al. (2001), are not useful in the study of these cancers, because they rarely lead to death. Only four cases have occurred in the small cohort ($N = 504$) of lumberjacks, and three in the nonexposed group (Thörn et al., 2000). The data on Chapaevsk compared with the Samara region at large suggest a 34% increase in incidence that is limited to women. The precision of that estimated difference and its consistency over time are unknown. Completeness of reporting is particularly problematic for nonmelanocytic skin cancers, and information on this issue would need to be obtained for the interpretation of the report by Revich et al. (2001).

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists

between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and basal-cell or squamous-cell cancers.

Biologic Plausibility

In mice treated with TCDD topically (on skin surface) for 2 years (Huff et al., 1991), under the conditions of the bioassay, fibrosarcomas occurred in the integumentary system of females. The data indicate that continuous dermal exposure to TCDD can induce skin tumors (fibrosarcomas, not squamous-cell carcinomas) in laboratory mice. Mechanistic data from in vitro and animal studies also support a role for TCDD as a promoter in the carcinogenic process. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The AFHS report reviewed in *Update 2000* indicates a higher incidence of nonmelanoma skin cancers among male, nonblack veterans than in the comparison group. However, analyses controlling for factors that might influence or be correlated with the incidence of skin cancers do not show a relationship between measures of exposure to the herbicides used in Vietnam and these cancers.

BREAST CANCER

Breast cancer (ICD-9 174.0–174.9 for females) is the second most common type of cancer (after nomelanocytic skin cancer) among women in the United States. The ACS estimates that about 203,500 women will be diagnosed with breast cancer in the United States in 2002 and that 39,600 will die from it (ACS, 2002). Overall, those numbers represent about 30% of the incidence of new cancers and 15% of cancer deaths among women. Among women 40–55 years old, breast cancer is the leading cause of cancer death. Incidence data on breast cancer are presented in Table 6-25.

Breast-cancer incidence generally increases with age. In the age groups of most Vietnam veterans, the incidence in whites is slightly higher than that in blacks. Risk factors other than age include personal or family history of breast cancer and some characteristics of reproductive history—specifically, early menarche, late onset of menopause, and either no pregnancies or first full-term pregnancy after the age of 30 years. 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

TABLE 6-25 Average Annual Incidence (per 100,000) of Breast Cancer in Females in United States[a]

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
200.0	202.5	194.5	273.0	280.8	261.8	328.1	338.0	297.4

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

personal behavioral and environmental factors in breast-cancer incidence is being studied extensively.

Most female Vietnam veterans who were potentially exposed to herbicides in Vietnam are approaching or have reached menopause and will experience an increasing risk of breast cancer. It is expected on the basis of demographics alone, therefore, that breast cancer will be a conspicuous and significant cause of death.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and breast cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Table 6-26 provides summaries of the results of the studies underlying the finding.

Update of the Scientific Literature

Occupational Studies

Since *Update 2000*, Duell and colleagues (2001) have published results on the reproducibility of self-reported data on farm exposures to potentially hazardous agents, such as pesticides. Using a 10% sample (comprising 30 cases and 31 controls) of the original case–control study (Duell et al., 2000), they show that farming-exposure information obtained from rural women by using detailed farm-by-farm exposure assessment is generally reproducible with an ever–never method. However, in some subgroups of cases (such as older women and low-educated women), they reported lower proportions of exact agreement. The overall pattern in reproducibility across questions was different between cases and

TABLE 6-26 Selected Epidemiologic Studies—Breast Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2000*			
Duell et al., 2000	Used pesticides in the garden	228	2.3 (1.7–3.1)
	Laundered clothes for pesticide user	119	4.1 (2.8–5.9)
Studies Reviewed in *Update 1998*			
Kogevinas et al., 1997	IARC cohort, female; identical with 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 US farmers in 23 states		
	White	71	1.0 (0.8–1.3)
	Nonwhite	30	0.7 (0.5–1.0)
Kogevinas et al., 1993	Female herbicide spraying and production workers	7	0.9 (0.4–1.9)
	Probably 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 ($p < 0.05$)
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			
Aronson et al., 2000	Female patients from Ontario, Canada—highest exposures to dioxin-like congeners		
	PCB 105	44	3.2 (1.5–6.7)
	PCB 118	49	2.3 (1.1–4.8)
Demers et al., 2002	Female patients from Quebec, Canada—analyzed for specific PCB congeners		
	PCB 118		
	All women	104	1.6 (1.0–2.5)
	Premenopausal women	11	2.9 (1.1–7.3)
	PCB 156		
	All women	101	1.8 (1.1–2.9)
	Premenopausal women	17	2.9 (1.2–7.2)
Holford et al., 2000	Patients at Yale-New Haven hospital with breast-related surgery; dioxin-like congener 156	*	0.9 (0.8–1.0)

TABLE 6-26 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of breast cancer in females		69.6 in Chapaevsk; 50.7 in Samara region[c]
	Mortality standardized to Samara region		
	Females	58	2.1 (1.6–2.7)
Warner et al., 2002	Seveso women	981	
	Seveso women with breast cancer who had a 10-fold increase in TCDD level.	15	2.1 (1.0–4.6)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A females	2	0.8 (0.2–3.1)
	Zone B females	12	0.7 (0.4–1.3)
Bagga et al., 2000	Women receiving medical care in Woodland Hills, California	73	NS
Demers et al., 2000	Women in Quebec City newly diagnosed	315	NS
Høyer et al., 2000	Female participants of Copenhagen City Heart Study	195	Overall survival RR 2.8 (1.4–5.6)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	1	0.6 (0.1–3.9)
	Zone B females	9	0.8 (0.4–1.5)
	Zone R females	67	0.8 (0.6–1.0)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone A females	1	0.6 (0.0–3.1)
	Zone B females	9	0.8 (0.4–1.5)
	Zone R females	67	0.8 (0.6–1.0)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone A females	1	0.5 (0.1–3.3)
	Zone B females	10	0.7 (0.4–1.4)
	Zone R females	106	1.1 (0.9–1.3)
Studies Reviewed in *VAO*			
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B females	5	0.9 (0.4–2.1)
	Zone R females	28	0.6 (0.4–0.9)

continues

TABLE 6-26 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
VIETNAM VETERANS			
New Studies			
Kang et al., 2000	Female Vietnam veterans	170	1.2 (0.9–1.5)
Studies Reviewed in *Update 2000*			
CDVA, 1998b	Australian Vietnam veterans—female	17[d]	5 expected (2–11)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	3	5.5 (1.1–16.1)
Studies Reviewed in *Update 1996*			
Dalager et al., 1995	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.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[d] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have breast cancer?"
* Information not provided by study authors.
ABBREVIATIONS: CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer; PCB, polychlorinated biphenyls; RR, relative risk; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

controls. But overall case–control differences in the kappa statistic, a statistical measure of agreement beyond chance, were small. The new results provide some assurance that the conclusion of "no overall excess risk of breast cancer" reported in Duell et al. (2000) is not due to measurement error in the exposure assessment.

Environmental Studies

Holford et al. (2000) studied the joint effects of all congeners of polychlorinated biphenyls (PCBs) with analytic methods that properly account for the high colinearity in the multiple components. On the basis of data on 490 patients with breast-related surgery at Yale-New Haven Hospital (304 cases and 186 controls), they showed that total PCB was not significantly associated with breast-cancer risk (OR = 1.0; 95% CI 0.9–1.1). However, significant protective effects were detected for potential antiestrogens and a dioxin-like congener, 156 (OR = 0.9; 95% CI 0.8–1.0). Although a higher risk of breast cancer was detected for congeners 180 and 183, the study underlined the need to analyze components of PCB

properly if biologically meaningful but potentially different effects of the multiple components of PCB were to be examined properly. This study used breast adipose tissue and hence required the use of female controls without breast cancer who had undergone breast surgery. That prevented the use of general-population controls and hence limited the external validity of the results.

Revich et al. (2001) studied the relationship between the relatively high dioxin concentrations in Chapaevsk, Russia (in air, soil, drinking water, and cows' milk because of pollution from a chemical plant in the area), and breast-cancer incidence and related mortality. The incidence rate of female breast cancer, age-adjusted to the Russian standard population, was higher in Chapaevsk in all age groups (69.6) than in Russia (46.2) and the Samara region (50.7) in 1998. The Chapaevsk women also had higher breast-cancer mortality (SMR = 2.1, 95% CI 1.6–2.7). One of the main weaknesses of this study is the lack of adjustment for such risk factors as family size (parity), breastfeeding, alcohol use, body-mass index (BMI), and fat consumption.

Aronson et al. (2000) reported results of a hospital-based case–control study in Ontario, Canada (Toronto and Kingston). The study used data on 217 breast-cancer cases and 213 benign controls that were frequency-matched by age (5-year age groups) and site to test whether breast-cancer risk is associated with breast adipose-tissue concentrations of several organochlorines. Questionnaire information was collected via mail or telephone interview on demographics, menopausal status, weight (at the age of 25 years and 2 years before interview), height, reproductive history, use of exogenous hormones, physical activity, diet, and family history of breast cancer. Using unconditional logistic-regression models with adjustments for age, study site, menopausal status, and other congener-specific additional confounders, the authors found statistically significant associations between risk of breast cancer and some PCB congeners with dioxin-like activity, albeit at much lower concentrations than dioxins or coplanar PCBs. Specifically, there was an increase in breast cancer in, for instance, the groups with the highest exposure (over 85th percentile) to PCB 105 (OR = 3.2, 95% CI 1.5–6.7) or PCB 118 (OR = 2.3, 95% CI 1.1–4.8) but not PCB 156 (OR = 1.4, 95% CI 0.7–2.7). Those associations showed a dose-response relationship and a stronger effect in premenopausal women. The study used breast adipose tissue and hence required as controls women who had biopsies that were negative for malignancy. That prevented the use of general-population controls and so limits the external validity of the results. It was also reported that the subjects excluded because of lack of information on organochlorine concentrations differed from subjects included in the study. The cases were older (by about 4 years), they had a higher BMI, a lower proportion of them were premenopausal, and more came from Kingston than the controls. Similar differences were also reported for controls. Those differences may further limit the external validity of the study.

Demers et al. (2002) conducted a case–control study on 314 breast-cancer cases and 523 hospital and population controls to test whether exposure to spe-

cific PCB congeners was associated with risk of breast cancer. The study focused on mono-*ortho* PCB congeners that are known to display dioxin-like activity. Cases and controls were matched with respect to age (within 5-year groups) and region of residence (rural vs urban). Risk of breast cancer was examined in all study subjects and separately by menopausal status. A telephone interview was conducted to collect information on lifestyle, dietary habits, and reproductive history. All study subjects were required to be 30–70 years old and to be residing in the Quebec City area. The association between risk of breast cancer and specific PCB congeners was examined with unconditional logistic-regression models. The results indicate that there was a significantly increased risk of breast cancer with increased plasma concentrations of PCB 118 (OR = 1.6, 95% CI 1.0–2.5) and PCB 156 (OR = 1.8, 95% CI 1.1–2.9) for the highest quartile compared with the first quartile. The associations were stronger for premenopausal women, with ORs of 2.9 (95% CI 1.1–7.3) and 2.9 (95% CI 1.2–7.2) for PCB 118 and 156, respectively. Effect modification by menopausal status, however, was not statistically significant. The risk of breast cancer was found to be statistically associated with the mean total concentration of mono-*ortho* congeners (PCB 105, PCB 118, and PCB 156), with ORs of 2.0 (95% CI 1.2–3.3), comparing the fourth with the first quartile. Again, the association was found to be stronger in premenopausal women (OR = 2.6, 95% CI 1.0–6.6). All the models were adjusted for age, region of residence, BMI, history of benign breast cancer, and duration of breastfeeding. The study was well conducted and appears to have used appropriate statistical methods. Its laboratory methods have smaller error rates than those used in other studies, such as the study by Aronson et al. (2000).

Warner et al. (2002) reported results from the Seveso Women's Health Study to test whether serum TCDD concentrations were associated with risk of breast cancer. As described in previous reports and elsewhere in this update, the Seveso cohort is a unique resource for studying the effect of TCDD because of an industrial explosion that occurred in the area in July 1976 and resulted in the highest TCDD concentrations known in human residential populations. The new historical cohort study differs from previous studies on the Seveso cohort in using individual TCDD concentrations as opposed to zones as indicators of exposure. The study involved 981 women (recruited from March 1996 to July 1998) who were infants to 40 years old in 1976, had resided in one of the most contaminated zones (zone A or B), and had adequate stored serum collected soon after the explosion. The 15 breast-cancer cases were diagnosed an average of 15.2 years after the explosion. On the basis of proportional-hazards models, there was a significant association between TCDD concentrations (treated as a continuous variable) and risk of breast cancer (crude hazard ratio = 2.1, 95% CI 1.0–4.6) over a 10-fold range of TCDD concentration. There was also a suggestion of a dose-response relationship, although not statistically significant, when TCDD concentrations were categorized into four groups. The associations did not appear to be confounded by gravidity, parity, age at first pregnancy, age at last pregnancy,

lactation, family history of breast cancer, age at menarche, current BMI, use of oral contraceptives, menarchial status at explosion, menopause status at diagnosis, weight, height, smoking, or alcohol consumption. Those potential confounders were entered into the model one by one because of the relatively small number of breast-cancer cases, a limitation of the study. The study has several strengths; it avoided potential biases associated with disease survival by examining the relationship between serum TCDD and breast-cancer incidence, it adjusted for risk factors (with information collected at the time of interview), and it used individual serum TCDD-concentration data.

Vietnam-Veteran Studies

In a study that included 4,140 female Vietnam veterans and 4,140 veteran controls that did not serve in Vietnam, Kang et al. (2000) concluded that Vietnam veterans had not experienced a significantly higher prevalence of breast cancer (OR = 1.2, 95% CI 0.9–1.5) or gynecologic cancer in the 3 decades since the conflict. Subjects were asked to complete a structured telephone interview to provide information on demographics, pregnancy history, pregnancy outcomes, menstrual history, military experience, smoking and drinking history, and general health. Information on history of gynecologic cancer was followed up with reviews of medical and hospital records. The prevalence of breast cancer was higher in female Vietnam veterans than in non-Vietnam veterans, but the difference was not statistically significant even after adjustment for demographic factors, lifestyle-related characteristics, and military experience. In a cancer-mortality study of the same 8,280 female veterans (based on a vital-status follow-up to December 31, 1991), there were 26 breast-cancer-related deaths, but differences from non-Vietnam veterans were not statistically significant (relative risk [RR] = 1.0, 95% CI 0.6–1.8). There were age differences between cases and controls. The study was well designed and appears to have adequate power. Given the paucity of data on Vietnam veterans, the study is highly relevant for the assessment of the effect of Agent Orange and other herbicides on breast-cancer risk in Vietnam veterans. But its usefulness may be limited because questions on exposure focused on the Vietnam experience as a whole instead of on exposures to Agent Orange, other herbicides, or their contaminants. Reanalysis of the dataset once the modeled exposures from the current study, which is being overseen by the NAS, become available could yield valuable information of the effects of Agent Orange on breast-cancer risk.

Synthesis

The studies published since *Update 2000* continue to support the conclusion that the evidence is inadequate or insufficient to determine whether there is an association between exposure to herbicides used in Vietnam or their contami-

nants and breast cancer. In a study that is perhaps the most relevant to the charge of the committee, Kang et al. (2000) have shown that there is no statistically significant increase in breast-cancer risk in Vietnam veterans. However, their study suffers from the use of nonspecific exposure information. The protective effect of some dioxin-like congeners reported in Holford et al. (2000) is consistent with results of previously reported studies. Results from Duell et al. (2001) rule out measurement error as a potential explanation of the previously reported lack of association between breast-cancer risk and farm exposures to potentially hazardous agents, such as pesticides. Recall bias is an important issue in the study. The results from Revich et al. (2001) give some evidence of increased risk of breast cancer and related mortality, but inadequate control for potential confounders and the use of the ecologic-study design limit the usefulness of the results. However, the study population promises to yield valuable information if better-designed studies are conducted with it. Results from Aronson et al. (2000) and Demers et al. (2002) yielded evidence of a relationship between development of breast cancer and increased concentrations of PCB congeners that have dioxin-like activity. The results from Warner et al. (2002) yielded further evidence of increased breast-cancer risk associated with increased serum TCDD. The Aronson et al. (2000), Demers et al. (2002), and Warner et al. (2002) studies were well conducted and free of major weaknesses in study design and potential biases. Therefore, they provide evidence of association between breast-cancer risk and exposures to TCDD and PCB congeners with dioxin-like activity.

Conclusions

Strength of Evidence from Epidemiologic Studies

There is some evidence of a protective effect of some dioxin-like congeners. The suggestive evidence of increased risk of breast cancer in Revich et al. (2001) probably needs to be followed up with better-designed studies. There is new evidence of increased risk of breast cancer associated with increased concentrations of PCB congeners with dioxin-like activity (Aronson et al., 2000; Demers et al., 2002) and with increased TCDD concentrations in the Seveso cohort (Warner et al., 2002). But PCB congeners 105, 118, and 156 also have non-dioxin-like components, and the observed effects may be attributable to those components. Moreover, the results of Warner et al., (2002) are based on only 15 cases. Therefore, on the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is still inadequate or insufficient evidence to determine whether there is an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and risk of breast cancer.

Biologic Plausibility

All experimental evidence indicates that 2,4-D, 2,4,5-T, and TCDD are not genotoxic or at most are weakly genotoxic. However, TCDD has been demonstrated to be a carcinogen in animals and is classified as a human carcinogen because of its ability to act as a strong tumor promoter. The promoting activity may take place by a number of biochemical mechanisms, including the altered expression of genes involved in tissue differentiation and the increase in enzymes responsible for the metabolic activation of procarcinogens to metabolites that are themselves genotoxic. The AhR, which mediates the actions of TCDD, is present in animal and human breast tissue and some evidence suggests that it is necessary for the normal development of said tissue. One study observed that activation of the AhR pathway and metabolism of benzo[a]pyrene, a constituent of tobacco smoke, are necessary for the repression of the BRCA-1 gene by this chemical. Repression of the gene is thought to be a predisposing event in the onset of sporadic breast cancer. Other studies have shown that TCDD includes the c-myc promoter and the production of TGF-α, and these may modulate the proliferation and tumorigenesis of mammary cells. Lifetime exposure to estrogen is a risk factor for human breast cancer, and under some conditions TCDD may have antiestrogenic properties. However, some studies suggest that TCDD exposure may facilitate the transition of breast-cancer cells from estrogen dependence to estrogen independence; this has been demonstrated to be one key step in the progression of breast cancer. In addition, studies have shown that prenatal exposure to TCDD increases the number of mammary tumors induced by other chemicals. Thus, experimental data indicate biologic plausibility of an association between exposure to TCDD and TCDD-containing herbicides and breast cancer. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

There are no data on which to base a conclusion concerning whether Vietnam veterans are at increased risk for breast cancer because of exposure to herbicides or TCDD.

CANCERS OF THE FEMALE REPRODUCTIVE SYSTEM

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). It also presents statistics on other cancers of the female reproductive system. ACS estimates of the numbers of new female reproductive system cancers in the United States in 2002 are presented in Table 6-27 (ACS,

TABLE 6-27 Estimates of the Numbers of New Cancers of Female Reproductive System in United States[a]

Site	New Cases	Deaths
Cervix	13,000	4,100
Endometrium	39,300	6,600
Ovary	23,300	13,900
Other genital organs	2,000	800

[a]ACS, 2002.

2002). Taken together, the numbers represent roughly 6% of new cancer diagnoses and 5% of cancer deaths in women.

Incidence patterns of and risk factors for these diseases vary (see Table 6-28). Cervical cancer occurs more often in black women than in whites, whereas whites are more likely to develop endometrial and ovarian cancers. The incidence of endometrial and ovarian cancer also depends on age, with older women at greater risk. 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 cancer.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and female reproductive cancers. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Tables 6-29, 6-30, and 6-31 for summaries of the studies).

TABLE 6-28 Average Annual Incidence (per 100,000) of Female Genital System Cancers in United States[a]

	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	16.5	14.8	24.6	14.7	12.8	18.0	14.3	11.7	25.6
Endometrium	23.9	24.1	10.3	50.6	53.4	26.3	69.7	74.5	41.1
Ovary	21.8	22.9	14.3	30.8	33.0	17.1	35.8	38.4	22.6
Other genital organs	3.7	3.6	3.6	4.9	5.2	3.4	5.9	6.0	6.0
Overall	65.8	65.4	52.8	101.0	104.3	64.8	125.6	130.6	95.4

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

TABLE 6-29 Selected Epidemiologic Studies—Cervical Cancers

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
Kogevinas et al., 1997	IARC cohort	0	0 (0.0–3.8)
Studies Reviewed in *Update 1996*			
Blair et al., 1993	US farmers in 23 states		
	Whites	6	0.9 (0.3–2.0)
	Nonwhites	21	2.0 (0.3–3.1)
Lynge, 1993	Danish female production workers	7	3.2 (1.3–6.6)
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 (0.4–0.8)[b]
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of cancers of the cervix		20.7 in Chapaevsk; 11.7 in Samara region[c]
	Mortality standardized to Samara region	13	1.8 (1.0–3.1)
VIETNAM VETERANS			
New Studies			
Kang et al., 2000	Female Vietnam veterans	57	1.1 (0.7–1.7)
Studies Reviewed in *Update 2000*			
CDVA, 1998b	Australian Vietnam veterans—female	8[b]	1 expected (0–5)

[a] Given when available.
[b] 99% CI.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[d] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have cancer of the cervix?"
* Information not provided by study authors.
ABBREVIATIONS: CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer.

TABLE 6-30 Selected Epidemiologic Studies—Uterine Cancers

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
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	US 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 (0.4–0.8)[b]
ENVIRONMENTAL			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B females	2	0.5 (0.1–2.1)
Weiderpass et al., 2000	Swedish females	154	1.0 (0.6–2.0)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B females	1	0.3 (0.0–2.4)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B females	1	0.3 (0.0–1.9)
	Zone R females	27	1.1 (0.8–1.7)
VIETNAM VETERANS			
New Studies			
Kang et al., 2000	Female Vietnam veterans	41	1.0 (0.6–1.6)
Studies Reviewed in *Update 2000*			
CDVA, 1998b	Australian Vietnam veterans—female	4[c]	1 expected (0–5)
Studies Reviewed in *Update 1996*			
Dalager et al., 1995	Women Vietnam veterans	4	2.1 (0.6–5.4)

[a] Given when available.

[b] 99% CI.

[c] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have uterine cancer?"

* Information not provided by study authors.

ABBREVIATIONS: CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer.

TABLE 6-31 Selected Epidemiologic Studies—Ovarian Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
Kogevinas et al., 1997	IARC cohort	0	0 (0.0–2.6)
Studies Reviewed in *Update 1996*			
Kogevinas et al., 1993	IARC cohort	1	0.7 (*)
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			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A females	1	1.6 (0.2–11.2)
	Zone B females	2	0.5 (0.1–2.0)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	1	2.3 (0.3–16.5)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone A females	1	2.3 (0.0–12.8)
	Zone R females	21	1.0 (0.6–1.6)
VIETNAM VETERANS			
New Studies			
Kang et al., 2000	Female Vietnam veterans	16	1.8 (0.7–4.6)
Studies Reviewed in *Update 2000*			
CDVA, 1998b	Australian Vietnam veterans—female	1[b]	0 expected (0–4)

[a] Given when available.

[b] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have ovarian cancer?"

* Information not provided by study authors.

ABBREVIATIONS: CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer.

Update of the Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2000*.

Environmental Studies

An investigation of female reproductive cancers and dioxin exposure was conducted in Chapaevsk, a town in Russia where dioxin contamination of air, water, cow's milk, and human serum and breast milk has been documented, with clear declines in exposure with increasing distance from the factory. Cancer mortality in Chapaevsk in 1995–1998 was compared with that in the surrounding Samara region. The SMR for cervical cancer was 1.8 (95% CI 1.0–3.1) on the basis of 13 cases. Age-standardized cervical-cancer incidences in Chapaevsk were 1.6 times higher than those in Russia at large and 1.8 times higher than those in Samara at large. To the extent that Russian mortality and morbidity data are complete and valid, the risk of cervical cancer appears to be increased, but the number of cases on which the rates were calculated was not provided and may have been small. Incidence and mortality data for a longer period would be more informative. Moreover, a comparison of demographic and socioeconomic descriptors of the Chapaevsk residents and those in surrounding Samara or in Russia at large would aid in the interpretation of health and mortality differences.

Vietnam-Veteran Studies

Kang et al. (2000) report on gynecologic cancers among female Vietnam veterans. Army veterans were identified from a list obtained by the US Army and Joint Services Environmental Support group. The Air Force, Navy, and Marine Corps provided computerized lists. Military-service data were abstracted from personnel records. After record review, 4,643 women met the eligibility criteria of having had a permanent tour of duty from July 4, 1965, through March 28, 1973, a period of substantial US military involvement in Vietnam. Of those, 4,390 were found to be alive as of January 1, 1992. A comparison group of female veterans whose tour of duty did not include service in Vietnam but who were assigned to a military unit in the United States during the Vietnam War was identified, and 4,390 people were randomly selected from among those still alive on January 1, 1992. After exclusion of 250 from each group who participated in a pilot study, an attempt was made to locate the remaining 4,140 in each group, for a total eligible cohort of 8,280. Various location strategies were used, and fewer than 5% (370) were not located; another 339 were deceased. A full telephone interview was conducted on 6,430, after 775 refused (13% of Vietnam veterans and 17% of non-Vietnam veterans) and another 336 completed only a short written questionnaire. The questionnaire collected information on demographic background, lifestyle factors, reproductive history, military experience, use of oral contraceptives and hormone replacement therapies, and health status. A self-reported history of gynecologic cancers (defined by the authors as cancers of the breast, ovary, uterus, and cervix) was collected. The authors attempted to "retrieve hospital records on all reported cancers as far back as 30 years." Of

records successfully found, 99% of the breast cancers were confirmed and overall 90% of cancers were confirmed. The authors did not provide data on validation of the three sites other than breast, but stated that Vietnam status was not associated with verification of outcome.

A description of the two cohorts shows that the age distribution of the Vietnam veteran group has more older women than the non-Vietnam veteran group. The racial distribution was similar in the two, but the branch and duration of military service differed. Logistic-regression models indicated no significant excess of the four cancer sites combined (OR = 1.1, 95% CI 0.9–1.4) or for any single site: breast cancer OR = 1.2 (95% CI 0.9–1.5); ovarian cancer OR = 1.8 (95% CI 0.7–4.6); uterine cancer OR = 1.0 (95% CI 0.6–1.6); and cervical cancer OR = 1.1 (95% CI 0.7–1.7). Those ORs were adjusted for age, race, branch of service, pay grade, marital status, nursing occupation, smoking, drinking, family history of cancer, use of birth-control pills, and postmenopausal estrogen and progestin use. However, given that the two groups differed in their age distribution, standard adjustment might be inadequate to control for confounding. A survival analysis with age as the time scale and with age at diagnosis, rather than at interview, would be the most appropriate way to analyze these cohorts. The direction of bias induced by the analytic strategy used by the authors could be toward the null.

The study has several strengths, including the success in locating over 95% of the cohort about 25 years since the Vietnam War and the high response rate. The grouping of breast cancer with cervical, uterine, and ovarian cancer is not justified, inasmuch as some risk factors have opposite effects in the breast from those in the other organs, but the analysis by organ site remains valid. Although it is not a large study, the numbers are adequate for most of the outcomes. Assuming that there is no residual confounding by age, this investigation provides evidence that service in Vietnam does not substantially increase the risk of uterine or cervical cancer. The increased risk of ovarian cancer, although not significant, may be of concern in that it could be downwardly biased because of deficiencies in the statistical analysis. It should also be noted that the report made no attempt to examine exposures to herbicides or TCDD in Vietnam. An analysis linking the locations of military service of these Vietnam veterans to spraying missions may make it possible to evaluate Agent Orange exposure in relation to the outcomes.

Synthesis

The study of female Vietnam veterans provides some evidence that female reproductive cancers—namely, neoplasms of the cervix, uterus, and ovary—are not increased in this cohort. Because service in Vietnam may not be a good surrogate for exposures to herbicides or their contaminants, however, the results cannot be interpreted as evidence of no effect of these chemicals on gynecologic

cancers. Interpretation of the Russian study, in which TCDD exposures were clearly increased, is difficult because the potential for confounding by socioeconomic factors was not addressed, temporal patterns were not presented, and the number of cases of cervical cancer appeared to have been small. Overall, no strong studies addressing female reproductive cancers in relation to herbicides or their contaminants have been conducted since *Update 2000*.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and uterine, ovarian, or cervical cancer.

Biologic Plausibility

No animal studies have found an increased incidence of female reproductive cancer after exposure to the chemicals of interest. One study (Kociba et al., 1978), however, has found a reduced incidence of uterine tumors in rats fed TCDD at 0.1 mg/kg diet for 2 years. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The sparse data on increased risk of female reproductive cancers in Vietnam veterans come from an Australian study. Although the proportion of women with uterine and cervical cancers was higher than expected, the small number of cases and the possibility of confounding by marital status preclude drawing definitive conclusions. Furthermore, a study in female US Vietnam veterans did not indicate any such increased risk.

PROSTATE CANCER

According to ACS estimates, 189,000 new cases of prostate cancer (ICD-9 185) will be diagnosed in the United States in 2000, and 30,200 men will die from the disease (ACS, 2002). That makes prostate cancer the second-most common cancer among men (after nonmelanocytic skin cancers). Among men, it is expected to account for about 29% of new cancer diagnoses and 11% of cancer

TABLE 6-32 Average Annual Incidence (per 100,000) of Prostate Cancer in United States[a]

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
32.0	28.3	79.2	127.5	120.9	254.3	310.2	299.9	573.4

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

deaths in 2002. The average annual incidence of prostate cancer is shown in Table 6-32.

The incidence of prostate cancer varies dramatically with age and race. The risk increases by a factor of 4 between the ages of 45–49 years and 50–54 years, and nearly doubles between the ages of 50–54 years and 55–59 years. As a group, American black men have the highest recorded incidence of prostate cancer in the world (Miller et al., 1996). Their risk is roughly 2 times that of whites in the United States, 5 times that of Alaskan natives, and nearly 8.5 times that of 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.

The study of the incidence of and mortality from prostate cancer is complicated by trends in screening for the disease. The recent introduction and widespread adoption of prostate-specific antigen (PSA) for screening purposes have led to increased reports of incidence in the United States because of improved detection. The long-term impact of screening on incidence and mortality, however, is difficult to predict for any country or population and will depend on the rapidity with which the screening tool is adopted, its differential use in men of various ages, and the aggressiveness of tumors detected early with this test (Gann, 1997). Differences among countries in the rate of use of PSA could cause more variability in the results of studies in different countries.

Prostate cancer tends not to be fatal in the overwhelming majority of cases, so studies of mortality may be unable to detect an increased incidence of the disease. Findings showing an association between an exposure and prostate cancer mortality should be examined closely to determine whether the exposed group might have had poorer access to treatments that would decrease the likelihood of death.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was limited or suggestive evidence to determine whether an association exists between exposure to the

chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and prostate cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Table 6-33 provides summaries of the results of the studies underlying the finding. It is important to note that the table contains both morbidity and mortality studies. As discussed previously, the type of study must be taken into consideration when interpreting and weighing the evidence and, therefore, simply examining all the estimated risks in the table together will not provide a good assessment of the risks.

Update of the Scientific Literature

In an occupational study, Burns et al. (2001) updated data on mortality in the Dow Chemical Company cohort, extending follow-up to 1994. Further details about methods of data collection and exposure assignment are provided in Chapter 5. During the entire follow-up of 1,517 men, seven deaths due to prostate cancer occurred; 5.2 were expected. The SMR of 1.3 had a wide confidence interval (0.5–2.8). Studies of mortality are problematic when a disease is not necessarily fatal and multiple factors related to diagnosis and treatment may influence survival. Often, those factors include socioeconomic status, education, and access to health care (including health-insurance status and coverage), distance to medical facilities, and individual health-care seeking behavior. As discussed above, those issues are especially problematic when studying prostate cancer.

A cohort of lumberjacks in Sweden was examined for mortality and for incidence of various cancers. The cohort consisted of males and females employed by a forestry company at any time from 1954 to 1967. The pay slips of the company included information about the number of working hours or days in different work tasks and were used to construct a measure of exposure to phenoxy herbicides. The cohort was divided into two exposure groups, one with more than 5 working days of phenoxy-herbicide exposure and the other with 5 days or less. The authors excluded those exposed to pesticides other than phenoxy herbicides or DDT. They excluded nonexposed women because there were few in this group; exposed women were included. Data for foremen were analyzed separately. The cohort consisted of 261 exposed and 243 nonexposed persons. The mean exposure time among exposed workers was 30 days (range, 6–114 days); foremen had a mean exposure time of 176 days. Follow-up was from 1954 through 1994 for mortality and from 1958 through 1992 for incidence. The number of person-years of follow-up was not provided. SMRs and SIRs were calculated from death and cancer registration information to derive expected rates. Two cases of prostate cancer occurred among 15 foremen, whereas 0.4 was expected (for a highly unstable SIR of 4.7). Among the 139 other exposed workers, three cases occurred, whereas 3.5 were expected. Given the small sample,

TABLE 6-33 Selected Epidemiologic Studies—Prostate Cancer Morbidity and Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	7	SMR = 1.3 (0.5–2.8)
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides		
	Foremen	2	SIR = 4.7 (*)
	Male lumberjacks	3	SIR = 0.9 (*)
Studies Reviewed in *Update 2000*			
Sharma-Wagner et al., 2000	Swedish citizens		
	Agriculture and stock raising	6,080	1.1 (1.0–1.1)
	Farmers, foresters, and gardeners	5,219	1.1 (1.0–1.1)
	Paper mill workers	304	0.9 (0.8–1.0)
	Pulp grinding	39	1.4 (1.0–1.9)
Fleming et al., 1999a	Florida pesticide appliers	353	1.9 (1.7–2.1)
Fleming et al., 1999b	Florida pesticide appliers	64	2.4 (1.8–3.0)
Steenland et al., 1999	NIOSH cohort	28	1.2 (0.8–1.7)
Dich and Wiklund, 1998	Swedish pesticide appliers	401	1.1 (1.0–1.2)
	Born 1935 or later	7	2.0 (0.8–4.2)
	Born before 1935	394	1.1 (1.0–1.2)
Studies Reviewed in *Update 1998*			
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 from 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 Rafnsson, 1996	Icelandic pesticide users	10	0.7 (0.3–1.2)
Studies Reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide appliers	5	0.8 (0.3–1.8)
Blair et al., 1993	US 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, 45–69 years old, 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 ($p < 0.05$)
Swaen et al., 1992	Dutch herbicide appliers	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)

continues

TABLE 6-33 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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 appliers	2	0.5 (*)
Burmeister et al., 1983	Iowa residents	4,827	1.2 ($p < 0.05$)
Wiklund, 1983	Swedish agricultural workers	3,890	1.0 (0.9–1.0)[b]
Burmeister, 1981	Iowa farmers	1,138	1.1 ($p < 0.01$)
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of prostate cancer		7.0 in Chapaevsk; 22.0 in Samara region[c]
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	8	1.2 (0.6–2.4)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	6	1.2 (0.6–2.8)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	6	1.2 (0.5–2.7)
	Zone R males	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 residents—10-year follow-up—morbidity		
	Zone R males	16	0.9 (0.5–1.5)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	4	1.4 (0.5–3.9)

TABLE 6-33 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zones A, B, R males	19	1.6 (1.0–2.7)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B males	3	2.2 (0.7–6.9)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	26	0.7 (0.4–1.3)
AIHW, 1999	Australian Vietnam veterans—male	212	147 expected (123–171)
CDVA, 1998a	Australian Vietnam veterans—male	428[c]	147 expected (123–171)
Studies Reviewed in *Update 1998*			
Clapp, 1997	Massachusetts Vietnam veterans		
	Exposed cancers	15	0.8 (0.4–1.6)
Crane et al., 1997a	Australian military Vietnam 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	Air Force Ranch Hand veterans	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% CI.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
[d] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have prostate cancer?"
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; USDA, US Department of Agriculture.

one might wish to combine the two exposed groups (foreman and other workers); in this case, the SIR would be 1.3. In contrast, the nonexposed lumberjacks experienced a deficit of cases: 4, whereas 9.4 were expected, for a SIR of 0.4 (0.1–1.1).

Environmental Studies

Revich et al. (2001) have reported on the incidence of cancer and other health conditions in Chapaevsk, a city in the Samara region of Russia. A variety of industries are located in Chapaevsk, and one major chemical plant appears to be responsible for dioxin contamination that has been measured in the air, water, and soil of the city. Incidence rates have been generated for Chapaevsk, the Samara region, and Russia during 1998. The age-adjusted incidence of prostate cancer for this year was markedly lower in Chapaevsk (7.0 per 100,000) than in the Samara region and Russia (22.0 and 19.6 per 100,000, respectively). No information was provided on the number of cases in the calculations of indicence or whether geographic differences in incidence were also observed in previous years. There was no discussion of the differences between Chapaevsk, the Samara region, and Russia in potential confounders or the completeness of cancer registration.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

As for previous updates, the new data are somewhat equivocal. The two recent occupational studies have rather small numbers of cases. The Dow Chemical Company cohort results show a small increase in risk of prostate cancer. Given the size of the study, the finding does not attain statistical significance, although it is quite consistent with results from many previous studies of exposure to TCDD. Inasmuch as the cohort included herbicide exposure, any observed association could be related to 2,4-D rather than to TCDD. Whether the exposed population had better access to care than the general population, which might reduce their mortality, should also be considered. Furthermore, factors that increase incidence might differ from those related to mortality among those with the disease. Screening with PSA results in earlier detection and may therefore reduce mortality. Differences across populations in the rate of use of PSA could alter the relationship between exposure and mortality from prostate cancer.

In contrast, the study of Swedish lumberjacks does address incidence but, given the study size, yields only weak evidence that those with the highest expo-

sures to phenoxy herbicides may have an increased risk of prostate cancer. No interpretaion of the data on Chapaevsk is possible without additional information on the precision of the differences in incidence rates, the stability of these differences over time, and the possible role of confounding and incomplete ascertainment of cases. Conclusions with regard to exposures to herbicides or their contaminants rely primarily on earlier studies of incidence.

Prostate cancer is a common condition in older men, so it is likely that multiple factors are responsible for it and unlikely that herbicide exposure has a major role. Still, even a small relative risk can mean a large number of cases. Therefore, if the observed increase of 13% in incidence among Swedish pesticide appliers were due solely to exposure to the pesticides or TCDD, it could translate into many cases. Generally speaking, for common conditions, such as prostate cancer and cardiovascular disease, relative risks are not expected to be high for any particular causative factor, because the background rates are already high; this situation is in contrast with that of rare diseases, for which one tends to observe higher RRs.

Although the data are generally mixed, it should be kept in mind that most Vietnam veterans have not yet reached the age when prostate cancer tends to appear and that morbidity is likely to represent a more sensitive outcome than mortality for this site of cancer.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and prostate cancer. Although the associations are not large, a number of studies provide evidence suggestive of a small increase in morbidity or mortality from prostate cancer. The evidence regarding association is drawn from occupational studies in which subjects were exposed to a variety of pesticides, herbicides, and herbicide components and from studies of Vietnam veterans.

Biologic Plausibility

No animal studies have found an increased incidence of prostate cancer after exposure to the chemicals of interest. The plausibility of a causal relationship could be argued on the basis that the prostate is hormonally responsive and that TCDD has been shown to be an endocrine disruptor, that is, a chemical that alters the production or metabolism of hormones. Data on the effect of TCDD on

hormone concentrations in occupationally exposed men are therefore relevant. Sweeney et al. (1997/98) examined 281 workers at two production facilities from the National Institute for Occupational Safety and Health (NIOSH) study and found a trend toward higher serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and a trend toward lower testosterone according to the serum concentration of lipid-adjusted 2,3,7,8-TCDD. Those results were seen in models adjusted for age, alcohol, smoking, and diabetes mellitus; the models for LH and testosterone were also adjusted for BMI. The data suggest that exposures of workers to TCDD, particularly above 20 picograms/gram (pg/g) serum lipids, are associated with alterations in male reproductive hormone concentrations. That the prostate may be a target organ for hormonally active xenobiotics lends biologic plausibility to an association with TCDD exposure. In addition, several studies have shown human prostate cell to be directly responsive to TCDD in terms of enzyme induction.

A summary of the biologic plausibility for the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The studies that have been conducted in Vietnam veterans have a low likelihood of detecting an increased risk of prostate cancer, if service in Vietnam is actually associated with this cancer, because of weak study design and the relative youth of Vietnam veterans. Continued follow-up of the Ranch Hand cohort for both biologic monitoring of PSA and verification of prostate-cancer incidence will be important for determining prostate-cancer risk. The statistically significant increase in prostate-cancer SMRs for Australian Vietnam veterans suggests that US 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.

TESTICULAR CANCER

ACS estimates that 7,500 men will be diagnosed with testicular cancer (ICD-9 186.0–186.9) in the United States in 2002 and that 400 men will die from it (ACS, 2002). The average annual incidence of testicular cancer is shown in Table 6-34.

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 4 times that for black men. Cryptorchidism, or undescended testes, 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 results of research regarding them are inconsistent (Bosl and Motzer, 1997).

TABLE 6-34 Average Annual Incidence (per 100,000) of Testicular Cancer in United States[a]

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
6.1	7.0	1.6	3.6	4.0	1.0	2.1	2.4	0.9

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and testicular cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-35 for a summary of studies).

Update of the Scientific Literature

In an update of a study of mortality in chemical workers potentially exposed to 2,4-D at Dow Chemical Company in 1945–1994, Burns et al. (2001) identified one death from testicular cancer among 1,517 male Dow employees, for a relative risk of 2.2 (0.0–12.5). They conclude that there is no significant risk of testicular cancer in this cohort.

No relevant environmental or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

The updated occupational-study analysis at Dow Chemical Company provides no evidence to suggest that chronic herbicide exposure increases the risk of testicular carcinoma.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there

TABLE 6-35 Selected Epidemiologic Studies—Testicular Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	1	2.2 (0.0–12.5)
Studies Reviewed in *Update 2000*			
Fleming et al., 1999b	Florida pesticide appliers	23	2.5 (1.6–3.7)
Hardell et al., 1998	Workers exposed to herbicides	4	0.3 (0.1–1.0)
Studies Reviewed in *Update 1998*			
Hertzman et al., 1997	British Columbia sawmill workers		
	Mortality	116[b]	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., 1996	Pentachlorophenol production workers	0	—
Studies Reviewed in *Update 1996*			
Blair et al., 1993	US 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)[c]
ENVIRONMENTAL			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	1	0.5 (0.1–3.7)
	Zone B males	16	1.1 (0.7–1.8)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	10	1.0 (0.5–1.8)
	Zone R males	73	1.0 (0.8–1.3)
Studies Reviewed in *Update 1998*			
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—10-year follow-up—morbidity		
	Zone B males	1	1.0 (0.1–7.5)
	Zone R males	9	1.4 (0.7–3.0)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	1	0.9 (0.1–6.7)
	Zone R males	9	1.5 (0.7–3.0)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	3	—
AIHW, 1999	Australian Vietnam veterans—male	59	110 expected (89–131)

TABLE 6-35 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
CDVA, 1998a	Australian Vietnam veterans—male	151[d]	110 expected (89–131)
Studies Reviewed in *Update 1998*			
Clapp, 1997	Massachusetts Vietnam veterans—incidence	30	1.2 (0.4–3.3)
Crane et al., 1997a	Australian military Vietnam veterans	4	(NS)
Crane et al., 1997b	Australian national service Vietnam 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	114	1.1 (*)
	Vietnam service, Marines	28	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, DC, area hospitals		2.3 (1.0–5.5)
Watanabe et al., 1991	Army Vietnam veterans	109	1.2 (NS)
	Marine Vietnam veterans	28	0.8 (NS)
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., 1986a	Wisconsin Vietnam veterans	11	1.0 (0.5–1.7)
Anderson et al., 1986b	Wisconsin Vietnam veterans	9	1.0 (0.5–1.9)

[a] Given when available.
[b] "Male genital cancers".
[c] 99% CI.
[d] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have cancer of the testis?"
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NS, not significant.

is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and testicular cancer.

Biologic Plausibility

No animal studies have found an increased incidence of testicular cancer after exposure to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

There are insufficient data on testicular cancer in Vietnam veterans to draw a specific conclusion as to whether they are at increased risk.

URINARY BLADDER CANCER

Urinary bladder cancer (ICD-9 188.0–188.9) is the most common of the genitourinary tract cancers. According to ACS estimates, 41,500 men and 15,000 women will be diagnosed with this cancer in the United States in 2002, and 8,600 men and 4,000 women will die from it (ACS, 2002). In males, in whom this cancer is about 3 times as likely in females, those numbers represent about 6% of new cancer diagnoses and 3% of deaths. Overall, bladder cancer is the fifth most common cancer and the fifth leading cause of cancer death in the United States. The average annual incidence of urinary bladder cancer is shown in Table 6-36.

Among men in the age groups that characterize most Vietnam veterans, bladder-cancer incidence is about twice as high in whites as in blacks. Bladder-cancer incidence increases greatly with age over 40 years. For men in the age groups shown in Table 6-36, the incidence in each 5-year group is roughly double that in the age group before it.

The most important known risk factor for bladder cancer is smoking; about 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), polycyclic aromatic hydrocarbons (PAHs), and some other organic chemicals used in the rubber, leather, textile, paint products, and printing industries is associated with higher incidence. High-fat diets and exposure to the parasite *Schistosoma haematobium* have been implicated as risk factors. Exposure to inorganic arsenic is also a risk factor for bladder cancer, and cacodylic acid is a metabolite of inorganic arsenic. As discussed in Chapter 3, however, the data remain insufficient to conclude that studies of inorganic arsenic exposure are

TABLE 6-36 Average Annual Incidence (per 100,000) of Urinary Bladder Cancer in United States[a]

	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	13.5	14.7	10.1	26.4	28.9	14.5	51.0	55.4	29.0
Females	3.7	4.2	2.4	9.1	10.4	5.4	14.9	16.3	11.3

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

directly relevant to exposure to cacodylic acid. Therefore, the literature on inorganic arsenic is not considered in this section.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committees responsible for *VAO* and *Update 1996* found that there was limited or suggestive evidence of *no* association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and urinary bladder cancer. Additional information available to the committee responsible for *Update 1998* led it to change that conclusion to one of inadequate or insufficient information regarding an association. *Update 2000* did not change the 1998 conclusion (see Table 6-37 for a summary of the studies).

Update of Scientific Literature

Occupational Studies

Burns et al. (2001) updated a study of mortality in Dow chemical workers potentially exposed to 2,4-D in 1945–1994. Among 1,517 male employees, there was one death from bladder cancer (SMR = 0.5, 95% CI 0.1–2.8) for the entire follow-up period compared with an expected of two. The authors conclude that there is no evidence of causal association between exposure to 2,4-D and mortality due to bladder cancer.

Environmental Studies

Revich et al. (2001) report on suspected dioxin exposure and resulting cancer risk in Chapaevsk, which is located in the Samara region of Russia. This region is the site of large petrochemical plants that produce crop-protection chemicals and chemical fertilizers. Dioxins were detected in the air (0.116 pg/m^3), in the soil (8.9–298 ng/kg), in the town's drinking water (28.4–74.1 pg/L), and in cow's milk and human milk. Overall male cancer mortality in Chapaevsk is 1.2 times as high as in the Samara region and 1.3 times as high as in Russia. Thirty-one deaths due to cancers of the urinary organs were observed in men of Chapaevsk, for an SMR of 2.6 (1.7–3.6). The age-adjusted bladder-cancer incidence in men is about twice that of the Samara region in general. Age-adjusted incidence of bladder cancer was also elevated in women of Chapaevsk relative to women of the Samara region (3.9 vs 2.3/100,000), but there was no excess in mortality from cancer of the urinary organs in women (SMR = 0.8). No data were available to assess and correct for confounding by smoking and occupation.

TABLE 6-37 Selected Epidemiologic Studies—Urinary Bladder Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	1	0.5 (0.1–2.8)
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers		
	Total cohort	16	2.0 (1.1–3.2)
	High-exposure cohort	6	3.0 (1.4–8.5)
Hooiveld et al., 1998	Dutch male production and contract workers		
	Total cohort	4	3.7 (1.0–9.5)
	Accidentally exposed subcohort	1	2.8 (0.1–15.5)
Studies Reviewed in *Update 1998*			
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 appliers—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[b]	6.8 (3.9–11.1)
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed farmers	300	0.6 ($p < 0.05$)
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 (0.0–5.6)
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 or 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	— (0.0–7.2)
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 et al., 1983	Florida pesticide appliers	3	1.6 (*)
Burmeister, 1981	Farmers in Iowa	274	0.9 (NS)

TABLE 6-37 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of bladder cancer in males		40.2 in Chapaevsk; 19.8 in Samara region[c]
	Age-adjusted incidence (100,000) of bladder cancer in females		3.9 in Chapaevsk; 2.3 in Samara region[c]
	Mortality standardized to Samara region (Urinary organs)		
	Males	31	2.6 (1.7–3.6)
	Females	17	0.8 (0.5–1.3)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	1	1.7 (0.2–12.0)
	Zone B males	5	1.1 (0.5–2.8)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	147	0.8 (0.7–1.0)
	Males—counties with wheat acreage ≥111,000	129	0.9 (0.7–1.1)
	Females—counties with wheat acreage 23,000–110,999	67	1.1 (0.8–1.5)
	Females—counties with wheat acreage ≥111,000	59	1.1 (0.8–1.6)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A males	1	2.4 (0.3–16.8)
	Zone B males	3	0.9 (0.3–3.0)
	Zone R males	21	0.9 (0.6–1.5)
	Zone R females	4	0.6 (0.2–1.8)
Studies Reviewed in *Update 1998*			
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	5	1.3 (0.4–3.1)
	West coast	20	1.0 (0.6–1.6)
	Swedish fishermen—incidence		
	East coast	10	0.7 (0.4–1.3)
	West coast	55	0.9 (0.7–1.1)

continues

TABLE 6-37 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	10	1.6 (0.9–3.1)
	Zones A, B females	1	0.9 (0.1–6.8)
Lampi et al., 1992	Finnish community exposed to chlorophenols	14	1.0 (0.6–1.9)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	11	3.1 (0.9–11.0)
Studies Reviewed in *Update 1998*			
Clapp, 1997	Massachusetts Vietnam veterans	80	0.6 (0.2–1.3)
Crane et al., 1997a	Australian military Vietnam veterans	11	1.1 (0.6–2.0)
Crane et al., 1997b	Australian national service Vietnam 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.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

Vietnam-Veteran Studies

No relevant Vietnam veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

A new study of occupational exposure to herbicide production shows no relationship of chronic herbicide exposure to bladder-cancer mortality. An environmental study in Russia reported an increase in age-adjusted bladder-cancer incidence for men and women; however, the study does not control for occupation and smoking history, and there was no information on the number of cases

included in the analysis or the completeness of surveillance for cancer in Chapaevsk and the Samara region. The committee concludes that there is no evidence to support changing the "inadequate or insufficient" categorization for bladder cancer.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and urinary bladder cancer.

Biologic Plausibility

No studies have found an increased incidence of urinary bladder cancer in TCDD-treated animals. Cacodylic acid administered to laboratory animals has induced neoplasms of the urinary bladder. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Although the sparse data available on Vietnam veterans suggest that they may be at an increased risk for urinary bladder cancer, the estimated risk ratios are unstable. Further studies of Ranch Hand Air Force veterans may clarify whether exposure incurred in Vietnam, or exposure to TCDD in particular, is associated with altered risks of bladder cancer.

RENAL CANCER

Cancers of the kidney (ICD-9 189.0) and renal pelvis (ICD-9 189.1) are often grouped in epidemiologic studies; cancer of the ureter (ICD-9 189.2) is also sometimes included. Although diseases of those organs have different characteristics and may have different risk factors, there is logic to grouping them: the structures are all exposed to filterable compounds, such as PAHs, that appear in urine. ACS estimates that 19,100 men and 12,700 women will be diagnosed with renal cancers (ICD-9 189.0, 189.1) in the United States in 2002 and that 7,200 men and 4,400 women will die from them (ACS, 2002). These figures represent

TABLE 6-38 Average Annual Incidence (per 100,000) of Kidney and Renal Pelvis Cancer in United States[a]

	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	13.4	13.2	17.1	21.7	21.2	33.5	34.9	35.1	43.4
Females	6.0	5.8	8.1	9.8	9.6	15.1	16.1	16.5	22.6

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

2–3% of all new cancer diagnoses and deaths. The average annual incidence of renal cancer is shown in Table 6-38.

Renal cancer is twice as common in men as in women. In the age groups that represent most Vietnam veterans, black men have a higher incidence than white men. With the exception of Wilms' tumor (which is more likely to occur in children), renal cancer is more common in people over 50 years old.

Smoking is a well-established risk factor for renal cancer. Abuse of phenacetin-containing analgesics has also been implicated. People with some rare syndromes—notably, von Hippel-Lindau syndrome and tuberous sclerosis—are at higher risk. Other potential risk factors include diet, weight, and occupational exposure to asbestos and cadmium. Firefighters, who are routinely exposed to numerous pyrolysis products, make up a known higher-risk group.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and renal cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that conclusion (see Table 6-39 for a summary of the studies).

Update of the Scientific Literature

Occupational Studies

In an occupational study, Burns et al. (2001) updated the mortality of chemical workers potentially exposed to 2,4-D who worked at Dow Chemical Company in 1945–1994. In 1,517 male employees, there were two renal-cancer deaths

TABLE 6-39 Selected Epidemiologic Studies—Renal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	2	2.2 (0.1–3.3)
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	13	1.6 (0.8–2.7)
Hooiveld et al., 1998	Male Dutch production and contract workers		
	Total cohort—kidney cancer	4	4.1 (1.1–10.4)
	Total cohort—"urinary organs"	8	3.9 (1.7–7.6)
	Accidentally exposed subcohort	0	—
Studies Reviewed in *Update 1998*			
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	29	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	US 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 ($p < 0.05$)
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 et al., 1983	Florida pesticide appliers	1	0.5 (*)
Burmeister, 1981	Farmers in Iowa	178	1.1 (NS)

continues

TABLE 6-39 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) of kidney cancer in males		12.3 in Chapaevsk; 12.8 in Samara region[c]
	Age-adjusted incidence (100,000) of kidney cancer in females		6.1 in Chapaevsk; 7.3 in Samara region[c]
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	3	0.9 (0.3–3.0)
	Zone B females	3	2.1 (0.7–6.7)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	147	1.0 (0.8–1.2)
	Males—counties with wheat acreage ≥111,000	129	1.0 (0.8–1.3)
	Females—counties with wheat acreage 23,000–110,999	85	0.9 (0.7–1.2)
	Females—counties with wheat acreage ≥111,000	90	1.1 (0.8–1.4)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone R males	10	0.9 (0.4–1.7)
	Zone R females	7	1.2 (0.5–2.7)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	0	—
	Zones A, B females	1	1.1 (0.2–8.1)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	11	3.1 (0.9–11.0)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	22	1.2 (0.8–1.9)
Crane et al., 1997b	Australian national service Vietnam veterans	3	3.9 (*)
Studies Reviewed in *Update 1996*			
Visintainer et al., 1995	Michigan Vietnam veterans	21	1.4 (0.9–2.2)

TABLE 6-39 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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	—

[a] Given when available.
[b] 99% CI.
[c] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

(RR = 0.9) and 2.2 expected (95% CI 0.1–3.3). The authors conclude that there is no evidence of an association between exposure to 2,4-D and mortality due to renal cancer.

Environmental Studies

Revich et al. (2001) have evaluated the occurrence of cancer and other health outcomes in Chapaevsk, a city in the Samara region of Russia with documented levels of dioxin contamination in the air, soil, and water. The assessment of cancer in this study is based on comparisons with the Samara region and Russia; additional information on the design of this study and measurement of exposure can be found in Chapters 4 and 5 of this report. Age-adjusted incidence of renal cancer is reported for 1998; the levels of cancer in Chapaevsk and the Samara region are similar for men (12.3 vs 12.8 per 100,000) and for women (6.1 vs 7.3 per 100,000). The incidence rates of renal cancer in all of Russia for 1998 are slightly lower (11.8 per 100,000 for men, 5.8 per 100,000 for women). No information is given on the number of cases in the calculation of these incidence rates or the stability of these rates in previous years.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

There are no data to support changing the "insufficient or inadequate" categorization for renal cancer. The study of Dow Chemical workers has low precision due to the small number of deaths from renal cancer. The study of Chapaevsk does not report the number of cases included in the analysis or the completeness and accuracy of cancer surveillance, and does not consider confounders besides age. Accordingly, these studies offer very little evidence for the absence of an association.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and renal cancer.

Biologic Plausibility

No studies have found an increased incidence of renal cancer in TCDD-treated animals. Cacodylic acid administered to laboratory animals has induced neoplasms in the kidney. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The sparse data available on Vietnam veterans do not suggest that they are at an increased risk for renal cancer.

BRAIN TUMORS

According to ACS, about 9,600 men and 7,400 women will be diagnosed with new cases of brain and other nervous system cancers (ICD-9 191.0–191.9, 192.0–192.3, and 192.8–192.9) in the United States in 2002, and 7,200 men and 5,900 women will die from them (ACS, 2002). Those numbers represent about 1.4% of new cancer diagnoses and 2.4% of all cancer deaths. The average annual incidence of brain and other nervous system cancers is shown in Table 6-40.

Among people in the United States who are 45–59 years old, brain cancer is more common in men than in women and more common in whites than in blacks.

TABLE 6-40 Average Annual Incidence (per 100,000) of Brain and Other Nervous System Cancers in United States[a]

	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	7.1	4.9	11.0	12.1	6.1	13.4	14.7	10.3
Females	4.6	5.2	2.0	6.4	7.0	4.9	9.5	10.2	8.7

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

Exposure to ionizing radiation is an established risk factor for brain cancer. Several other potential factors have been examined, but ACS notes that most brain cancers are not associated with any known risk factors.

Cancers of and affecting the brain fall into numerous histologic types and subtypes. Meningiomas, which are cancers of the tissue surrounding the brain and spinal cord, do not arise from nerve tissue and do not share a similar risk profile with cancers that do. Metastases from cancers elsewhere in the body may be found in the brain and may be difficult to distinguish from primary brain cancers. Because of that diversity, the potential for misclassification, and the relative infrequency of brain cancers, this group of cancers has been difficult to study epidemiologically and to attribute to specific exposures.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was limited or suggestive evidence of *no* association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and brain cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that conclusion (see Table 6-41 for a summary of the studies).

Update of Scientific Literature

Two occupational studies published since *Update 2000* were located. A cohort of 1,517 male employees of the Dow Chemical Company who were involved in the manufacture or formulation of 2,4-D at some time in 1945–1994 demonstrated no excess mortality from brain cancer (SMR = 109) (Burns et al., 2001). However, the numbers in that study were small, and the study had limited power to detect an increase in an uncommon outcome.

A small study of 261 Swedish lumberjacks exposed to phenoxyacetic herbicides demonstrated no cases of brain cancer although one was seen among 241

TABLE 6-41 Selected Epidemiologic Studies—Brain Tumors

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	3	SMR = 109 (22–319)
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides	0	(*)
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	8	0.8 (0.4–1.6)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	4	0.9 (0.2–2.3)
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)
Becher et al., 1996	German chemical production workers—subcohort I	3	2.3 (0.5–6.8)
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 appliers	3	1.2 (0.3–3.6)
Dean, 1994	Irish farmers and farm workers		
	Males	195	—
	Females	72	—
Blair et al., 1993	US 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	Farmers in Canadian prairie province—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 appliers	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 or 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)

TABLE 6-41 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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 appliers	5	2.0 (*)
Burmeister, 1981	Farmers in Iowa	111	1.1 (NS)
ENVIRONMENTAL			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	1	0.5 (0.1–3.5)
	Zone B females	3	2.2 (0.7–7.0)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	131	0.9 (0.8–1.2)
	Males—counties with wheat acreage ≥111,000	130	1.1 (0.9–1.4)
	Females—counties with wheat acreage 23,000–110,999	94	1.0 (0.7–1.2)
	Females—counties with wheat acreage ≥111,000	95	1.2 (0.9–1.5)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	1	0.8 (0.1–5.5)
	Zone B females	3	3.2 (1.0–10.3)
	Zone R males	12	1.3 (0.7–2.5)
	Zone R females	8	1.1 (0.5–2.4)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	1	0.8 (0.0–4.2)
	Zone B females	3	3.2 (0.6–9.4)
	Zone R males	12	1.3 (0.7–2.3)
	Zone R females	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 residents—10-year follow-up—morbidity		
	Zone R males	6	0.6 (0.3–1.4)
	Zone R females	6	1.4 (0.6–3.4)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B females	1	1.5 (0.2–11.3)

continues

TABLE 6-41 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Bertazzi et al., 1989a	Seveso residents—10-year follow-up		
	Zones A, B, R males	5	1.2 (0.4–3.1)
	Zones A, B, R females	5	2.1 (0.8–5.9)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	1	—
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	39	1.1 (0.8–1.5)
Crane et al., 1997b	Australian national service Vietnam veterans	13	1.4
Dalager and Kang, 1997	Army Chemical Corps veterans	2	1.9[b] (—)
Studies Reviewed in *Update 1996*			
Dalager et al., 1995	Female 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 (NS)
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)

[a] Given when available.

[b] Crude rate ratio of Vietnam to non-Vietnam veterans.

* Information not provided by study authors.

— When information was denoted by a dash in the original study.

ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

nonexposed lumberjacks followed as a control group (Thörn et al., 2000). That study is much too small to derive stable estimates of risk that are generalizable to other populations.

No relevant environmental or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

Data from epidemiologic studies are inadequate or insufficient to link herbicide exposure to brain cancer; no new published information was found to change this opinion.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of *no* association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and brain cancer.

Biologic Plausibility

No new studies suggest that TCDD induces cancers of the brain. The evidence that exposure to 2,4-D in animals causes brain tumors remains questionable. In one study (Hazleton Laboratories America, 1986) in which Fischer 344 rats received 2,4-D at 45 mg/kg of body weight, six of 60 male rats developed brain tumors compared to one control rat. However, that study has been criticized for several reasons, and brain tumors have not occurred in other studies. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The sparse data available on Vietnam veterans do not suggest that they are at an increased risk for brain tumors.

NON-HODGKIN'S LYMPHOMA

Non-Hodgkin's lymphoma (ICD-9 200.0–200.8, 202.0–202.2, and 202.8–202.9) is the more common of the two primary types of cancer of the lymphatic system. ACS estimates that 28,200 men and 25,700 women will be diagnosed with this disease in the United States in 2002 and that 12,700 men and 11,700 women will die from it (ACS, 2002). 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. The average annual incidence of non-Hodgkin's lymphoma (NHL) is shown in Table 6-42.

NHL incidence is uniformly higher in males than in females and, in most age groups, higher in whites than in blacks. In the cohorts that characterize most Vietnam veterans, rates increase with age in whites and vary inconsistently in blacks.

The causes of NHL are poorly understood. People with suppressed or compromised immune systems are known to be at higher risk, and some studies show

TABLE 6-42 Average Annual Incidence (per 100,000) of Non-Hodgkin's Lymphoma in United States[a]

	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	20.2	20.1	26.2	28.8	29.1	33.8	37.4	39.3	33.6
Females	12.4	12.8	12.5	18.8	19.3	17.7	28.5	30.7	19.2

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

increased incidence in people with HIV, human T-cell lymphotropic virus, 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).

Although chronic lymphocytic leukemia shares many traits with NHL (immunohistochemical traits, B-cell origin, and progression to an acute aggressive form of NHL), it is discussed separately after the general section on leukemia.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was sufficient information to determine that an association exists between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and NHL. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that conclusion (see Table 6-43 for a summary of the studies).

Update of the Scientific Literature

Occupational Studies

Thörn et al. (2000) conducted an occupational cohort study of male and female lumberjacks employed in Sweden by a forestry company in 1954–1967. The company's pay slips included information about the number of working hours or days in different work tasks and were used to construct a measure of exposure to phenoxy herbicides. The cohort was divided into two exposure groups, one with more than 5 working days of phenoxy herbicide exposure and the other with 5 days or fewer. The authors excluded those exposed to pesticides other than phenoxy herbicides or DDT. They also excluded nonexposed women,

TABLE 6-43 Selected Epidemiologic Studies—Non-Hodgkin's Lymphoma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	3	SMR = 1.0
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides	2	2.3 (0.3–8.5)
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	12	1.1 (0.6–1.9)
Hooiveld et al., 1998	Dutch male production and contract workers	3	3.8 (0.8–11.0)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers		1.3 (0.3–3.3)
Keller-Byrne et al., 1997	Farmers in central United States		1.3 (1.2–1.6)
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	1.3 (0.9–1.8)
Becher et al., 1996	German chemical production workers	6	3.3 (1.2–7.1)
Nanni et al., 1996	Italian farming and animal-breeding workers	23[b]	1.8 (1.2–2.6)
Ramlow et al., 1996	Pentachlorophenol production workers	5[c]	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	10	1.9 (0.7–4.8)
	Exposed to TCDD	11	1.9 (0.7–5.1)
Asp et al., 1994	Finnish herbicide appliers	1	0.4 (0.0–2.0)
Dean, 1994	Irish farmers and farm workers		
	Males	244[b]	—
	Females	84[b]	—
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 three 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	US farmers from 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	10	2.3 (0.7–7.2)
	Occupation as lumberjack	9	6.0 (1.1–31.0)

continues

TABLE 6-43 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Zahm et al., 1993	Females in eastern Nebraska farms	119	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)
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 appliers	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	12	1.8 (0.7–4.1)
	USDA forest conservationists	10	2.5 (1.0–6.3)
Corrao et al., 1989	Italian farmers licensed to apply pesticides		
	Licensed pesticide users and nonusers	45[d]	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 appliers	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., 1988	Swedish agricultural and forestry workers		
	Workers in land or 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	33	1.0 (0.7–1.5)
	Fencing work	68	1.4 (1.0–2.0)
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 appliers	*	4.8 (1.2–19.4)

TABLE 6-43 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Hoar et al., 1986	Kansas residents		
	Farmers compared with nonfarmers	133	1.4 (0.9–2.1)
	Farmers using herbicides >20 days/year	7	6.0 (1.9–19.5)
Pearce et al., 1986	Male residents of New Zealand—agricultural sprayers	19[e]	1.5 (0.7–3.3)
Pearce et al., 1985	Male residents of New Zealand—agricultural occupations, 20–64 years old	224	1.4 (0.9–2.0)
Burmeister et al., 1983	Iowa residents		
	Farmers	1,101	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., 1982	Finnish herbicide appliers	0	—
Wiklund, 1983	Swedish agricultural workers	476	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)[d]
	Exposed to chlorophenols	50	4.3 (2.7–6.9)[d]
ENVIRONMENTAL			
New Studies			
Hardell et al., 2001	Case control study of NHL—TEQ >27.8 and EA >80	8	2.8 (0.5–1.8)
McDuffie et al., 2000	Case control study of NHL in Canada		
	Exposed to phenoxyherbicides	131	1.4 (1.1–1.8)
	2,4-D	111	1.3 (*)
	Mecoprop	53	2.3 (*)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A males	1	3.2 (0.4–23.0)
	Zone A females	1	3.3 (0.5–23.7)
	Zone B males	2	0.9 (0.2–3.8)
	Zone B females	3	1.6 (0.5–4.9)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	186	0.8 (0.7–1.0)
	Males—counties with wheat acreage ≥111,000	176	0.9 (0.8–1.1)

continues

TABLE 6-43 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Females—counties with wheat acreage 23,000–110,999	202	1.0 (0.8–1.2)
	Females—counties with wheat acreage ≥111,000	162	1.0 (0.8–1.2)
Viel et al., 2000	Residents near a French municipal solid-waste incinerator	286	1.3 (p = 0.00003)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	2	1.5 (0.4–6.0)
	Zone R males	10	1.1 (0.5–2.1)
	Zone R females	8	0.9 (0.4–1.8)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	2	1.5 (0.2–5.3)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone B males	3	2.3 (0.7–7.4)
	Zone B females	1	0.9 (0.1–6.4)
	Zone R males	12	1.3 (0.7–2.5)
	Zone R females	10	1.2 (0.6–2.3)
Studies Reviewed in *VAO*			
Lampi et al., 1992	Finnish community exposed to chlorophenols		
	Compared with two uncontaminated municipalities	16	2.8 (1.4–5.6)
	Compared with cancer-control region	16	2.1 (1.3–3.4)
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	3	1.9 (0.6–6.1)
	Zones A, B females	1	0.8 (0.1–5.5)
	Zone R males	13	1.4 (0.7–2.5)
	Zone R females	10	1.1 (0.6–2.2)
Bertazzi et al., 1989b	Seveso residents—10-year follow-up		
	Zone B females	2	1.0 (0.3–4.2)
	Zone R males	3	1.0 (0.3–3.4)
	Zone R females	4	1.6 (0.5–4.7)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	1	0.2 (0.0–2.6)
AIHW, 1999	Australian Vietnam veterans	62	48 expected (34–62)
CDVA, 1998a	Australian Vietnam veterans—male	137[f]	48 expected (34–62)
CDVA, 1998b	Australian Vietnam veterans—female	2[f]	0 expected (0–4)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans		1.3 (0.5–3.5)

TABLE 6-43 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Watanabe and Kang, 1996	Marine Vietnam veterans	46	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[g]	1.8 (*)
Thomas et al., 1991	Women Vietnam veterans	3	1.3 (0.3–1.8)
Watanabe et al., 1991	Army Vietnam veterans compared with Army non-Vietnam veterans	140	0.8 (*)
	Army Vietnam veterans compared with combined Army and Marine Vietnam-era veterans	140	0.9 (*)
	Marine Vietnam veterans compared with Marine non-Vietnam veterans	42	1.8 (1.3–2.4)
	Marine Vietnam veterans compared with combined Army and Marine Vietnam-era veterans	42	1.2 (*)
CDC, 1990	US men born 1921–1953		
	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 veteran mortality	0	(*)
Wolfe et al., 1990	Air Force Ranch Hand veteran 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	68	0.7 (0.5–0.9)
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., 1987	Australian Vietnam veterans	4	1.8 (0.4–8.0)
Anderson et al., 1986a	Wisconsin Vietnam veterans		
	Wisconsin Vietnam veterans compared with Wisconsin nonveterans	13	0.7 (—)
	Wisconsin Vietnam veterans compared with non-Vietnam-era veterans	13	0.6 (—)
	Wisconsin Vietnam veterans compared with Vietnam-era veterans	13	1.0 (—)
Anderson et al., 1986b	Wisconsin Vietnam veterans compared with general population	24	0.7 (—)
	Wisconsin Vietnam veterans compared with Wisconsin veterans	24	1.1 (—)

continues

TABLE 6-43 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Holmes et al., 1986	West Virginia Vietnam veterans compared with West Virginia Vietnam-era veterans	2	1.1 (*)
Lawrence et al., 1985	New York Vietnam veterans	10[d]	1.0 (0.4–2.2)

[a] Given when available.
[b] Includes NHL and chronic lymphocytic leukemia combined.
[c] Includes all lymphomas combined.
[d] Includes NHL and Hodgkin's disease.
[e] Only NHL other than lymphosarcoma and reticulosarcoma (ICD-9 202).
[f] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have NHL?"
[g] NHL, four living cases and three deaths originally listed in the CDC Vietnam Experience Study (Boyle et al., 1987).
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid, 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDC, Centers for Disease Control and Prevention; CDVA, Commonwealth Department of Veterans' Affairs; EA, Epstein-barr virus early antigen; IARC, International Agency for Research on Cancer; NHL, Non-Hodgkin's Lymphoma; SIR, standard incidence ratio; SMR, standardized mortality ratio; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, toxin equivalents; USDA, US Department of Agriculture.

because there were few in this group, but they included exposed women. The cohort consisted of 261 exposed and 243 nonexposed persons; the mean exposure time was 30 days (range, 6–114 days) among exposed workers and 176 days among foremen. Follow-up was from 1954 through 1994 for mortality and from 1958 through 1992 for incidence. The number of person-years of follow-up was not provided. SMRs and SIRs were calculated from death and cancer registration information to derive expected rates.

Of the three cases of NHL found, two were in the exposed group, for which the expected number of incident cases was 0.9, yielding an SIR of 2.3 (95% CI 0.3–8.5); and the third was among the nonexposed group, for which the expected number of cases was 1.2. The authors also noted rather high cancer incidence in the small group of highly exposed foremen (six of 15 persons). The strengths of this study were the detailed information on exposure to phenoxy herbicides, the nearly complete follow-up (98%) and the quality of the cancer registry in Sweden. Nevertheless, the sample size of this study was too small or the follow-up insufficient for evaluation of increased risks for such rare cancers as NHL. As in most occupational cohorts, data on nonworkplace confounders were not available.

An update on mortality in a cohort of 1,517 Dow Chemical Company employees was recently published (Burns et al., 2001), extending the follow-up to 1994. A job–exposure matrix assessing potential phenoxy herbicide exposure for 1945–1982 had been created by an industrial hygienist, with categories for time-weighted average concentrations of greater than 1.0 mg/m^3, 0.1–1.0 mg/m^3, and less than 0.1 mg/m^3. For follow-up from 1983 to 1994, a new category of "very low" was added for jobs that had nondetectable exposure-monitoring concentrations or in which less than 50% of time was spent in a low-exposure area. The limit of detection for that monitoring was not specified by the authors. A total of 495 workers worked for any period in either high-exposure or moderate-exposure jobs.

SMRs were calculated to compare the total male cohort with the US population. To control for the healthy-worker effect, comparisons were also made with an internal reference cohort of all other Dow employees at the Midland plant. For the exposed cohort, the total number of person-years of follow-up was 39,799, for an average of 26.2 years. For all sites of cancer, there were 83 deaths compared with 86.9 expected, for an SMR of 1.0 (95% CI 0.8–1.2). Three deaths due to NHL were observed, compared with the same number expected, for an SMR of 1.0. The internal comparisons yielded an adjusted RR of 2.6 (95% CI 0.9–8.3), reflecting a lower than expected mortality from NHL in the nonexposed cohort. The authors did note that two of the three deaths from NHL occurred in workers with moderate exposure; this suggested a dose-response relationship.

The finding of an increased RR from internal comparisons is interesting and raises several questions: Is there a healthy-worker effect for NHL? Why were the NHL deaths fewer than expected among those in the referent group at Dow Chemical Company? What exposures were prevalent in this referent group but not in the phenoxy-herbicide-exposed men? In any case, despite industrial-hygiene monitoring data that covered several decades, the study provides little information regarding phenoxy herbicides and their contaminants in relation to the risk of NHL. First, although larger than that in the study of Swedish lumberjacks, the cohort is small for detecting such rare outcomes as NHL. Second, as in most occupational mortality studies, lifestyle confounder information was lacking. Third, it is unclear whether exposure assignments were validated or even replicated by multiple hygienists. Fourth, mortality studies are problematic in that their outcome is influenced not just by the incidence of a disease but also by the stage at diagnosis and the treatment received.

Environmental Studies

In a small clinic-based case–control study of NHL, Hardell et al. (2001) collected adipose tissue for determination of TCDD and dibenzofuran exposure. The authors enrolled 33 cases of NHL and 39 patients undergoing surgery for benign lesions, from whom an adipose tissue sample was taken from the abdomi-

nal wall. One underlying concern in this study was whether Epstein-Barr virus (EBV), a risk factor for some subgroups of NHL, might predispose those with TCDD exposure to a higher risk, potentially resulting in a synergistic effect. EBV is a ubiquitous infection throughout the world, usually occurs initially during childhood, and is often subclinical. Growth transformation of B cells is induced by latent EBV infection. Antibodies to EBV nuclear antigens can be used to assess infection or the host's immunologic control of the infection. EBV titers were obtained for 23 cases and 32 controls. Organochlorines were measured with high-resolution gas chromatography and mass spectrometry, and lipids were measured gravimetrically. Body burdens were then expressed in picograms per gram of lipid, or parts per billion (ppb). Dioxin-like toxic equivalence was calculated to obtain the person's body burden of compounds operating through mechanisms similar to those of 2,3,7,8-TCDD.

The authors stated that "no significant differences were found between cases and controls." Although that statement is true for dioxins, controls actually had significantly higher concentrations of 1,2,3,7,8-pentachlorodibenzofuran and two other furans also were substantially higher in controls. All three of those congeners, however, were present at rather low concentrations, particularly relative to all polychlorinated dibenzo-*p*-dioxin (PCDD) concentrations, and possibly were in the range where laboratory quantitation may be unreliable (Willman et al., 2001). Cases and controls also differed little with respect to antibodies to EBV antigens. The interaction between higher TCDD or furan exposure and high titers for EBV early antigen (EA) IgG was analyzed. The study was too small to yield any definitive findings. Nevertheless, after stratification on NHL subtype, there is a clear association of EA titers greater than 80 with a higher risk for low-grade B-cell NHL, which is expressed at all levels of TCDD and furan exposure and is sometimes magnified at higher TCDD and furan exposure. Confidence intervals are extremely large (usually covering a factor of 80–100) because of the small numbers of cases and controls in all exposure groups. Furthermore, weight loss in people with NHL is a possible concern, in that it could result in at least a transient rise in concentrations of organochlorine compounds in circulating lipids. The authors attempted to address that concern by adjusting for BMI at the time of the adipose sampling and a year earlier. They do not indicate, however, whether BMI was measured or self-reported, particularly for the value a year earlier, and self-reported weight can be quite inaccurate (Villanueva, 2001). Although the hypothesis and findings are intriguing, this study is too small to shed much light on the risks of NHL posed by TCDD exposure.

A large population-based cross-Canada case–control study of NHL in relation to pesticides was recently reported (McDuffie et al., 2001). The report included the results of a multicenter study of incidence of NHL, soft-tissue sarcoma, Hodgkin's disease, and multiple myeloma among men 19 years old or older in six provinces. A pilot study was used to generate an efficient definition of pesticide exposure that distinguished incidental or bystander environmental

exposure from more intensive exposure and cases from controls. The cases and controls who participated in the pilot study were not included in the analysis reported from the full investigation. A validation study of the questionnaire for farmers was also conducted to evaluate concordance of self-reported agrochemical purchases with vendor and supplier records. Using an initial postal questionnaire, the authors obtained confounder data and some exposure history. A list of pesticides was mailed to those with pesticide exposure of 10 h/year or more, according to the screening questions, and for a 15% random sample of the remainder, and a telephone interview was conducted later to collect detailed exposure information. HIV-positive subjects and persons with Kaposi's sarcoma were excluded. A pathologist reviewed slides from cases to validate NHL; 84% (*N* = 436) were validated; tumor blocks could not be obtained for the others, for financial reasons. Controls were selected with age stratification from health-insurance records, computerized telephone listings, or voter lists. The final sample size was 517 cases of NHL and 1,506 controls.

On the basis of the postal questionnaire screening questions, the authors observed an OR, adjusted for age and province, of 1.2 for those with 10 h/year or more of exposure. Analysis of the detailed exposure data with unconditional logistic regression showed an adjusted OR of 1.4 (95% CI 1.1–1.8) for phenoxy herbicides, with 131 exposed cases. Two of four phenoxy herbicides (including any with 1% or more prevalence of exposure) also showed increased risks: OR = 1.3 for 2,4-D and 2.33 for mecoprop, with 111 and 53 exposed cases, respectively. The only other individual herbicides associated with NHL were the dicamba compounds (Banvel and Target), which showed an OR of 1.7 (95% CI 1.0–2.8) based on 26 cases exposed to one or the other. Those models were adjusted for age, province of residence, and several medical variables that significantly predicted either a reduced or increased NHL risk: history of measles, mumps, cancer, and allergy desensitization shots and a positive family history of NHL in a first-degree relative. Several classes of insecticides and fungicides also were associated with an increased NHL risk. In a model that contained multiple pesticides, mecoprop and aldrin were both associated with higher risk. When data on frequency of exposure were analyzed, individuals exposed more frequently to 2,4-D, or mecoprop did not show the highest risks for NHL.

That study provides fairly strong data supporting an association between NHL and pesticides. First, it is a large study—over 500 cases. Second, investigation of pesticides was the primary focus of the study; for this reason, the exposure data were obtained from detailed exposure histories and covered a wide array of occupational and environmental exposures. The authors seem to have paid meticulous attention to the development of the questionnaire, including modification of questionnaires used by others, pilot validation, a strategy of mailing a list of pesticides in advance of the interview, and a sequential approach from broad categories to major pesticide classes to chemical groups and finally to individual compounds. Third, cases were systematically validated. Nevertheless, the study

has several important weaknesses: low response rates (among those contacted, 67% of cases and 48% of controls); the lack of a clear dose–response relationship in connection with exposure days per year (this may be explained by the modest range of frequency of exposure and the lack of information on exposure magnitude or body burdens); the possibility of recall bias; the lack of examination of cell lines or histologic subtypes of NHL; and the inability to determine independent effects of individual herbicides or classes of herbicides in NHL etiology. As the authors warn, few men were exposed to only one pesticide or one class of pesticides. Still, the authors did show that the association of NHL with mecoprop is independent of that with aldrin. It is also possible that combinations of compounds, perhaps in multiple chemical classes, play a role. Despite those limitations, the study adds weight to previous findings in the scientific literature and suggests specific chemical compounds that might be responsible.

Veitnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

In previous reports, the evidence was found to be sufficient to support a conclusion of an association between NHL and exposures to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid). Most of the evidence suggests that 2,4-D or 2,4,5-T, rather than TCDD, is responsible for the associations observed in occupational cohorts. For instance, the main cohorts with TCDD exposure do not have increased rates of NHL. None of the five studies reviewed for this report provide strong evidence contradicting the previous finding of IOM committees. The two occupational studies are too small to be useful for evaluating the hypothesis of interest, and the same applies to the clinic-based case–control study of the interaction with Epstein-Barr virus. The exposure data from the childhood NHL study were inadequate. Finally, the large Canadian case–control study, despite some limitations, provided supportive evidence of an association between phenoxy herbicides and NHL.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is sufficient evidence to conclude that an association exists between exposure to

at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and NHL. 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

Increased rates of lymphoma have been reported to occur in female B6C3F mice exposed to TCDD at 1 mg/kg of body weight via gavage twice a week for 2 years (NTP, 1982). Other animal studies have not noted an increase in lymphoma in TCDD-exposed animals. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Analysis in the Ranch Hand study was limited by the small numbers of veterans with NHL, and no conclusions could be drawn. However, data from a survey study of male Vietnam veterans from Australia indicated a possibly increased risk of NHL.

HODGKIN'S DISEASE

Hodgkin's disease (HD) (ICD-9 201.0–201.9) is distinct from NHL in its cell of origin, demographics, and genetics. According to ACS estimates, 3,700 men and 3,300 women will be diagnosed with the disease in the United States in 2002, and 800 men and 600 women will die from it (ACS, 2002). The average annual incidence of Hodgkin's disease is shown in Table 6-44.

TABLE 6-44 Average Annual Incidence (per 100,000) of Hodgkin's Disease in United States[a]

	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	3.1	3.2	3.8	2.7	2.9	2.0	3.7	4.1	4.2
Females	1.9	2.0	2.0	2.2	2.3	3.4	2.1	2.2	1.8

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

HD is less common in people in the age groups that characterize most Vietnam veterans than in younger or older people. Among people over 40 years old, the incidence in males generally exceeds that in females, and the incidence in whites exceeds that in blacks. However, the very small numbers of cases indicate that care should be exercised in interpreting the figures.

The potential infectiousness of HD has been a topic of discussion since its earliest description. An increased incidence in people 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 people with suppressed or compromised immune systems.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was sufficient information to conclude that an association exists between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and HD. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-45 for summary of studies).

Update of the Scientific Literature

The update of the Dow Chemical Company cohort (Burns et al., 2001), which extended follow-up to 1994 for 1,517 workers, reported one case of HD; 0.6 case was expected (SMR = 1.5, 95% CI 0.04–8.6). No other studies of this outcome were found.

Synthesis

The relatively low incidence of HD complicates the evaluation of epidemiologic studies addressing this lymphoreticular tumor. Newly published studies report small numbers of cases and are imprecise although the pattern is one of excess risk in nearly all exposed study populations. However, earlier studies carried out in Sweden (for example, the work of Hardell and colleagues) were well conducted, were based on good exposure characterization, and have not been contradicted by later work. The committee believes that data available for review in this report, when combined with information available to previous *Veterans and Agent Orange* committees, demonstrate a pattern of increased mortality and morbidity risk. Although it has not been demonstrated as clearly as that related to NHL, a positive association between TCDD and the development of HD is biologically plausible because of their common lymphoreticular origin and common risk factors.

TABLE 6-45 Selected Epidemiologic Studies—Hodgkin's Disease

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	1	SMR = 1.5 (0.04–8.6)
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	3	1.1 (0.2–3.2)
Hooiveld et al., 1998	Dutch chemical production workers	1	3.2 (0.1–17.6)
Rix et al., 1998	Danish paper mill workers		
	Men	18	2.0 (1.2–3.2)
	Women	2	1.1 (0.1–3.8)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	1	0.7 (0.1–3.6)
Kogevinas et al., 1997	IARC cohort	10	1.0 (0.5–1.8)
Becher et al., 1996	German chemical production workers	0	—
Ramlow et al., 1996	Pentachlorophenol production workers	0	—
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 appliers	2	1.7 (0.2–6.0)
Blair et al., 1993	US 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)[b]
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 appliers	1	3.3 (0.04–18.6)
Fingerhut et al., 1991	NIOSH cohort	3	1.2 (0.3–3.5)
	20-year latency, 1+ years of exposure	1	2.8 (0.1–15.3)
Green, 1991	Ontario herbicide sprayers	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 or soil conservationists	4	2.2 (0.6–5.6)
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)

continues

TABLE 6-45 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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 appliers	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	1	2.7 (0.03–14.2)
Dubrow et al., 1988	Ohio farmers	3	2.7 (*)
Wiklund et al., 1988	Swedish agricultural and forestry workers		
	Workers in land or 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)	28	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, 20–64 years old	107	1.0 (0.6–2.0)
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., 1982	Finnish herbicide appliers	0	(*)
Wiklund, 1983	Swedish agricultural workers	226	1.0 (0.9–1.2)[c]
Burmeister, 1981	Farmers in Iowa	47	1.2 (NS)
Hardell et al., 1980	Umea Hospital patients		
	Exposed to phenoxy acids	41	4.8 (2.9–8.1)[d]
	Exposed to chlorophenols	50	4.3 (2.7–6.9)[d]
ENVIRONMENTAL			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	2	3.0 (0.7–12.4)
	Zone B females	2	4.3 (1.0–18.3)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	32	1.8 (1.1–2.9)
	Males—counties with wheat acreage ≥111,000	14	0.8 (0.4–1.5)
	Females—counties with wheat acreage 23,000–110,999	19	1.0 (0.6–1.9)

TABLE 6-45 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Females—counties with wheat acreage ≥111,000	14	0.9 (0.4–1.7)
Viel et al., 2000	Residents around a French municipal solid-waste incinerator	9	1.5 (NS)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	2	3.3 (0.8–14.0)
	Zone B females	2	6.5 (1.5–29.0)
	Zone R females	4	1.9 (0.6–5.8)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	2	3.3 (0.4–11.9)
	Zone B females	2	6.5 (0.7–23.5)
	Zone R females	4	1.9 (0.5–4.9)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone B males	1	1.7 (0.2–12.8)
	Zone B females	1	2.1 (0.3–15.7)
	Zone R males	4	1.1 (0.4–3.1)
	Zone R females	3	1.0 (0.3–3.2)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	1	0.3 (0.0–3.2)
Studies Reviewed in *Update 1998*			
Watanabe and Kang, 1996	Marine Vietnam veterans	25	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 with Army non-Vietnam veterans	116	1.0 (*)
	Marine Vietnam veterans compared with Marine non-Vietnam veterans	25	1.9 (*)
	Army Vietnam veterans compared with non-Vietnam veterans	116	1.1 (*)
	Marine Vietnam veterans compared with non-Vietnam veterans	25	1.0 (*)
CDC, 1990	US men born 1921–1953		
	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; Wolfe et al., 1990	Air Force Ranch Hand veteran mortality	0	—

continues

TABLE 6-45 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Breslin et al., 1988	Army Vietnam veterans compared with Vietnam-era Army veterans	92	1.2 (0.7–1.9)
	Marine Vietnam veterans compared with 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 with Wisconsin nonveterans	6	0.5 (0.2–1.2)
	Wisconsin Vietnam veterans compared with non-Vietnam-era veterans	6	1.0 (0.4–2.2)
	Wisconsin Vietnam veterans compared with 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] 90% CI.
[c] 99% CI.
[d] Includes both NHL and HD.
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; CDC, Centers for Disease Control and Prevention; IARC, International Agency for Research on Cancer; NHL, non-Hodgkin's lymphoma; NIOSH, National Institute for Occupational Safety and Health; PMR, proportionate-mortality ratio; USDA, US Department of Agriculture.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is sufficient evidence to conclude that an association exists between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and HD.

Biologic Plausibility

No animal studies have found an increased incidence of HD after exposure to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility. As mentioned previously, the common lymphoreticular origin and common risk factors between NHL and HD add to its biological plausibility.

Increased Risk of Disease Among Vietnam Veterans

The available data on HD in Vietnam veterans are too limited to form the basis of a conclusion regarding increased risk.

MULTIPLE MYELOMA

Multiple myeloma (MM) (ICD-9 203.0, 203.2–203.8) is characterized by the proliferation of bone marrow stem cells that results in an excess of neoplastic plasma cells and the production of excess abnormal proteins, usually fragments of immunoglobulins. ACS estimates that 7,800 men and 6,800 women in the United States will be diagnosed with this disease in 2002 and that 5,500 men and 5,300 women will die from it (ACS, 2002). The average annual incidence of MM is shown in Table 6-46.

MM incidence is highly age-dependent, with a relatively low rate in people under 40 years old and most cases occurring at the ages 55–70 years. Rates in blacks are at least twice those in whites. Incidence in males is slightly higher than in females, with the difference becoming much more pronounced with age.

An 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). People

TABLE 6-46 Average Annual Incidence (per 100,000) of Multiple Myeloma in United States[a]

	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	3.3	2.8	8.2	7.5	6.9	13.9	12.5	10.5	36.9
Females	2.8	2.4	6.4	4.7	4.0	13.4	7.6	6.5	18.5

[a]SEER nine standard registries, crude age-specific rates, 1995–1999.

with high exposure to ionizing radiation are also at greater risk. The evidence regarding other risk factors is mixed.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and MM. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-47 for summary of studies).

Update of the Scientific Literature

In an occupational study, mortality from multiple causes was analyzed in a cohort of 1,517 male workers involved in the manufacture or formulation of 2,4-D in 1945–1994 (Burns et al., 2001). Mortality in the cohort was compared with mortality for all white US males. (Further information about the design of the study, including the measurement of exposure and the collection and analysis of data, is provided in Chapter 4.) Only one death due to myeloma was observed in the cohort (SMR = 0.8, 95% CI 0.0–4.5). The value for the SMR was unchanged in an additional analysis that assumed an induction period of 20 years (SMR = 0.8).

Cancer incidence and mortality were assessed in a cohort of 504 forestry workers in Sweden employed in 1954–1967 (Thörn et al., 2000). The cohort included 261 workers exposed to phenoxy herbicides and 243 nonexposed workers. Data on cause-specific mortality were collected for the period 1954–1994, and cancer incidence was identified from the Swedish Cancer Register for the period 1958–1992. Expected rates of cancer were based on population data from Sweden (additional information about study design is summarized in Chapter 4). No cases of MM occurred in exposed workers, and one case occurred among the nonexposed (SIR = 1.5, 95% CI 0.0–8.6).

No relevant environmental or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

The new information on exposure to herbicides and the incidence of MM comes from two relatively small occupational cohort studies (Burns et al. 2001; Thörn et al. 2000). These studies have very limited power for the assessment of an association with MM. Accordingly, they yielded no suggestion of an increased risk of MM from exposure to herbicides—but also no evidence to dismiss the possibility of such a risk.

TABLE 6-47 Selected Epidemiologic Studies—Multiple Myeloma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers	1	SMR = 80 (2–446)
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides	0	—
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	10	2.1 (1.0–3.8)
Hooiveld et al., 1998	Dutch chemical production workers	0	0.0 (*)
Studies Reviewed in *Update 1998*			
Gambini et al., 1997	Italian rice growers	0	—
Kogevinas et al., 1997	IARC cohort		
	Workers exposed to TCDD (or higher-chlorinated dioxins)	9	1.2 (0.6–2.3)
	Workers not exposed to TCDD (or higher-chlorinated dioxins)	8	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 appliers	3	2.6 (0.5–7.7)
Dean, 1994	Irish farmers and farm workers	170	1.0 (*)
Semenciw et al., 1994	Farmers in Canadian prairie provinces	160	0.8 (0.7–1.0)
Blair et al., 1993	US 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 US states		
	White males	233	1.2 (*)
	White females	12	2.6 (*)
Brown et al., 1993	Iowa male users of pesticides or herbicides	111	1.2 (0.8–1.7)
Lynge, 1993	Danish production workers		
	Male	0	0
	Female	2	12.5 (1.5–45.1)
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 appliers	3	8.2 (1.6–23.8)

continues

TABLE 6-47 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Fingerhut et al., 1991	NIOSH cohort	5	1.6 (0.5–3.9)
	20-year latency, 1+ years of 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 or soil conservationists	6	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)
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 use	*	1.4 (0.8–2.3)
Burmeister et al., 1983	Iowa residents (farmers in counties with highest herbicide use)		
	Born 1890–1900	*	2.7 ($p < 0.05$)
	Born after 1900	*	2.4 ($p < 0.05$)
Riihimaki et al., 1982	Finnish herbicide appliers	1	2.5 (0.3–14.0)
ENVIRONMENTAL			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	1	0.7 (0.1–5.0)
	Zone B females	4	3.7 (1.3–10.2)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	108	1.0 (0.8–1.3)
	Males—counties with wheat acreage ≥111,000	75	0.8 (0.6–1.0)
	Females—counties with wheat acreage 23,000–110,999	91	1.0 (0.8–1.3)
	Females—counties with wheat acreage ≥111,000	77	1.0 (0.7–1.3)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	1	1.1 (0.2–8.2)
	Zone B females	4	6.6 (2.3–18.5)
	Zone R males	5	0.8 (0.3–2.0)
	Zone R females	5	1.0 (0.4–2.5)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B females	4	6.6 (1.8–16.8)

TABLE 6-47 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone B males	2	3.2 (0.8–13.3)
	Zone B females	2	5.3 (1.2–22.6)
	Zone R males	1	0.2 (0.0–1.6)
	Zone R females	2	0.6 (0.2–2.8)
Studies Reviewed in *VAO*			
Pesatori et al., 1992	Seveso residents		
	Zones A, B males	2	2.7 (0.6–11.3)
	Zones A, B females	2	4.4 (1.0–18.7)
	Zone R males	1	0.2 (0.0–1.5)
	Zone R females	3	0.9 (0.3–3.1)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	2	0.7 (0.1–5.0)
Studies Reviewed in *Update 1998*			
Crane et al., 1997a	Australian military Vietnam veterans	6	0.6 (0.2–1.4)
Crane et al., 1997b	Australian military Vietnam veterans	0	(*)
Watanabe and Kang, 1996	Army Vietnam veterans	36	0.9 (*)
	Marine Vietnam veterans	4	0.6 (*)
Studies Reviewed in *VAO*			
Breslin et al., 1988	Army Vietnam veterans	18	0.8 (0.2–2.5)
	Marine Vietnam veterans	2	0.5 (0.0–17.1)

[a] Given when available.

* Information not provided by study authors.

— When information was denoted by a dash in the original study.

ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; ACS, American Cancer Society; AFHS, Air Force Health Study; IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; SEER, Surveillance, Epidemiology, and End Results (SEER) Program; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and MM. The evidence regarding association is drawn from earlier occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

Biologic Plausibility

No animal studies have found an increased incidence of MM after exposure to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

There are insufficient data on MM in Vietnam veterans to draw a specific conclusion as to whether they are at increased risk.

LEUKEMIA

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 ACS estimates, 17,600 men and 13,200 women will be diagnosed with some form of the disease in the United States in 2002, and 12,100 men and 9,600 women will die from it (ACS, 2002). Collectively, leukemias were expected to account for 2.5% of all new cancer diagnoses and nearly 4% of cancer deaths in 2002. The different forms of leukemia have different patterns of incidence and in some cases different risk factors. The incidences of the various forms of leukemia are presented in Table 6-48.

Acute lymphocytic leukemia (ALL) is a disease of the young and of people over 70 years old, and it 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 blacks and in males than females. Exposure to high doses of ionizing radiation is a known risk factor for this form of leukemia; the evidence on other factors is inconsistent.

Acute myeloid leukemia (AML) is the most common acute leukemia among adults; its incidence increases steadily with age in people over 40 years old. In the Vietnam-veteran age groups, AML accounts for roughly one-fourth of cases of leukemia in men and one-third in women. Overall, AML is slightly more common in males than in females. White males have a higher incidence than white females; the lifetime incidence in black 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 (such as melphalan). Fanconi's anemia and Down syndrome are associated with an increased risk of AML, and tobacco-smoking has been suggested as a risk factor.

Chronic lymphocytic leukemia (CLL) is the most common of the four primary types of leukemia in men. Because CLL shares many traits with lymphomas

TABLE 6-48 Average Annual Incidence (per 100,000) of Leukemias in United States[a]

	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	8.0	8.1	7.5	14.1	14.7	12.5	19.4	19.9	14.9
Females	6.1	5.9	7.5	9.8	10.0	8.6	12.5	13.3	9.8
Acute Lymphocytic Leukemia									
Males	0.6	0.5	1.2	1.0	1.1	0.3	0.8	0.8	0.5
Females	0.4	0.4	0.4	0.7	0.4	0.3	0.7	0.7	0.8
Chronic Lymphocytic Leukemia									
Males	2.2	2.4	1.6	5.2	5.4	5.1	8.2	8.7	7.0
Females	1.2	1.2	1.2	2.7	2.8	2.9	3.9	4.4	1.9
Acute Myeloid Leukemia									
Males	2.2	2.1	2.1	3.8	4.0	3.0	4.7	4.9	2.3
Females	2.5	2.3	3.2	3.2	3.3	1.7	4.5	4.6	4.2
Chronic Myeloid Leukemia									
Males	1.3	1.3	1.4	1.8	1.8	3.4	2.7	2.6	2.8
Females	1.2	1.2	1.2	1.9	1.9	2.6	1.9	1.8	1.5
All Other Leukemia[b]									
Males	1.2	1.3	0.9	1.8	2.0	0.7	2.0	2.1	1.4
Females	0.7	0.7	1.0	0.7	0.7	0.9	1.0	1.1	1.1

[a]SEER nine standard registries, crude age-specific rate, 1995–1999.

[b]Includes leukemic reticuloendotheliosis (hairy-cell leukemia), plasma-cell leukemia, monocytic leukemia, and acute and chronic erythremia and erythroleukemia.

(immunohistochemical, B cell origin, progression to an acute aggressive form of non-Hodgkin's lymphoma), the committee has reviewed CLL separately from the other leukemias; the committee's review of CLL is presented in the next section.

The incidence of chronic myeloid leukemia (CML) increases steadily with age in people over 30 years old. Its lifetime incidence is roughly equal in whites and blacks and is slightly higher in males than in females. Among people in the age groups that characterize most Vietnam veterans, CML accounts for about one-fifth of cases of leukemia. It 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.

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*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and leukemia. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding (see Table 6-49).

Update of the Scientific Literature

Occupational Studies

Burns et al. (2001) reported data on a cohort of 1,517 male workers involved in the manufacture or formulation of 2,4-D in 1945–1994. A job–exposure matrix was developed to assign workers to exposure categories based on measurements of time-weighted average exposure. Mortality in the study cohort was compared with mortality for all white US males and for an internal reference population of nonexposed manufacturing workers in the same company (see Chapter 4 for study details). Mortality from leukemia in the entire cohort was similar to rates in all US males (four deaths; SMR = 1.3, 0.4–3.3). Similar results were obtained in an analysis based on a 20-year induction period. In the comparison with non-exposed workers, an excess in lymphopoietic mortality was noted in workers with high-cumulative-dose exposure to 2,4-D (four deaths; RR = 2.1 for 0-year induction and 2.7 for 20-year induction). That subgroup of exposed workers did not experience deaths from non-Hodgkin's lymphoma but may have included deaths from Hodgkin's disease and MM in addition to leukemia.

Cancer incidence and mortality were assessed in a cohort of 504 forestry workers in Sweden who were characterized by presence or absence of exposure to phenoxy herbicides in 1954–1967 (Thörn et al., 2000; see Chapter 4 for study details). Follow-up for mortality was completed through 1994, and data on cancer incidence were available from 1958–1992. No cases of leukemia occurred in the exposed members of this cohort.

Environmental Studies

Revich et al. (2001) analyzed data on cancer incidence and mortality in Chapaevsk, a city of 83,000 residents in the Samara region of Russia. A number of industries are in Chapaevsk, and production at one major chemical plant appeared to be responsible for dioxin contamination that was documented in the air, soil, and water of the city. Mortality due to leukemia during the years 1995–1998 was compared with mortality in the Samara region as a whole; the SMR was 1.5 for both men and women (0.8–2.7 in men; 0.8–2.4 in women), on the basis of 11

TABLE 6-49 Selected Epidemiologic Studies—Leukemia

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Burns et al., 2001	Dow 2,4-D production workers—lymphopoietic mortality in workers with high 2,4-D exposure	4	2.1 (0-yr induction) 2.7 (20-yr induction)
Thörn et al., 2000	Swedish lumberjack workers exposed to phenoxyacetic herbicides	0	—
Studies Reviewed in *Update 2000*			
Steenland et al., 1999	US chemical production workers	10	0.8 (0.4–1.5)
Hooiveld et al., 1998	Dutch chemical production workers	1	1.0 (0.0–5.7)
Rix et al., 1998	Danish paper mill workers		
	Males	20	0.8 (0.5–1.2)
	Females	7	1.3 (0.5–2.7)
Studies Reviewed in *Update 1998*			
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—cohort 1	4	1.8 (0.5–4.7)
Ramlow et al., 1996	Pentachlorophenol production workers	2	1.0 (0.1–3.6)
Waterhouse et al., 1996	Residents of Tecumseh, Michigan		1.4 (1.0–1.9)
Studies Reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide appliers	2	(*)
Semenciw et al., 1994	Farmers in Canadian prairie provinces	357	0.9 (0.8–1.0)
Blair et al., 1993	US 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—all other types of leukemia	3	1.2 (0.3–3.6)
	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	145	0.9 (*)
	Danish male employees	33	1.0 (*)
	Italian self-employed farmers	12	0.7 (*)
	Italian male employees	8	0.9 (*)
Fingerhut et al., 1991	US chemical workers	6	0.7 (0.2–1.5)
Saracci et al., 1991	Chemical workers		
	Exposed	18	1.2 (0.7–1.9)
	Probably exposed	0	0 (0.0 –11.2)
	Nonexposed	3	0.9 (0.2–2.6)
	Unknown exposure	0	0 (0.0–10.3)

continues

TABLE 6-49 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Brown et al., 1990	Residents of Iowa and Minnesota		
	All types of leukemia, ever farmed		1.2 (1.0–1.5)
	All types of leukemia, any herbicide use		1.2 (0.9–1.6)
	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)
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
ENVIRONMENTAL			
New Studies			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Mortality standardized to Samara region		
	Males	11	1.5 (0.8–2.7)
	Females	15	1.5 (0.8–2.4)
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone B males	9	2.4 (1.2–4.7)
	Zone B females	3	1.1 (0.4–3.5)
Schreinemachers, 2000	Rural or farm residents of Minnesota, Montana, North Dakota, South Dakota		
	Males—counties with wheat acreage 23,000–110,999	246	1.0 (0.8–1.1)
	Males—counties with wheat acreage ≥111,000	248	1.1 (1.0–1.3)
	Females—counties with wheat acreage 23,000–110,999	183	1.0 (0.8–1.2)
	Females—counties with wheat acreage ≥111,000	146	0.9 (0.8–1.2)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone B males	7	3.1 (1.4–6.7)
	Zone B females	1	0.6 (0.1–4.0)
	Zone R males	12	0.8 (0.4–1.5)
	Zone R females	12	0.9 (0.5–1.6)
Studies Reviewed in *Update 1998*			
Bertazzi et al., 1997	Seveso residents—15-year follow-up		
	Zone B males	7	3.1 (1.3–6.4)
	Zone B females	1	0.6 (0.0–3.1)

TABLE 6-49 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—morbidity		
	Zone B males	2	1.6 (0.4–6.5)
	Zone B females	2	1.8 (0.4–7.3)
Studies Reviewed in *VAO*			
Bertazzi et al., 1992	Seveso residents—10-year follow-up		
	Zones A, B, R males	4	2.1 (0.7–6.9)
	Zones A, B, R females	1	2.5 (0.2–27.0)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	2	0.7 (0.1–5.0)
AIHW, 1999	Australian Vietnam veterans	27	26 expected (16–36)
CDVA, 1998a	Australian Vietnam veterans—male	64[b]	26 expected (16–36)
CDVA, 1998b	Australian Vietnam veterans—female	1[b]	0 expected (0–4)
Studies Reviewed in *Update 1998*			
Dalager and Kang, 1997	Army Chemical Corps veterans		1.0 (0.1–3.8)
Crane et al., 1997b	Australian military Vietnam 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.
[b] Self-reported medical history. Answer to question: "Since your first day of service in Vietnam, have you been told by a doctor that you have leukemia?"
* Information not provided by study authors.
— When information was denoted by a dash in the original study.
ABBREVIATIONS: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid, 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs; IARC, International Agency for Research on Cancer; USDA, US Department of Agriculture.

deaths in men and 15 deaths in women. Age-adjusted incidences during 1998 were reported for leukemia and lymphoma combined. The rates in Chapaevsk were lower than in the Samara region in men (2.4 vs 14.6 per 100,000) but higher in women (19.0 vs 13.9). The actual number of cases was not given, *p* values and confidence intervals were not calculated, and there was no adjustment for factors other than age.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

Studies of leukemia reviewed for the first time in this report offer no compelling or consistent evidence of an association with exposure to herbicides. No cases occurred among exposed workers in the cohort of lumberjacks in Sweden. Four workers from the Dow Chemical Company with high cumulative exposure to 2,4-D did die from lymphopoietic cancer, but at least some of the deaths may have been due to HD or MM, forms of cancer that have already been causally linked with a history of exposure to herbicides. The relationship between mortality from leukemia and residence in Chapaevsk is suggestive but somewhat imprecise. The possible excess incidence of leukemia in Chapaevsk is difficult to interpret, in that only 1 year of data are presented, the incidences of leukemia and lymphoma are reported together, and the incidence in Chapaevsk is higher than that in the Samara region and Russia in women but substantially lower in men. Further information on the quality of cancer registration in Chapaevsk, the Samara region, and Russia is needed to understand fully the meaning of these data.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and leukemias other than CLL.

Biologic Plausibility

No animal studies have found an increased incidence of leukemia after exposure to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The limited data available on Vietnam veterans do not suggest that they are at increased risk for leukemias other than CLL.

CHRONIC LYMPHOCYTIC LEUKEMIA

In the proposed World Health Organization classification of non-Hodgkin's lymphoid neoplasms, CLL and its lymphomatous form, small lymphocytic lym-

phoma, are mature B-cell neoplasms (IARC, 2001). About 7,000 new cases (4,100 in men, 2,900 in women) of CLL will be diagnosed in the United States in 2002 (ACS, 2002). Nearly all cases occur after the age of 50. The rate per 100,000 in persons 50-74 years old is 18.7 and in persons 75 years old and older 38.8. For average annual incidence information, see the previous section on leukemia.

The requirements for diagnosis of CLL include an absolute peripheral-blood lymphocyte count of more than 10×10^9 per liter, a predominant population of mature-looking lymphocytes, and a hypercellular or normal cellular bone marrow containing more than 30% lymphocytes. The malignant cells in CLL exhibit a characteristic membrane phenotype with coexpression of pan-B-cell antigens, including CD19, CD20, and CD23 along with CD5. However, the cell-surface membranes express only weak surface membrane immunoglobulin.

Patients with CLL are staged according to the Rai classification: Stage 0, clinical features of lymphocytes in the blood and marrow only; Stage I or II (intermediate risk), lymphocytosis, lymphadenopathy, and splenomegaly with or without hepatomegaly; and Stage III or IV (high risk), lymphocytosis and anemia and/or thrombocytopenia. The most consistent abnormal finding at initial diagnosis is lymphadenopathy—from small lymph nodes to nodes as large as an orange. Patients with large lymphadenopathy, white-cell counts over 100×10^9 per liter, or thrombocytopenia require therapy. The disease is complicated by autoimmune anemias and recurrent infection because of hypogammaglobulinemia.

Diffuse small-cell lymphocytic lymphoma is the term for the condition of patients with lymphomatous presentation of CLL. Patients seek medical attention for painless generalized lymphadenopathy that in many cases has lasted for several years. Unlike the situation in CLL, the peripheral blood may be normal or reveal only mild lymphocytosis. However, the bone marrow is positive in 75–95% of cases. Both small-cell lymphocytic lymphoma and CLL can transform into aggressive NHL known as Richter's syndrome. Richter's syndrome is characterized by diffuse large-cell lymphoma or its immunoblastic variant. It is resistant to current therapies, and the median survival is about 6 months.

Summary of Studies on CLL

In response to a request from the Department of Veterans Affairs and because CLL shares more traits (immunohistochemical characteristics, B-cell origin, and progression to an acute aggressive form of NHL) with NHL than with other types of leukemia, the committee reassessed the available epidemiologic data to determine whether CLL merited reclassification regarding association with exposure to herbicides. The relevant data are summarized in Table 6-50.

Six studies reported in previous updates in which CLL was specifically investigated were reviewed. Waterhouse et al. (1996) performed a prospective study of site-specific cancer incidence in 7,016 males and females in a rural farming community in Michigan in 1959–1987. There was a significant increase

TABLE 6-50 Selected Epidemiologic Studies—Chronic Lymphocytic Leukemia

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
Waterhouse et al., 1996	Residents of Tecumseh, Michigan—CLL	10	SIR = 1.8 (0.8–3.2)
Amadori et al., 1995	CLL in Italian workers		
	Farming or animal-breeding workers	15	2.3 (0.9–5.8)
	Farming	5	1.6 (0.5–5.2)
	Animal-breeding	10	3.1 (1.1–8.3)
Studies Reviewed in *VAO*			
Hansen et al., 1992	Danish gardeners		
	All gardeners—CLL	6	2.5 (0.9–5.5)
	Male gardeners—CLL	6	2.8 (1.0–6.0)
Brown et al., 1990	Residents of Iowa and Minnesota		
	CLL, ever farmed	156	1.4 (1.1–1.9)
	CLL, any herbicide use	74	1.4 (1.0–2.0)
Blair and White, 1985	Residents of Nebraska		
	All cases, all leukemia—farming	1,084	1.3
	CLL	248	1.7 (CI did not include 1.0)
Burmeister et al., 1982	Residents of Iowa		
	CLL in white male farmers using herbicides		1.9 (1.2–3.1)
ENVIRONMENTAL			
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up—lymphatic leukemia		
	Zone A	0	—
	Zone B	2	1.1 (0.3–4.4)
	Total	2	1.0 (0.2–3.9)

[a]Given when available.
— When information was denoted by a dash in the original study.
Abbreviations: USDA, United States Department of Agriculture.

in the lymphopoietic neoplasms, NHL, Hodgkin's disease, and CLL (combined SIR = 1.40, 95% CI 1.0–1.9, $p = 0.03$). Waterhouse et al. (1996) also conducted a case–control study of risk factors in 74 patients with lymphoma and leukemia matched with four controls each. Family history was the only covariant significantly associated with disease; there was a correlation with pesticide use but the

database did not distinguish herbicides from insecticides and fungicides. Amadori et al. (1995) conducted a population-based case–control study in an agricultural area of Italy. Subjects working in agriculture denoted as farmers or farmers with animal breeding had a high risk of CLL (OR = 1.6, 95% CI 0.5–5.2, and 3.1, 95% CI 1.1–8.3, respectively). If both groups were combined the OR was 2.3 (95% CI 0.9–5.8). There was no information on herbicide exposure. The study of cancer risk in Danish male gardeners highly exposed to pesticides (Hansen et al., 1992) showed a significant increase in CLL (standardized morbidity ratio, SMbR = 2.8, 95% CI 1.0–6.0) on the basis of 6 exposed cases. The paper states that gardeners holding outdoor jobs were exposed primarily to herbicides through the growing season but also to insecticides and fungicides; however, data on exposure were not presented for CLL cases. Blair and White (1985) reported that CLL mortality among farmers in Nebraska in 1957–1974 was significantly increased (OR = 1.7). The higher risk occurred among farmers residing in counties associated with cattle and dairy products; however no information was provided on herbicide exposure of the CLL group.

Two of the epidemiologic studies reported on herbicide exposure and CLL. In a study of 1,675 white Iowa males who died of leukemia (Blair and White, 1985). CLL and nonspecific nonacute lymphocytic leukemia were significantly increased in farmers (OR = 1.7). Further analysis showed a strong relationship of CLL deaths in counties with acres producing soybeans and acres treated with herbicides. Brown et al. (1990) carried out a population-based case–control interview study of 578 white men with leukemia and 1,245 controls living in Iowa and Minnesota. CLL mortality (244 cases) was higher in farmers than in nonfarmers (OR = 1.4). When risk was calculated for CLL subtype, ORs were significantly increased for use of any herbicide (OR = 1.4), any insecticide (OR = 1.3), and any animal insecticide (OR = 1.3). The risk of CLL in farmers who ever handled 2,4-D was 1.3. The risk of CLL in men who first handled 2,4,5-T at least 20 years before interview was significantly increased (OR = 3.3, 95% CI 1.2–8.9).

Bertazzi et al. (2001) evaluated lymphocytic leukemia in the 20-year follow-up of Seveso residents. No increased risk was seen, with relative risks of 1.1 (95% CI, 0.3–4.4) and 1.0 (95% CI, 0.2–3.9) for Zone B residents and the total of Zone A and B residents, respectively.

No relevant Vietnam-veteran studies that specifically investigate CLL have been published since *Update 2000* (IOM, 2001).

Synthesis

A reanalysis of the epidemiologic studies indicates that farming occupation, especially where there is exposure to the herbicides 2,4-D and 2 4,5-T, is associated with significant risk of CLL mortality. Many more studies support the hypothesis that herbicide exposure can contribute to NHL risk. Most cases of CLL

and NHL reflect malignant transformation of B-lymphocyte progenitor cells, so these diseases could have a common etiology.

Conclusions

Strength of the Evidence

On the basis of its evaluation, the committee concludes that there is sufficient evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and CLL.

Biologic Plausibility

No animal studies have found an increased incidence of CLL after exposure to the chemicals of interest. A summary of the biologic plausibility of the carcinogenicity of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

The limited data available on Vietnam veterans do not suggest that they are at increased risk for CLL.

SUMMARY

On the basis of the occupational, environmental, and veteran studies reviewed, the committee has reached one of four standard conclusions about the strength of the evidence regarding an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and each of the kinds of cancer studied. As explained in Chapter 2, the distinctions reflect the committee's judgment that if an association between exposure and a given outcome is "real," it would have been found in a large, well-designed epidemiologic study in which exposure to herbicides or TCDD was sufficiently high, well characterized, and appropriately measured. For consistency 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 the four conclusions are based on statistical association, not on causality. The committee used the same criteria to categorize diseases according to the strength of the evidence as were used in *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*.

Health Outcomes with Sufficient Evidence of an Association

For outcomes in this category, a positive association with one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) 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 were free of bias and confounding and that showed an association that is consistent in magnitude and direction as sufficient evidence of an association.

In previous reports, the committees found sufficient evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and three cancers: soft-tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease. The scientific literature continues to support the classification of those three cancers in the category of sufficient evidence. In this update the committee considers the available data on CLL separate from other leukemias. On the basis of those data, the committee classifies CLL in this category.

Health Outcomes with Limited or Suggestive Evidence of Association

For outcomes in this category, the evidence must be suggestive of an association with at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid), 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; often several studies provide positive results, but the results of other studies are inconsistent.

In previous reports, the committees found limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and: laryngeal cancer, lung cancer, bronchial (tracheal) cancer, prostatic cancer, and multiple myeloma. The scientific literature continues to support the classification of those diseases in the category of limited or suggestive evidence. On the basis of the literature, no additional cancers satisfy the criteria for inclusion in this category.

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

The scientific data on many of the kinds of cancer reviewed by the committee were inadequate or insufficient to determine whether an association exists. For those cancers, the available studies are of insufficient quality, consistency, or statistical power to support a conclusion of the presence or absence of an association. For example, some studies fail to control for confounding or have inadequate exposure assessment. This category includes hepatobiliary cancers (can-

cers of the liver and intrahepatic bile duct), nasopharyngeal cancer, bone cancer, skin cancer (including basal-cell carcinoma, squamous-cell carcinoma, and non-melanocytic skin cancer), breast cancer, cancers of the female reproductive system (including cancer of the cervix, endometrium, and ovaries), testicular cancer, urinary bladder cancer, renal cancer (cancers of the kidney and renal pelvis), and the various forms of leukemia other than CLL.

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

For outcomes in this category, several adequate studies covering the full range of exposure that human beings are known to encounter are consistent in *not* showing a positive association with exposure to one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid). The studies have relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, magnitude of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small increase in risk associated with the exposure studied can never be excluded.

In previous reports, the committees found a sufficient number and variety of well-designed studies to conclude that there is limited or suggestive evidence of *no* association between a small group of cancer types and exposure to herbicides or TCDD: gastrointestinal tumors (of the colon, rectum, stomach, and pancreas) and brain tumors. The most recent scientific evidence continues to support the classification of such kinds of cancers in this category. On the basis of evaluation of the scientific literature, no additional cancers satisfy the criteria for inclusion in this category.

Biologic Plausibility

Chapter 3 presents details of the committee's evaluation of recent toxicologic studies relevant to the biologic plausibility of a connection between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and various forms of cancer. Some of the preceding discussions of cancer outcomes include references to papers relevant to specific types of cancer.

Although evidence suggests that TCDD is not genotoxic, data on animals indicate that TCDD has carcinogenic activity. A number of animal species—such as rats, mice, and hamsters—have been exposed to TCDD and examined for increases in tumor incidence and cancer. They have included studies in which TCDD was fed to animals, applied to their skin, injected under their skin, or injected into their abdominal cavity. The research indicates that TCDD can both cause cancers or tumors and act as a promoter (that is, enhance the incidence of some cancers or tumors in the presence of known carcinogens). Increased cancer rates have been observed at several sites in the body, notably the thyroid gland,

skin, and lungs. Studies have demonstrated an increased incidence of liver cancer after TCDD exposure but only after other adverse changes in the liver were observed. TCDD is also an extremely potent promoter of neoplasia in laboratory rats. Decreased rates of some cancers—including those of the uterus, pancreas, and pituitary and mammary glands—have also been reported. The sites at which effects were observed and the exposure needed to induce them varied considerably from species to species.

The mechanism by which TCDD exerts its carcinogenic effects is not established. TCDD has a wide array of effects on growth regulation, hormone systems, and other factors associated with the regulation of cellular processes involving growth, maturation, and differentiation; those effects could influence tumor formation. Data on female rats suggest that complex hormonal interactions are involved in TCDD-induced carcinogenesis.

Studies are consistent with the hypothesis that the effects of TCDD are mediated by the aryl hydrocarbon receptor (AhR), a protein in animal and human cells to which TCDD can bind. After the binding of TCDD, the TCDD–AhR complex has been shown to bind DNA and lead to changes in transcription (that is, genes are differentially regulated). In many cases, the differential gene regulation leads to transformation of a normal cell into an abnormal cell. Furthermore, data on animals genetically modified not to express the AhR suggest that the AhR plays a role in normal growth, and this supports the hypothesis that TCDD could affect cell growth.

The transcriptional alterations induced by TCDD result in alterations in some forms of cellular regulation and metabolism at a very basic level. For example, TCDD has been shown to induce cytochrome P4501A1 (CYP1A1) and other metabolizing enzymes. Those changes result in altered cell metabolism and could be involved in TCDD's carcinogenic activity, especially as this may involve the metabolic activation of other chemicals to carcinogenic intermediates. An accumulation of data also indicates that genes and pathways modulating cell cycle, altering the pattern of cell death, involved in the production and activity of hormones and growth factors, and involved in cellular oxidative stress appear to be predominantly affected. Those data are consistent with findings that TCDD alters cell pathways involving growth, maturation and differentiation, all of which could modulate processes involved in the tissue-specific formation of tumors. On the other hand, tissue-specific protective cellular mechanisms can affect the response to TCDD, further complicating the carcinogenic effects of this chemical.

There are differences among various experimental animals in susceptibility to TCDD-induced effects; the sites at which tumors are induced also vary from species to species. Modulated gene expression by TCDD is also highly specific for species and cell type. Differences in the induction or repression of responsive genes likely play a role in the different responses seen in different cell types and species.

Although structural differences in the AhR have been identified among different species, this receptor operates in a similar manner in animals and humans. Therefore, a common mechanism is likely to underlie the carcinogenic effects of TCDD in humans and animals, and data on animals support a biologic basis of the carcinogenic effects of TCDD in humans. Because of the many species and strain differences in TCDD responses, however, controversy remains regarding the magnitude of TCDD exposure that is carcinogenic.

Fewer studies have been conducted on the carcinogenicity of the herbicides. Several studies of the carcinogenicity of 2,4-D, 2,4,5-T, and picloram have been performed in laboratory animals. In general, the results were negative. However, some studies do not meet present-day standards for cancer bioassays, and others produced equivocal results. Thus, it is not currently possible to have confidence in the conclusions regarding the carcinogenicity of these compounds. With respect to genotoxicity, however, most of the evidence indicates that 2,4-D is genotoxic only at very high concentrations. Although 2,4,5-T increased the formation of DNA adducts by cytochrome P450-derived metabolites of benzo-[*a*]pyrene, most available evidence indicates that 2,4,5-T is genotoxic only at high concentrations.

There is some evidence that cacodylic acid (also known as dimethylarsinic acid, DMA) is carcinogenic. DMA may induce DNA modifications that sensitize it to free-radical injury. Other studies concluded that it is a promoter of urinary bladder, kidney, liver, and thyroid gland carcinogenesis in rats; causes pulmonary neoplasms in mice; and causes bladder hyperplasia and tumors in rats. Another exposure study in mice, however, produced negative results.

The evidence suggests that a connection between TCDD and cancer in humans is, in general, biologically plausible. However, differences in sensitivity and susceptibility among individual animals, strains, and species; the lack of strong evidence of organ-specific effects among species; and differences in route, dose, duration, and timing of exposure complicate any more definitive conclusions about the presence or absence of a mechanism of induction of site-specific cancers by TCDD. Experiments on 2,4-D, 2,4,5-T, and picloram in animals and cells do not provide a strong biologic basis for the presence or absence of carcinogenic effects of these compounds. Some animal data might support a carcinogenic effect of DMA, but these data alone are not sufficient to draw conclusions on the carcinogenicity of this compound in humans.

Considerable uncertainty remains about how to apply this information to the evaluation of potential health effects of herbicide or TCDD exposure in Vietnam veterans. Scientists disagree about the extent to which information derived from animals and cellular studies predicts human health outcomes and about the comparability of health effects resulting from high-dose and low-dose exposure. Investigating the biologic mechanisms underlying TCDD's carcinogenic effects continues to be an active field of research, and future updates of this report might

have more and better information on which to base conclusions, at least for that compound.

Increased Risk of Disease Among Vietnam Veterans

Under the Agent Orange Act of 1991, the committee was asked to determine (to the extent that available scientific data permit meaningful determinations) the increased risk of various diseases it studies among those exposed to herbicides during their service in Vietnam. As discussed under the specific cancers, for most cancer outcomes there is insufficient data to quantitate the increased risk to Vietnam veterans.

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7

Reproductive and Developmental Effects

This chapter summarizes the scientific literature published since *Veterans and Agent Orange: Update 2000* (hereafter, *Update 2000*; IOM, 2001) on the association between exposure to herbicides and adverse reproductive or developmental effects. The categories of association and the committee's approach to categorizing the health outcomes are discussed in Chapters 1 and 2. The literature discussed in this chapter includes papers that describe environmental, occupational, and Vietnam-veteran studies that evaluate herbicide exposure and the risk of birth defects, declines in sperm quality and fertility, spontaneous abortion, stillbirths, neonatal and infant mortality, low birthweight and preterm birth, childhood cancer, and alterations in sex ratio. Besides studies of herbicides and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), studies of populations exposed to polychlorinated biphenyls (PCBs) are also reviewed when relevant, because TCDD is sometimes a contaminant of PCBs.

The primary emphasis of this chapter is on the potential adverse reproductive effects of herbicide exposure in men, because the vast majority of Vietnam veterans are men. Because about 8,000 women served in Vietnam (H. Kang, US Department of Veterans Affairs, personal communication, December 14, 2000), findings relevant to female reproductive health are also included.

BIRTH DEFECTS

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 abnormalities that are present at birth and are severe enough to interfere with viability or physical well-being. Major birth defects are seen in about 2–3% of live births. Birth defects can be detected in an additional 5% of babies with follow-up through the first year of life. The causes of most birth defects are unknown. In addition to genetic factors, a number of exposures—including medications and environmental, occupational, and lifestyle factors—have long been implicated in the etiology of birth defects (Kalter and Warkany, 1983). Historically, most etiologic research focused on the effect of maternal and fetal exposures, but some work has addressed paternal exposures. Paternally mediated exposures could occur via several routes, and therefore exert an effect in various ways. One is through direct genetic damage to the male germ cell that is transmitted to the offspring and expressed as a birth defect. A second is through transfer of chemicals from the work, home, or general environment via seminal fluid with subsequent fetal exposure during gestation. A third route is via indirect exposure from household contamination by take-home exposures.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and birth defects among offspring. Additional information available to the committee responsible for *Update 1996* led it to conclude that there was limited or suggestive evidence of an association between at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and spina bifida in the children of veterans; there was no change in the conclusions regarding other birth defects. There was no change in those findings in *Update 1998* or *Update 2000*. Reviews of the studies underlying the findings may be found in the earlier reports (see Tables 7-1 and 7-2).

Update of the Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2000* (IOM, 2001).

Environmental Studies

In a pilot study of 30 Vietnamese women who were known or whose spouses were known to be exposed to Agent Orange, Le and Johansson (2001) reported

TABLE 7-1 Selected Epidemiologic Studies—Birth Defects

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
Dimich-Ward et al., 1996	Sawmill workers (paternal exposure)		
	Cataracts	11[b]	5.7 (1.4–22.6)
	Genital organs	105[b]	1.3 (0.9–1.5)
Garry et al., 1996	Private pesticide appliers (paternal exposure)		
	Circulatory–respiratory	17	1.7 (1.0–2.8)
	Gastrointestinal	6	1.7 (0.8–3.8)
	Urogenital	20	1.7 (1.1–2.6)
	Musculoskeletal–integumental	30	
	Maternal age < 30 years	11	0.9 (0.5–1.7)
	Maternal age > 30 years	19	2.5 (1.6–2.1)
	Chromosomal	8	1.1 (0.5–2.1)
	Other	48	
	Maternal age < 35 years	36	1.1 (0.8–1.6)
	Maternal age > 35 years	12	3.0 (1.6–5.3)
	All births with anomalies	125	1.4 (1.2–1.7)
Kristensen et al., 1997	Offspring of Norwegian farmers (maternal and paternal exposure)	4,189[c]	1.0 (1.0–1.1)
Studies Reviewed in *VAO*			
Townsend et al., 1982	Follow-up of Dow Chemical plant workers (paternal exposure)	30	0.9 (0.5–1.4)
Smith et al., 1982	Follow-up of 2,4,5-T sprayers (paternal exposure)—sprayers compared with nonsprayers	13	1.2 (0.5–3.0)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	18	1.1 (0.5–2.2)
Moses et al., 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	11	1.3 (0.5–3.4)
ENVIRONMENTAL			
New Studies			
Loffredo et al., 2001	Infants exposed to herbicides during the first trimester (maternal exposure)	66	2.8 (1.3–7.2)
Revich et al., 2001	Residents of Chapaevsk, Russia—congenital malformations	*	(*) NS
ten Tusscher et al., 2000	Infants born in Zeeburg, Amsterdam, clinics in 1963–1965 with orofacial cleft (maternal exposure)		
	Births in 1963	5	(*) SS
	Births in 1964	7	(*) SS

continues

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Studies Reviewed in *VAO*			
Fitzgerald et al., 1989	Follow-up of an electric transformer fire—total birth defects (maternal and paternal exposure)	1	SIR = 212 (5.4–1,185.1)
Hanify et al., 1981	All birth malformations[d]	164	1.7 (1.4–2.2)
	All heart malformations	20	3.9 (1.7–8.9)
	Hypospadias, epispadias	18	5.6 (2.1–15.1)
	Talipes	52	1.7 (1.1–2.4)
	Anencephaly	10	1.4 (0.6–3.3)
	Spina bifida	13	1.1 (0.6–2.3)
	Cleft lip	6	0.6 (0.2–1.5)
	Isolated cleft palate	7	1.4 (0.5–3.8)
Mastroiacovo et al., 1988	Reproductive outcomes of Seveso, Italy, residents (maternal, paternal, and in utero exposure)		
	Zones A, B total defects	27	1.2 (0.8–1.8)
	Zones A, B, R total defects	137	1.0 (0.8–1.2)
	Zones A, B mild defects	14	1.4 (0.9–2.6)
Stockbauer et al., 1988	TCDD soil contamination in Missouri (all exposures)		
	Total birth defects	17	0.8 (0.4–1.5)
	Major defects	15	0.8 (0.4–1.7)
	Midline defects	4	0.6 (0.2–2.3)
	Central nervous system defects	3	3.0 (0.3–35.9)
Studies Reviewed in *Update 2000*			
Garcia et al., 1998	Infants born with various birth defects in agricultural areas in Spain	21	
	Index based on months of work in agriculture and intensity of exposure to chlorophenoxy herbicides		3.1 (0.6–16.9)
VIETNAM VETERANS			
New Studies			
Kang et al., 2000	Female Vietnam veterans	4,140	
	"Llikely" birth defects		1.7 (1.2–2.2)
	"Moderate-to-severe" birth defects		1.5 (1.1–2.0)
Studies Reviewed in *Update 2000*			
AIHW, 1999	Australian Vietnam veterans—Validation Study (paternal exposures)		
	Down syndrome	67	92 expected (73–111)
	Tracheoesophageal fistula	10	23 expected (14–32)
	Anencephaly	13	16 expected (8–24)

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
	Cleft lip or palate	94	64 expected (48–80)
	Absent external body part	22	34 expected (23–45)
	Extra body part	74	74 expected (*)
Michalek et al., 1998	Children with birth defects born to Air Force Ranch Hand veterans (paternal exposures)		
	Pre-Southeast Asia	*	0.7 (*)
	Post-Southeast Asia	*	1.5 (*)
Studies Reviewed in *Update 1996*			
Wolfe et al., 1995	High-exposure Ranch Hands relative to comparisons (paternal exposure)		
	Nervous system	3	(*)
	Eye	3	1.6 (0.4–6.0)
	Ear, face, and neck	5	1.7 (0.6–4.7)
	Circulatory system, heart	4	0.9 (0.3–2.7)
	Respiratory system	2	(*)
	Digestive system	5	0.8 (0.3–2.0)
	Genital system	6	1.2 (0.5–3.0)
	Urinary system	7	2.1 (0.8–5.4)
	Musculoskeletal	31	0.9 (0.6–1.2)
	Skin	3	0.5 (0.2–1.7)
	Chromosomal anomalies	1	(*)
	All anomalies	57	1.0 (0.8–1.3)
Studies Reviewed in *VAO*			
Erikson et al., 1984a	Birth defects study (paternal exposure)		
	Any major birth defects	428	1.0 (0.8–1.1)
	Multiple birth defects with reported exposure	25	1.1 (0.7–1.7)
	EOI-5: spina bifida	1	2.7 (1.2–6.2)
	EOI-5: cleft lip with or without cleft palate	5	2.2 (1.0–4.9)
CDC, 1989	Vietnam Experience Study (paternal exposure)		
	Interview study		
	Any congenital anomaly	826	1.3 (1.2–1.4)
	Nervous system defects	33	2.3 (1.2–4.5)
	Ear, face, neck defects	37	1.6 (0.9–2.8)
	Integument	41	2.2 (1.2–4.0)
	Musculoskeletal	426	1.2 (1.1–1.5)
	Hydrocephalus	11	5.1 (1.1–23.1)
	Spina bifida	9	1.7 (0.6–5.0)
	Hypospadias	10	3.1 (0.9–11.3)
	Multiple defects	71	1.6 (1.1–2.5)
	Defects with high exposure	46	1.7 (1.2–2.4)

continues

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
CDC, 1989	General Birth Defects Study (paternal exposure)		
	Birth defects	130	1.0 (0.8–1.3)
	Major birth defects	51	1.2 (0.8–1.9)
	Black Vietnam veterans with children with birth defects	21	3.4 (1.5–7.6)
	Digestive system defects	18	2.0 (0.9–4.6)
Aschengrau and Monson, 1990	Birth defects and father's Vietnam service		
	Vietnam veterans compared with men without known military service	55	1.3 (0.9–1.9)
	Vietnam veterans compared with non-Vietnam veterans	55	1.2 (0.8–1.9)
	Major malformations		
	Vietnam veterans compared with men without known military service	18	1.8 (1.0–3.1)
	Vietnam veterans compared with non-Vietnam veterans	18	1.3 (0.7–2.4)
Donovan et al., 1984	Birth defects and father's Vietnam service (Australia)		
	Vietnam veterans vs all other men National Service veterans	127	1.02 (0.8–1.3)
	Vietnam service vs no Vietnam service	69	1.3 (0.9–2.0)
AFHS, 1992	Follow-up of Air Force Ranch Hand personnel		
	Birth defects in conceptions after service in Southeast Asia		
	Congenital anomalies	229	1.3 (1.1–1.6)
	Nervous system	5	1.9 (0.5–7.2)
	Respiratory system	5	2.6 (0.6–10.7)
	Circulatory system or heart	19	1.4 (0.7–2.6)
	Urinary system	21	2.5 (1.3–5.0)
	Chromosomal	6	1.8 (0.6–6.1)
	Other	5	2.6 (0.6–10.7)

[a] Given when available.
[b] Number of workers with maximal index of exposure (upper three quartiles) for any job held up to three months prior to conception.
[c] 95% confidence intervals contained one for all outcomes. Anencephaly and spina bifida included in this calculation.
[d] Excludes stillbirths, neonatal death, or dislocated or dislocatable hip.
* Information not provided by study authors.
ABBREVIATIONS: AFHS, Air Force Health Study; AIHW, Australian Institute of Health and Welfare; CDC, Centers for Disease Control and Prevention; CI, confidence interval; EOI, exposure opportunity index; NS, not significant; SIR, standardized incidence ratio; SS, statistically significant.

TABLE 7-2 Selected Epidemiologic Studies—Neural Tube Defects

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 1998*			
Blatter et al., 1997	Offspring of Dutch farmers—spina bifida (paternal exposure)		
	Pesticide use (moderate or heavy exposure)	9	1.7 (0.7–4.0)
	Herbicide use (moderate or heavy exposure)	7	1.6 (0.6–4.0)
Kristensen et al., 1997	Offspring of Norwegian farmers—spina bifida (paternal exposure)		
	Tractor spraying equipment	28	1.6 (0.9–2.7)
	Tractor spraying equipment, orchards or greenhouses	5	2.8 (1.1–7.1)
Dimich-Ward et al., 1996	Sawmill workers (paternal exposure)		
	Spina bifida or anencephaly	22[b]	2.4 (1.1–5.3)
	Spina bifida	18[b]	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[c]			
Studies Reviewed in *VAO*			
Stockbauer et al., 1988	TCDD soil contamination in Missouri—central nervous system defects (all exposures)	3	3.0 (0.3–35.9)
Hanify et al., 1981	Spraying of 2,4,5-T in New Zealand (all exposures)		
	Anencephaly	10	1.4 (0.6–3.3)
	Spina bifida	13	1.1 (0.6–2.3)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AIHW, 1999	Australian Vietnam veterans—validation study, spina bifida (paternal exposure)	50	1.5 (NR)
Studies Reviewed in *Update 1996*			
Wolfe et al., 1995	Follow-up of Air Force Ranch Hands (paternal exposure)		
	Neural tube defects among Ranch Hand personnel children[d]	4	(*)
	Neural tube defects among comparison children	0	(*)
Studies Reviewed in *VAO*			
CDC, 1989	Vietnam Experience Study (paternal exposure)		
	Spina bifida among Vietnam veterans' children	9	1.7 (0.6–5.0)
	Spina bifida among non-Vietnam veterans' children	5	(*)

continues

TABLE 7-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Anencephaly among Vietnam veterans' children	3	(*)
	Anencephaly among non-Vietnam veterans' children	0	(*)
Erickson et al., 1984a,b	Birth Defects Study (paternal exposure)		
	Vietnam veterans: spina bifida	19	1.1 (0.6–1.7)
	Vietnam veterans: anencephaly	12	0.9 (0.5–1.7)
	EOI-5: spina bifida	19[e]	2.7 (1.2–6.2)
	EOI-5: anencephaly	7[e]	0.7 (0.2–2.8)
Australia Department of Veterans Affairs, 1983	Australian Vietnam veterans—neural tube defects (paternal exposure)	16	0.9

[a] Given when available.
[b] Number of workers with maximal index of exposure (upper three quartiles) for any job held up to 3 months before conception.
[c] Either or both parents potentially exposed.
[d] Four neural tube defects among Ranch Hand offspring include two spina bifida (high dioxin), one spina bifida (low dioxin), and one anencephaly (low dioxin). Denominator for Ranch Hand group is 792 and for comparison group 981.
[e] Number of Vietnam veterans fathering a child with a neural tube defect given any exposure opportunity index.
*Information not provided by study authors.
ABBREVIATIONS: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; AIWI, Australian Institute of Health and Welfare; CDC, Centers for Disease Control and Prevention; EOI, exposure opportunity index; NR, not reported.

that two-thirds of their children had congenital malformations or developed disabilities within the first few years of life. However, by the authors' own admission, the study subjects were purposely selected to be those who had given birth to at least one disabled child. So, although suggestive for future research, the results may not be amenable to serious interpretation.

Loffredo et al. (2001) reported results from the Baltimore-Washington Infant Study. This case–control study of congenital heart defects in liveborn infants, conducted in 1981–1989, included 1,832 cases of congenital heart defects—66 with transdisposition of the great arteries (TGA) and 114 with non-TGA outflow-tract anomalies—and 771 controls. In 1987–1989, information on exposure was obtained by expanding the original questionnaire to include questions on pesticide exposure for the 1987–1989 period. Information was obtained on type, mode, location, frequency, and time of exposure. A mother was said to be exposed to pesticides if exposure occurred during the 3 months preceding pregnancy or the first trimester, a period of pregnancy considered critical for cardiovascular devel-

opment. The mothers were further classified into four mutually exclusive groups: mothers not exposed to pesticides at any time 6 months before pregnancy or during pregnancy, mothers exposed to any pesticides 4–6 months before pregnancy, mothers exposed to pesticides during the 3 months preceding pregnancy or during the first trimester, and mothers exposed during the last 4–9 months of pregnancy (late gestational exposures).

The authors report significant associations between TGA and exposures to any pesticide (odds ratio [OR] = 2.0, 1.2–3.3), herbicides (OR = 2.8, 1.3–7.2), or rodenticidal chemicals (OR = 4.7, 1.4–12.1) but not insecticides (OR = 1.5, 0.9–2.6). Of those, only the models for herbicides and rodenticidal chemicals were adjusted for race of infant, socioeconomic status, maternal age, maternal smoking and alcohol use, family history of heart defects, maternal diabetes, maternal solvent exposures, and paternal pesticide exposures. Non-TGA cardiac outflow-tract anomalies were significantly associated with pesticide exposure (OR = 3.8, 1.4–10.6). All other associations were not statistically significant, and ORs ranged from 0.8 to 1.5. The effect on TGA was stronger among those who used pellet, powder, or food imitators (OR = 4.0, 0.7–9.8); and effects generally were also stronger with more frequent use of pesticides. For both herbicides and rodenticides, the effects on TGA were pronounced if they occurred during the critical period of pregnancy for cardiovascular development, i.e., the 3 months preceding pregnancy and the first trimester. No data were collected on specific chemicals used, although the authors specifically mention chlorophenoxy herbicides being sold commercially during the period of interest. A major strength of this study is the high response rate, even among controls. Also notable are the specificity for exposures early but not late in pregnancy and the lack of association of herbicides with other heart defects.

ten Tusscher et al. (2000) reported results of a retrospective observational epidemiologic study that compared trends in incidence of nonsyndromal orofacial clefts during 1961–1969 in clinics in the communities of Zeeberg, Amsterdam, and Wilhelmina Gasthuis, Amsterdam. The two locations were different. Zeeberg was highly exposed to potentially toxic chemicals, including dioxins, because of its proximity to open chemical combustion in a nearby incinerator. In fact, the area was still a prohibited terrain as late as 1998 because of the toxic chemical present. Wilhelmina Gasthuis was a clean control location. The authors showed that the trend in incidence of orofacial clefts was consistently higher in the exposed communities than in the control community. There was also a suggestion of a dose–response relationship in that a higher volume of combustion tended to be followed by higher incidence. Note, however, that information on combusted quantities was sketchy. Combusted quantities were generally underreported (by up to 70%) and unknown for 5 of the 10 years of interest (1960–1969). In 1961–1969, the incidence in Zeeberg averaged about 2.4 per 1,000 births, with a peak of about 7.1 per 1,000 births in 1963–1965; it later plateaued at 1.7 per 1,000 births (a figure that is still higher than that in the control commu-

nity in that period). In Wilhelmina, the maximal incidence over the entire 10-year period was 2.3 per 1,000 births. The differences in incidence between the Zeeberg and Wilhelmina Gasthuis clinics were found to be statistically significant for 1963 and 1964.

The results are not based on multivariate models that account for confounding, but the authors outline the comparability of the two populations. Both clinics served communities with low socioeconomic status; hence the need for delivery at the clinics instead of at home. In addition to the social indications that were common to the two clinics, the Wilhelmina clinic handled all pathologic pregnancies. That indicates that the Zeeberg clinic handled healthier pregnancies, despite the observed higher incidence of orofacial clefts. The authors argue, but do not present data, that the two populations are comparable with respect to smoking, socioeconomic status, and alcohol consumption. The study is relevant to the charge of the committee in that chemical emissions from incineration are known to contain TCDD and dioxin-like compounds. Moreover, eels and rabbits from the vicinity of the incinerator were found to have very high concentrations of dioxins in their bodies. The combustion processes, however, were likely to produce multiple chemicals with potential adverse health effects. The nonspecific nature of the exposure information and lack of direct information on potential confounders limit the usefulness of the results of this study for the charge of this committee.

Revich et al. (2001) studied the relationship between the relatively high dioxin concentrations in the air, soil, drinking water, and cows' milk of Chapaevsk, Russia, due to pollution from a chemical plant in the area and its effects on the reproductive health status of the study population. In 369 children born in 1990–1995, the average number of congenital morphogenetic conditions (CMGCs) per child was higher in Chapaevsk, Russia (4.5 in boys and 4.4 in girls), than reported previously in other comparably polluted industrial towns. The most frequent CMGCs were sandals chack, epicanthus, shawl scrotum (in boys), clinodactyly, and broad first fingers. Fequencies of congenital malformations in Chapaevsk were not statistically significantly different from the data reported for the entire continent in the European register.

Vietnam-Veteran Studies

In a historical cohort study of 4,140 female Vietnam veterans and 4,140 female non-Vietnam (but contemporary) veterans, Kang et al. (2000) assessed potential associations between various self-reported pregnancy outcomes and the Vietnam experience or lack thereof. Statistically significant associations were detected only with "likely" (OR = 1.7, 1.2–2.2) and "moderate-to-severe" (OR = 1.5, 1.1–2.0)] birth defects. Here, "likely" birth defects were defined as congenital anomalies—including structural, metabolic, or hereditary defects—based on an initial 11-category grouping of birth defects. "Moderate-to-severe" birth de-

fects were defined according to the severity of the diagnosis or were conditions that had any history of surgical or medical treatment or functional impairment or were related to death. The "moderate-to-severe" category was constructed to analyze possibly teratogenic defects. When analysis was restricted to the nonnurse veterans in both groups, the associations between serving in Vietnam and birth defects became stronger for both "likely" (OR = 3.1, 1.8–5.6) and "moderate-to-severe" (OR = 2.6, 1.4–4.9) birth defects. This study has very high relevance to the assessment of effects of Agent Orange and other herbicides used in the Vietnam era, but its usefulness is somewhat limited by the definition of exposure as service in Vietnam. In addition, a serious attempt to validate the self-reported pregnancy outcomes was not generally successful, possibly because of the long gap between the reported events and data collection. However, the rather strong association with birth defects, in the absence of a significant association with any other pregnancy outcome, is rather convincing. Reanalysis of the data in conjunction with forthcoming data from a current study (which is being overseen by the National Academies) characterizing herbicide exposure in Vietnam could yield information valuable for understanding the effects of Agent Orange and other herbicides on birth defects.

Synthesis

The interpretation of the increased risk of CMGCs due to high concentrations of dioxin that was reported in Revich et al. (2001) suffers from poor study design (it used an ecologic study design) and inadequate control for confounding factors. Similarly, the findings of Le and Johansson (2001) suffer from an admitted bias in selection of study subjects.

Of the three relatively well-designed recent studies (Kang et al., 2000; Loffredo et al., 2001; ten Tusscher et al., 2000), Loffredo et al. (2001) and ten Tusscher et al. (2000) do not deal with effects that are directly associated with Vietnam veterans. The positive associations between TGA and use of pesticides and herbicides give some evidence of increased risks that may be relevant to exposure of Vietnam veterans to Agent Orange and other herbicides. However, the stronger results that were associated with exposures during critical periods may not be relevant to Vietnam veterans unless pregnancies occurred during periods of active duty, although the persistence of TCDD implies that direct exposure during pregnancy might not be required.

The results of ten Tusscher et al. (2000) provide evidence of increased risk of nonsyndromal orofacial clefts and a possible dose–response relationship with exposures to potentially high concentrations of dioxins. This study suffers from lack of adjustment for confounders, but its two populations appear to be comparable with respect to potential confounders, such as sociodemographic factors. It is also not possible to rule out confounding by the effects of exposures other than

TCDD and dioxin-like compounds. The nonspecific nature of the exposure information might limit the usefulness of the results of the study.

Perhaps the evidence most relevant to the charge of the committee is that reported by Kang et al. (2000). The statistically significant association of the "Vietnam experience" with increased risks of birth defects, in the absence of associations with any of the other pregnancy outcomes, needs further attention. The importance of the results is attenuated by the nonspecific nature of the exposure; until more specific information on exposure is available, the results will remain intriguing but inconclusive.

Conclusions

Strength of Evidence from Epidemiologic Studies

There were no new relevant studies on the association between exposure to herbicides (2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram) and spina bifida in offspring. The committee believes that the evidence is still limited or suggestive of an association between exposure the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and spina bifida. On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there remains inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, and cacodylic acid) and all other birth defects. Although there are reports of increased risks of TGA, nonsyndromal orofacial clefts, and CMGCs in the various studies reviewed for this update, those studies suffer from various pitfalls with respect to study design, sample size, and nonspecific exposure ascertainment.

Biologic Plausibility

Laboratory studies of potential male-mediated developmental toxicity of TCDD and herbicides, specifically with regard to birth defects, are too limited to permit conclusions. Research on chemical production workers with TCDD exposure suggests that some hormonal changes are associated with such exposure, but it is unclear whether the changes could be responsible for an increase in spina bifida or other birth defects. Notably, one recent investigation did not show evidence that paternal exposure to a herbicide formation containing 2,4-D and picloram caused birth defects or any other adverse reproductive outcomes in experimental animals.

A summary of the biologic plausibility of reproductive effects of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties about the exposure of Vietnam veterans and the magnitude of potential risk, if any, of the various birth defects, it is not possible for the committee to quantify the degree of risk to Vietnam veterans of having children born with birth defects.

FERTILITY

Male reproductive function is a complex system under the control of several components whose proper coordination is important for normal fertility. Several components and end points related to male fertility, including reproductive hormones and sperm characteristics, can be studied as indicators of fertility. Briefly, the reproductive neuroendocrine axis involves the central nervous system, the anterior pituitary gland, and the testis. In the central nervous system, the hypothalamus integrates neural inputs from the central and peripheral nervous systems and regulates the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Both those hormones are secreted in episodic bursts by the anterior pituitary gland into the circulation and are necessary for normal spermatogenesis. In the testis, LH interacts with receptors on Leydig cells, where it leads to increased testosterone synthesis. FSH and the testosterone from the Leydig cells interact with the Sertoli cells in the seminiferous tubule epithelium to regulate spermatogenesis. More-detailed reviews of the male reproductive hormones can be found elsewhere (Knobil et al., 1994; Yen and Jaffe, 1991). 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*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and altered sperm characteristics or infertility. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Reviews of the studies underlying those findings may be found in the earlier reports (see Table 7-3 for summaries of studies).

TABLE 7-3 Selected Epidemiologic Studies—Fertility

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in Update 2000			
Abell et al., 2000	Fecundibility ratios in female greenhouse workers in Denmark (maternal exposure)		
	>20 hours of manual contact per week	220	0.7 (0.5–1.0)[b]
	Never used gloves	156	0.7 (0.5–1.0)[b]
	High exposure	202	0.6 (0.5–0.9)[b]
Larsen et al., 1998	Fecundibility ratios in Danish farmers who used any potentially spermatotoxic pesticides, including 2,4-D (paternal exposure)	523	1.0 (0.8–1.4)[b]
	Used three or more pesticides		0.9 (0.7–1.2)[b]
	Used manual sprayer		0.8 (0.6–1.1)[b]
Studies Reviewed in *Update 1998*			
Heacock et al., 1998	Standardized fertility ratios in workers at sawmills using chlorophenates (paternal exposure)	18,016 (births)	0.9 (0.8–0.9)[c]
	Mantel-Haenszel rate ratio estimator in workers at sawmills using chlorophenates (paternal exposure)	18,016 (births)	0.7 (0.7–0.8)[c]
	Cumulative exposure (hours)		
	120–1,999	7,139	0.8 (0.8–0.9)[c]
	2,000–3,999	4,582	0.9 (0.8–0.9)[c]
	4,000–9,999	4,145	1.0 (0.9–1.1)[c]
	≥10,000	1,300	1.1 (0.9–1.2)[c]
VIETNAM VETERANS			
New Studies			
Staessen et al., 2001	Delays in sexual maturity in adolescents from highly exposed areas		
	In Antwerp, Belgium	15	4 (*)
	In Wilrik, Belgium	8	1.7 (*)
Studies Reviewed in *Update 1996*			
Henriksen et al., 1996	Effects on specific hormone levels in Ranch Hands (paternal exposure)		
	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)

TABLE 7-3 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	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 LH		
	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)
Studies Reviewed in *VAO*			
CDC, 1989	Vietnam Experience Study (paternal exposure)		
	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 (paternal exposure)		
	Difficulty in having children	349	1.3 ($p < 0.01$)

[a] Given when available.
[b] For this study, relative risk has been replaced with the fecundability ratio, a value of which less than 1.0 indicates an adverse effect.
[c] Standardized fertility ratio, for which a value less than 1.0 indicates an adverse effect.
* Information not provided by study authors.
ABBREVIATIONS: CDC, Centers for Disease Control and Prevention; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Update of the Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2000* (IOM, 2001).

Environmental Studies

Staessen et al. (2001) used biomarkers to assess whether exposure to heavy metals, PCBs, volatile organic compounds, and polycyclic aromatic hydrocarbons is related to early reproductive effects. They compared 100 17-year-old

lifetime residents of two highly exposed suburbs of Antwerp, Belgium, with 100 17-year-old lifetime residents of a rural control community. The two suburban locations (Hoboken and Wilrijk) are 11–13 km southeast of the chemical industry of Antwerp and were characterized by pollution from a lead smelter and two waste incinerators. The control town of Peer is far from any busy highways and lies 15–25 km east of the nearest nonferrous-metal smelters and chemical plants. Information was collected on medical history, Tanner staging was conducted and testicular volume measured (in boys), and questionnaires were used to collect data on lifestyle, use of tobacco and alcohol, food intake, dietary habits, medication use and socioeconomic status. Concentrations of several environmental agents, including dioxin-like compounds in serum samples, were found to be higher in the two suburban locations than in the control community, after adjustment for sex, body-mass index (BMI), weeks of breastfeeding, parental social class, and dietary fat intake. The results indicate that children in the two suburban locations experienced substantial and statistically significant delays in sexual maturation and (in boys) lower testicular volume, after adjustment for age, BMI, parental social class, and use of oral contraceptives (by girls).

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

In a recent review article, Figà-Talamanca et al. (2001) summarize the most up-to-date evidence on the relationship between male fertility and occupational exposure to pesticides, metals, and solvents. After a careful review of the literature, they conclude that there is insufficient evidence to conclude that use of pesticides leads to significantly higher risk to human reproduction. More specifically, results on the effects of dioxins, including TCDD, appear to be conflicting and open to debate.

That the delayed sexual maturation and (in boys) lower testicular volume in adolescents is associated with higher concentrations of dioxin-like compounds (Staessen et al., 2001) supports a potential effect on male reproductive capacity, but the implication for Vietnam veterans remain unclear, inasmuch as most veterans were past their pubertal development during their terms of duty. Moreover, there were numerous exposures of the Belgian adolescents, and attribution of effects to TCDD is not possible. However, the overall effect of dioxin-like compounds on human reproduction increases the evidence of adverse effects of dioxin-like compounds. The new findings since *Update 2000* do not appear to be strong enough to change the overall conclusions of the previous reports regarding fertility.

Conclusions

Strength of Evidence from Epidemiological Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and altered hormone concentrations, decreased sperm counts or quality, subfertility, or infertility.

Biologic Plausibility

Experimental-animal evidence suggests that TCDD can alter testosterone synthesis, generally at relatively high doses, but does not provide direct clues to the reproductive significance of alterations in hormone concentrations of the magnitude found in available studies.

A summary of the biologic plausibility of reproductive effects of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of exposures in Vietnam and about the potential risk, if any, for altered hormones, semen quality, and subfertility or infertility, it is not possible for the committee to quantify the degree of risk of infertility likely to be experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

SPONTANEOUS ABORTION

Spontaneous abortion refers to the expulsion of a nonviable fetus, generally before 20 weeks of gestation, not induced through physical or pharmacologic means. The background risk of recognized spontaneous abortion is generally around 7–15% (Hertz-Picciotto and Samuels, 1988), but it is established that many more pregnancies terminate before the woman is aware that she has become pregnant (Wilcox et al., 1988); the latter are known as subclinical pregnancy losses and are generally not included in studies of spontaneous abortion. Estimates of the risk of recognized spontaneous abortion vary with the design and method of analysis. Major types of study design include cohorts of women asked retrospectively about their pregnancy history, cohorts of pregnant women (usually those receiving prenatal care), and cohorts of women who are monitored for future pregnancies. Retrospective reports can be limited by memory loss, particu-

larly of spontaneous abortions that took place a long time before. Studies enrolling women who appear for prenatal care require the use of life tables and specialized statistical techniques to account for differences in the times at which women seek medical care during pregnancy. Enrollment of women before pregnancy provides the theoretically most valid estimate of risk, but it may attract nonrepresentative study groups because the protocols are demanding.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and spontaneous abortion. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change this finding. Reviews of the studies underlying these findings may be found in the earlier reports (see Table 7-4 for summary of studies).

Update of the Scientific Literature

Occupational Studies

Schnorr and colleagues (2001) at the National Institute for Occupational Safety and Health (NIOSH) conducted an investigation of pregnancy outcomes of the wives and partners of men in the NIOSH cohort (see Chapter 4 for a description of the cohort). A brief interview was conducted with the male study subjects, and contact information on current and former wives and partners was collected. In-depth telephone interviews with these partners were conducted to collect reproductive history and medical, lifestyle, and occupational data. All pregnancies after the date of the father's first exposure at the plant were considered exposed. Pharmacokinetic models applied to paternal serum TCDD were used to estimate exposure at the time of each conception. Serum TCDD was measured in 79 of the referents (controls). Those concentrations were assumed to represent lifetime background environmental exposures and were assumed for all pregnancies. The median serum TCDD concentration of the 79 referents (6 ppt) was assigned to the remaining referents. Statistical analyses examined exposure as the log of TCDD at the time of the conception or in five categories (referents, and exposed at <20, at 20 to <255, at 255 to <1,120, and at ≥1,120 ppt).

Multiple births, induced abortions, tubal and other ectopic pregnancies, and pregnancies exposed to oral contraceptives, intrauterine devices, or injections to induce menstruation were excluded. All remaining pregnancies were classified as live birth, stillbirth, or spontaneous abortion (termination no later than 20 weeks

TABLE 7-4 Selected Epidemiologic Studies—Spontaneous Abortion

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Schnorr et al., 2001	NIOSH cohort		
	Levels of Exposure		
	<20 ppt	29	0.8 (0.5–1.2)
	20 to <255 ppt	11	0.8 (0.4–1.6)
	255 to <1120	11	0.7 (0.3–1.6)
	≥1120 ppt	8	1.0 (0.4–2.2)
Arbuckle et al., 2001	Ontario farm families		
	Phenoxyacetic acid herbicide exposure in the pre-conception period and risk of first-trimester spontaneous abortion	48	1.5 (1.1–2.1)
Studies Reviewed in *Update 2000*			
Driscoll, 1998	Women employed by US Forest Service—pregnancies ending in miscarriage	141	2.0 (1.1–3.5)
Studies Reviewed in *VAO*			
Townsend et al., 1982	Wives of men employed at Dow involved in chlorophenol processing (paternal exposure)	85	1.0 (0.8–1.4)
Smith et al., 1982	Follow-up of 2,4,5-T sprayers—sprayers compared to non-sprayers (paternal exposure)	43	0.9 (0.6–1.5)
Carmelli et al., 1981	Spontaneous abortion among wives of men occupationally exposed to 2,4-D (paternal exposure)		
	All reported work exposure to herbicides (high and medium)	63	0.8 (0.5–1.2)
	Farm exposure	32	0.7 (0.3–1.8)
	Forest and commercial exposure	31	0.9 (0.5–1.6)
	Exposure during conception period		
	Farm exposure	15	1.0 (0.4–2.1)
	Forest and commercial exposure	16	1.6 (0.7–3.3)
	All exposures, father 18–25 years old		
	Forest and commercial exposure	8	3.1 (0.9–9.6)
	Exposure during conception period		
	Father 31–35 years old, farm exposure	10	2.9 (0.8–10.9)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	69	0.9 (0.6–1.2)
Moses et al., 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	14	0.9 (0.4–1.8)

continues

TABLE 7-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Tuyet and Johansson, 2001	Women whose husbands were exposed to Agent Orange	*	(*)
Revich et al., 2001	Residents of Chapaevsk, Russia	*	(*)
	Spontaneous abortion per 100 full-term pregnancies for "the last seven years"		
	Chapaevsk 24.4%		
	Samara 15.2%		
	Toliatti 10.6%		
	Syzran 15.6%		
	Novokuibyshevsk 16.9%		
	Other small towns 11.3%		
Studies Reviewed in *Update 2000*			
Petrelli et al., 2000	Wives of pesticide appliers	26	3.8 (1.2–12.0)
Axmon et al., 2000	Wives of Swedish fishermen		
	Miscarriages before week 12		0.5 (0.3–1.0)
	East coast	12	(*)
	West coast	54	(*)
VIETNAM VETERANS			
New Studies			
Kang et al., 2000	Female Vietnam veterans		
	Vietnam veteran spontaneous abortions or stillbirths (1,665 pregnancies)	278	(*)
	Non-Vietnam spontaneous abortions or stillbirths (1,912 pregnancies)	317	(*)
Studies Reviewed in *Update 2000*			
Schwartz, 1998	Female Vietnam veterans—miscarriages	63	(*)
Studies Reviewed in *Update 1996*			
Wolfe et al., 1995	Air Force Ranch Hand veterans	157	
	Background		(*) (0.8–1.5)
	Low-level exposure		(*) (1.0–1.7)
	High-level exposure		1.0 (0.7–1.3)
Studies Reviewed in *VAO*			
CDC, 1989	Vietnam Experience Study (paternal exposure)	1,566	1.3 (1.2–1.4)
	Self-reported low exposure	489	1.2 (1.0–1.4)
	Self-reported medium exposure	406	1.4 (1.2–1.6)
	Self-reported high exposure	113	1.7 (1.3–2.1)

TABLE 7-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Stellman et al., 1988	Assessment of reproductive effects among American Legionnaires who served in Southeast Asia (1961–1975), (paternal exposure)		
	Vietnam veterans compared with Vietnam-era veterans		
	All Vietnam veterans	231	1.4 (1.1–1.6)
	Low exposure	72	1.3 (1.0–1.7)
	Medium exposure	53	1.5 (1.1–2.1)
	High exposure	58	1.7 (1.2–2.4)
	Herbicide handlers compared with Vietnam-era veterans	9	1.6 (0.7–3.3)
	Vietnam veterans with medium or high exposure compared to Vietnam veterans with low exposure		
	Medium exposure	53	1.2 (0.8–1.7)
	High exposure	58	1.4 (0.9–1.9)
Aschengrau and Monson, 1989	Spontaneous abortion and husband's Vietnam service—		
	spontaneous abortions	*	0.9 (0.4–1.9)
	First-trimester abortions	10	1.2 (0.6–2.8)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans (paternal exposure)	195	1.6 (1.3–2.0)

[a] Given when available.

* Information not provided by study authors.

ABBREVIATIONS: CDC, Centers for Disease Control and Prevention; CI, confidence interval; NIOSH, National Institute for Occupational Safety and Health.

after the last menstrual period). Workers' wives and partners had a total of 332 pregnancies during or after the workers' exposure, and 300 conceived before exposure; 707 pregnancies in the wives and partners of the referent group were included. Of those, 35, 25, and 89 miscarriages, respectively, occurred by 20 weeks of gestation, and four, three, and six stillbirths (after 20 weeks gestation) occurred, respectively. Generalized estimating equations were used to adjust for multiple pregnancies per woman. Many covariates were evaluated for inclusion as confounders. For the four levels of exposure, the adjusted ORs were 0.8, 0.8, 0.7 and 1.0, respectively; the confidence intervals had widths of 2.5–5 and included the null in all cases. Early and late spontaneous abortions did not differ with respect to the OR for TCDD exposure.

The strengths of this study include the pharmacokinetically based quantita-

tive exposure data on the fathers, the wide range of paternal exposure, the confirmation of all pregnancies, and the collection of and adjustment for many potential confounders. Limitations include the moderate response rate (73% and 78% among referents' and workers' wives, respectively) and the relatively small number of exposed cases of fetal loss (35 spontaneous abortions and four stillbirths). The literature on retrospective interviews for recall of pregnancies suggests that live births are remembered well but some spontaneous abortions are not recalled and that recall worsens as time elapses (Wilcox and Horney, 1984; Heidam and Olsen, 1985). Most of the pregnancies in this study occurred during the 1950s and 1960s, so the women were recalling events that occurred 3 or 4 decades previously. No measure of take-home contamination was available, and the results should not be construed as providing evidence regarding the effect of maternal exposure on pregnancy outcome.

Environmental Studies

Tuyet and Johansson (2001) conducted a series of semistructured interviews with women who were or whose husbands were exposed to Agent Orange. The goal was to determine the impact of Agent Orange exposure on the women's lives. This was a case series and provides insight into the lives of individual Vietnamese women and their children, many of whom had congenital malformations or developed disabilities within the first years of life. For those whose husbands came back from the war as invalids, the hardship was both physical and emotional. The paper does not attempt to generate quantitative data.

The Ontario Farm Family Health Study conducted by Arbuckle et al. (2001) collected data on pesticide use, medical and reproductive history, and lifestyle retrospectively from farm operators and couples living on farms. Eligible couples were those in which the wife was 44 years old or younger and at least one member was working on the farm. In this analysis, the outcome of interest was self-reported spontaneous abortion at less than 20 weeks of gestation. The husband, wife, and farm operator (if other than husband or wife) were all interviewed, with both open-ended and checklist questions, and the resulting information was pooled to construct a history of monthly agricultural and residential pesticide use. The active ingredients of each pesticide were identified and grouped into use categories and chemical families. For each pregnancy, exposure to pesticides was analyzed for two periods: preconception, representing the 4-month period from 3 months before conception to the calendar month of conception, and postconception, the period from the first calendar month after conception to the end of the pregnancy or through the end of the first trimester, whichever came first. Crude ORs were calculated, comparing those exposed to the pesticide of interest during the period with those not exposed to that pesticide during the same period. The authors found that no variables confounded the associations and therefore reported only crude analyses. In addition, the classification and regres-

sion tree (CART) method was used to examine interactions between pesticides and other factors.

Phenoxyacetic acid herbicide exposure in the preconception period was associated with a higher risk of first-trimester spontaneous abortion (OR = 1.5, 1.1–2.1, based on 48 exposed cases). The authors note that in an earlier publication on the same cohort, a referent group consisting of those with no pesticide exposure during the period yielded a higher OR of 2.3. The "use" class of herbicides (78 exposed cases) also showed a modestly increased risk (OR = 1.4, 1.1–1.9). For exposure in the postconception period in relation to first-trimester spontaneous abortion, all ORs but one (miscellaneous pesticides) were less than 1.0. For exposure in the postconception period in relation to second-trimester spontaneous abortion, 2,4-D was associated with a moderately increased risk (OR = 1.6, 0.9–2.7, based on 16 exposed cases). In the analysis of interactions, the authors found that women who were 35 years old or older were most susceptible to the effects of pesticides: those exposed to both carbaryl and 2,4-D in the preconception period were 27 times more likely to have a spontaneous abortion than those with only carbaryl exposure, although the confidence interval was extremely wide (2.0–368). An analysis of chemical classes demonstrated an interaction of phenoxy herbicides with triazines.

Several methodologic concerns affect the interpretation of those results. One is the assignment of postconception exposure, a time-dependent variable: the opportunity to be exposed is longer among noncases than among cases (Hertz-Picciotto et al., 1996). Exposure for a first-trimester pregnancy termination could occur only before the spontaneous abortion, but for noncases it could occur at any time during the full first trimester, so it is not surprising that all ORs for this period were less than 1.0. In other words, those ORs are downwardly biased estimates of the effect of exposure. A survival analysis could have eliminated the problem if the information on timing of exposure had been exact (Hertz-Picciotto et al., 1989). This concern would apply only to the analysis of first-trimester spontaneous abortions and not to the analysis of late spontaneous abortions (weeks 12–19) or the analysis of preconception exposure in relation to first- or second-trimester losses, in that all pregnancies would have the same opportunity for exposure in this period. Overall, the study was based on a thorough interview to develop exposure indexes and involved a large cohort (3,936 pregnancies among 2,000 farm couples). Self-reports of spontaneous abortion have been found to have high validity (Wilcox and Horney, 1984; Lindbohm and Hemminki, 1988), but some are not remembered, and memory worsens as time elapses (Wilcox and Horney, 1984; Heidam and Olsen, 1985). Also, it is not clear whether self-reports of the timing of pregnancy loss are accurate. The authors did not account for the multiple pregnancies per woman, and the reported standard errors are downwardly biased in that the propensity to abort is known to cluster in pregnancies of the same woman (Watier et al., 1997). Generalized estimating equations should have been used to produce correct confidence limits, but the effect on precision of

not using those equations is usually not large in populations with small family sizes. The point estimates of the OR would not be affected. The authors did not address how induced abortions were handled. In spite of some concerns about the analysis, this study suggests reproductive toxicity of phenoxy herbicides. Spontaneous abortions occur at a higher rate in women exposed to them during the preconception period, including the month of conception, and this effect is particularly strong in women 35 years old or older. The findings are characterized by internal consistency in that the individual pesticide (2,4-D), the chemical class (phenoxyacetic acids), and the "use" category of herbicides all produce similar results.

Numerous studies of populations with high dietary intake of PCBs from consumption of fatty fish in the Baltic Sea have examined reproductive outcomes. Axmon et al. (2000) collected information on miscarriages and stillbirths from a cohort of fishermen's wives from the Swedish east coast ($N = 438$) and a referent cohort of west coast fishermen's wives ($N = 983$) with a retrospective self-administered questionnaire. Demographic, lifestyle, and occupational information was also collected. To avoid using nonindependent observations resulting from multiple pregnancies per woman, only the first planned pregnancy was included in the analysis. Only physician-confirmed or home-pregnancy-test-confirmed pregnancies were included. Initial comparisons were between the east and west coast women. Fish consumption was also assessed for the current period, and the 179 women who consumed at least two meals of fish per week were compared with the 73 who consumed no fatty fish.

After adjustment for confounders, the east coast fishermen's wives experienced a deficit of miscarriages in the first trimester (OR = 0.5, 0.3–1.0). A similarly lower risk was observed for high consumers of fatty fish compared with nonconsumers. Those results suggest that the risk of spontaneous abortion or stillbirth is not increased in women who consume fatty fish contaminated with PCBs. Whether there is no adverse effect of PCBs and other concomitant contaminants or some adverse effect is outweighed by the benefits of fatty-fish consumption remains unclear.

The association between TCDD exposure and spontaneous abortion was examined in a study conducted in Chapaevsk, a town in Russia that is the site of a chemical plant that produced hexachlorocyclohexane (lindane) and its derivatives in 1967–1987. Mustard gas and other chemical blister agents were produced there previously, and crop-protection chemicals now (Revich et al., 2001). Contamination of air, water, cows' milk, and human serum and breast milk has been documented; exposure declines with increasing distance from the factory. In a report that surveyed a variety of exposure and public-health indicators, Revich and colleagues obtained official medical statistics for Chapaevsk, for the surrounding region of Samara, and for several other areas. The mean frequency, defined as number of spontaneous abortions per 100 full-term pregnancies in the preceding 7 years, was 24.4 in Chapaevsk, 15.2 in Samara, 10.6 in Toliatti, 15.6

in Syzran, 16.9 in Novokuibyshevsk, and 11.3 in small towns. It is difficult to interpret those results, because there are no demographic or lifestyle data comparing Chapaevsk with other areas.

Vietnam-Veteran Studies

Kang et al. (2000) conducted a survey of female Vietnam veterans. A cohort was assembled on the basis of records from all branches of the military and consisted of both Vietnam veterans and women who served in the military during the Vietnam War but were stationed at bases in the United States. Military-service data were abstracted from personnel records. After record review, 4,643 women met eligibility criteria of having had a permanent tour of duty in Vietnam in the period July 4, 1965, through March 28, 1973, a period of substantial US military involvement in Vietnam. Of them, 4,390 were alive on January 1, 1992. A comparison group of female veterans whose tour of duty did not include service in Vietnam but who were assigned a military unit in the United States during the Vietnam War was identified, and 4,390 were randomly selected from among those still alive on January 1, 1992. After exclusion of 250 from each group who participated in a pilot study, an attempt was made to locate the remaining 4,140 in each group, for a total eligible cohort of 8,280. Various location strategies were used; 370 (less than 5%) were not located, and another 339 were deceased. A full telephone interview was conducted on 6,430, after 775 refused (13% of Vietnam veterans and 17% of non-Vietnam veterans) and another 336 completed only a short written questionnaire, which collected information on demographic background, lifestyle factors, reproductive history, military experience, use of oral contraceptives and hormone-replacement therapy, and health status.

For each pregnancy, information on smoking, drinking, complications, infections, medications, exposure to x-rays, occupational history, and exposure to anesthetic gases, ethylene oxide, herbicides, and pesticides was collected. An index pregnancy was defined for each woman. For the Vietnam veterans, it was the first pregnancy after entry into Vietnam service; for the non-Vietnam veterans, it was the first pregnancy after July 4, 1965, the starting date for US ground-troop involvement in Vietnam, or after the entrance date into military service, whichever came later. Fetal loss included both spontaneous abortion and stillbirth; these were not separated. The logistic-regression models for those adverse outcomes of pregnancy vs a live birth adjusted for age at conception, education, race, marital status, military characteristics of the mother's service (branch, rank, and military nursing), and lifestyle factors (smoking, drinking, and average number of hours worked during pregnancy). There were 278 spontaneous abortions or stillbirths among 1,665 Vietnam-veteran index pregnancies, and 317 out of 1,912 among the non-Vietnam-veteran pregnancies, representing rates of 16.7% and 16.6%. Although a smaller number of Vietnam than non-Vietnam veterans re-

ported ever having been pregnant, that was because more of them never tried; the main reasons for never having tried to become pregnant were "never married" and "never wanted children." Those data do not suggest an association between service in Vietnam and pregnancy loss. However, because the authors had no indexes of exposure to the chemicals of interest, the study does not provide evidence on whether TCDD or the herbicides 2,4-D and 2,4,5-T affect the likelihood of spontaneous abortion or stillbirth. The authors did not state how they handled induced abortions.

Synthesis

Because the literature on TCDD shows different results with regard to spontaneous abortion than does the evidence on herbicides, this discussion addresses the two exposures separately. In light of two well-conducted studies that were recently published (Schnorr et al., 2001; Arbuckle et al., 2001), the committee reexamined in detail all the studies relevant to spontaneous abortion and the exposures of interest in that light.

The strongest evidence to date regarding preconception paternal exposures to TCDD in relation to spontaneous abortion comes from the occupational study by Schnorr et al. (2001). That study was able to assign exposures and corresponding pregnancies to a sizable number of men. Despite a large range of exposures, no association was observed, even for the most highly exposed. Some earlier studies have shown slight increases in risk of spontaneous abortion among those who served in Vietnam (Stellman et al., 1988; CDC, 1989), among the low- but not the high-exposure Ranch Hand Air Force personnel (Wolfe et al., 1995), and in a study of Tasmanian men who served in the Australian services in Vietnam (Field and Kerr, 1988). However, control selection and outcome definition were weak in the Australian veteran study. The Stellman and Centers for Disease Control (CDC) studies, although stronger, used paternal reports of pregnancy outcomes of partners, which are known to be error-prone and the errors could be in the direction of creating artifactual associations. CDC also conducted a validation substudy of reported birth defects and found evidence of reporting bias in comparisons of Vietnam with non-Vietnam veterans. In the analysis of miscarriages, the association was stronger among those who reported herbicide exposure, and this was interpreted by the authors as evidence of reporting bias due to the inaccuracy of self-reports of herbicide exposure. Given the lack of an increased risk among highly exposed men in the Air Force Health Study, the other studies are not of sufficient quality to strengthen the evidence of an effect of TCDD or herbicides on spontaneous abortion.

The strengths of the study by Schnorr et al. (2001) provide convincing evidence that TCDD is unlikely to increase the risk of spontaneous abortion. That does not, however, rule out the possibility that herbicides used in Vietnam, such as 2,4-D and 2,4,5-T, are associated with an increased risk. The results of

Arbuckle et al. (2001), who used thorough exposure assessment, are remarkably consistent for various measures of phenoxy-herbicide exposures during the preconception period. Increased risks of spontaneous abortion were observed for the use category of "herbicides" and specifically for phenoxyacetic acid, and the numbers of exposed cases were 78 and 48, respectively. Whether maternal or paternal exposure or both played a role remains unclear, in that the analysis pooled the information for an overall "farm" exposure. An earlier study of women working for the Forest Service also showed an increased risk in those with herbicide exposure although it suffered from a low response rate (Driscoll, 1998).

In contrast, a case–control study of an occupational cohort constructed to include a high proportion of workers with 2,4-D exposure observed no association with paternal exposure either during the 2–3 years before conception or during the 2-month period consisting of the month before conception and the month of conception (Carmelli et al., 1981). This study also suffered from a low response rate. No association was observed with maternal occupational or environmental exposure.

Overall, it is difficult to determine whether the biases in those two studies would have attenuated or magnified an association. In a small study, Petrelli et al. (2000) found that pesticide appliers reported more spontaneous abortions among their wives than did a comparison group of food retailers, but confounders were not all controlled. The list of pesticides applied during the period of employment of these workers included several that may have been contaminated by TCDD, but the published report did not address particular exposures.

Several other studies were too weak to provide evidence of an association or of *no* association. The present report reviews the negative study by Kang et al. (2000), which compared women who served in Vietnam with those who served elsewhere but had no direct measures of exposure to TCDD or herbicides, and the ecological comparisons by Revich et al. (2001), which failed to provide any data on potential confounding variables. Earlier inadequate investigations that the committee reviewed include a report on the wives of men who were employed by Dow Chemical Company, in which an obscure, nonstandard method of analysis was used (the overall results showed no association, but an association was observed in a specific stratum of women with high gravidity) (Townsend et al., 1982); a small study of 2,4,5-T sprayers, which had very crude data on the timing of exposure and failed to adjust for Maori ethnicity in spite of a difference between exposed and nonexposed (Smith et al., 1982); and a report on 2,4,5-T production workers, which relied on men's reports of their spouses' pregnancy outcomes (Suskind and Hertzberg, 1984).

On the basis of the NIOSH study, the evidence is therefore strong that paternal exposure to TCDD is not associated with an increased risk of spontaneous abortion among partners. Despite the strengths of the study by Arbuckle et al. (2001), the evidence regarding herbicides and spontaneous abortion remains inadequate to determine whether an association exists, primarily because of the

limitations of the numerous studies that have attempted to evaluate pesticides and spontaneous abortion.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic literature examining spontaneous abortion reviewed in this and previous *Veterans and Agent Orange* reports, the committee notes that the evidence on TCDD and the evidence on herbicides used in Vietnam are divergent. For TCDD, the data suggest that paternal exposure to TCDD is not associated with risk of spontaneous abortion, but the data are inadequate to determine whether an association with maternal exposure to TCDD exists. For herbicides—namely 2,4-D and 2,4,5-T—the committee finds that there is inadequate or insufficient evidence to determine whether an association exists. Overall, the committee finds that the data are inadequate or insufficient to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and the risk of spontaneous abortion in pregnancies that begin after exposure.

Biologic Plausibility

Experimental-animal evidence suggests that TCDD can alter hormones after low-dose exposure and can cause fetal lethality after high doses. However, the reproductive significance of those effects and the risk of recognized pregnancy loss before 20 weeks of gestation in humans are not clear. There is no evidence to suggest a relationship between paternal exposure to TCDD and spontaneous abortion. In experimental animals, 2,4-D and 2,4,5-T have been shown to cause fetal toxicity and lethality following maternal exposure. However, this occurs only at high doses and in the presence of maternal toxicity. No fetal toxicity or lethality has been found following paternal exposure to 2,4-D.

A summary of the biologic plausibility of the reproductive effects of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Studies of the outcomes of pregnancies of female Vietnam veterans or women whose partners were Vietnam veterans have provided uncertain results because of methodologic limitations. It is therefore not possible for the committee to quantify the degree of risk of spontaneous abortion due to exposure to herbicides

or the contaminant TCDD in pregnancies that began after such exposure in Vietnam.

STILLBIRTH, NEONATAL DEATH, AND INFANT DEATH

Stillbirth or *late fetal death* typically refers to the delivery of a fetus at or after 20 weeks of gestation that shows no signs of life, although a more recent definition includes deaths among all fetuses that weigh more than 500 g at birth, regardless of gestational age at delivery (Kline et al., 1989). *Neonatal death* refers to the death of a liveborn infant within the first 28 days after birth.

Because the causes of stillbirth and early neonatal death overlap considerably, they are commonly analyzed as one group, referred to as *perinatal mortality* (Kallen, 1988). Stillbirths occur in less than 1% of all births (CDC, 2000). Among low-birthweight (500–2,500 g) liveborn and stillborn infants, 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 g at birth, the most common causes of perinatal death are complications of the cord, placenta, and membranes and lethal congenital malformations (Kallen, 1988).

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, and cacodylic acid) and stillbirth, neonatal death, and infant death. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Reviews of the studies underlying those findings may be found in the earlier reports.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant

TCDD, picloram, and cacodylic acid) 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 summary of the biologic plausibility of reproductive effects of TCDD and the herbicides in general is presented at the end of this chapter. Chapter 3 discusses recent toxicologic studies that concern biologic plausibility.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk of stillbirth, neonatal death, and infant death, it is not possible for the committee to quantify the degree of risk likely to be experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

LOW BIRTHWEIGHT AND PRETERM DELIVERY

The World Health Organization (WHO) recommends a cut point of 2,500 g for the determination of low birthweight (Alberman, 1984). Low infant weight at birth is one of the most important predictors of neonatal mortality and morbidity in the United States, and preterm delivery is one of the most important causes. The concept of low birthweight actually encompasses two different causal pathways often treated as a single entity: low birthweight secondary to intrauterine growth retardation (IUGR), in which case a fetus or baby is referred to as small for gestational age, and low birthweight secondary to preterm delivery (PTD), which may have other long-term consequences. The concept of IUGR represents birthweight adjusted for gestational age. The current definition of PTD is delivery at less than 259 days, or 37 completed weeks, of gestation, calculated on the basis of the date of the first day of the last menstrual period (Bryce, 1991). About 7% of live births have low birthweight. The incidence of IUGR is much more difficult to quantify because there are no universally applied standards for distributing birthweight by gestational age. When no distinction is made between the causes of low birthweight (IUGR vs 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 weight, birth order, maternal complications during pregnancy (such as severe preeclampsia) and obstetric history, job stress, and cocaine or caffeine use during pregnancy (Kallen, 1988). Established risk factors for PTD include race (black), marital status (single), low SES, previ-

ous low birthweight or PTD, multiple gestations, cigarette-smoking, and cervical, uterine, or placental abnormalities (Berkowitz and Papiernik, 1993).

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and low birthweight. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Reviews of the studies underlying those findings may be found in the earlier reports.

Update of Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2000* (IOM, 2001).

Environmental Studies

The possible association between TCDD exposure and spontaneous abortion was examined in a study conducted in Chapaevsk, a town in Russia that is the site of a chemical plant that produced hexachlorocyclohexane (lindane) and its derivatives in 1967–1987. Mustard gas and other chemical blister agents were produced there previously, and crop-protection chemicals now (Revich et al., 2001). Contamination of air, water, cows' milk, and human serum and breast milk has been documented; exposure declines with increasing distance from the factory. In a report that surveyed a variety of exposure and public-health indicators, Revich and colleagues obtained official medical statistics for Chapaevsk, for the surrounding region of Samara, and for several other areas. The average rate of PTD was 45.7 per 1,000 deliveries in Chapaevsk, 39.9 in Samara, 45.8 in Toliatti, 36.5 in Novokuibyshevsk, and 30.0–38.4 in small towns. The authors report a prevalence of low birthweight of 7.1%, compared with 5.1–6.2% in Russia and in most of the Samara towns. It is difficult to interpret those results, because there are no demographic or lifestyle data comparing Chapaevsk with the other areas. The rate of PTD in Chapaevsk, however, is not high by US standards.

Vietnam-Veteran Studies

Kang et al. (2000) conducted a survey of female Vietnam veterans. A cohort was assembled on the basis of records from all branches of the military and

consisted of both Vietnam veterans and women who served in the military during the Vietnam War but were stationed at bases in the United States. Military-service data were abstracted from personnel records. After record review, 4,643 women met eligibility criteria of having had a permanent tour of duty in Vietnam in the period July 4, 1965, through March 28, 1973, a period of substantial US military involvement in Vietnam. Of them, 4,390 were alive on January 1, 1992. A comparison group of female veterans whose tour of duty did not include service in Vietnam but who were assigned to a military unit in the United States during the Vietnam War was identified, and 4,390 were randomly selected from among those still alive on January 1, 1992. After exclusion of 250 from each group who participated in a pilot study, an attempt was made to locate the remaining 4,140 in each group, for a total eligible cohort of 8,280. Various location strategies were used; 370 (less than 5%) were not located, and another 339 were deceased. A full telephone interview was conducted on 6,430, after 775 refused (13% of Vietnam veterans and 17% of non-Vietnam veterans) and another 336 completed only a short written questionnaire, which collected information on demographic background, lifestyle factors, reproductive history, military experience, use of oral contraceptives and hormone-replacement therapy, and health status.

For each pregnancy, information on smoking, drinking, complications, infections, medications, exposure to x-rays, occupational history, and exposure to anesthetic gases, ethylene oxide, herbicides, and pesticides was collected. An index pregnancy was defined for each woman. For the Vietnam veterans, it was the first pregnancy after entry into Vietnam service; for the non-Vietnam veterans, it was the first pregnancy after July 4, 1965, the starting date for US ground-troop involvement in Vietnam, or after the entrance date into military service, whichever came later. Low birthweight was defined as <2,500 g (5 lb, 8 oz) in a singleton delivery. PTD was defined as delivery at no more than 37 weeks or 8 months (if reported in months) of gestation. Logistic-regression analyses were adjusted for age at conception, education, race, marital status, military characteristics of the mother's service (branch, rank, and military nursing), lifestyle factors (smoking, drinking, and average number of hours worked during pregnancy), and pregnancy complications (toxemia, diabetes, high blood pressure, bleeding, or threatened miscarriage).

Of 1,229 index live births among Vietnam veterans, the rates of low birthweight and PTD were 6.3% and 9.1%, respectively; of 1,460 non-Vietnam-veteran pregnancies, the figures were 6.6% and 8.6%. Adjusted ORs were 1.1 (95% CI 0.8–1.5) for low birthweight and 1.2 (95% CI 0.9–1.6) for preterm delivery.

Synthesis

Of the two newly published studies on low birthweight and PTD, the environmental study does not adjust for possible differences among communities, and

the Vietnam-veteran study shows small non-significant elevations in risk of these outcomes between women who served in Vietnam and women who served elsewhere.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and low birthweight or PTD.

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. Regarding female-mediated developmental toxicity, TCDD and herbicides are found in follicular fluid (Tsutsumi et al., 1998), suggesting exposure of embryos, and are known to cross the placenta and lead to direct exposure of the fetus. A more detailed discussion of biologic plausibility is found at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Given the uncertainties about the exposure of Vietnam veterans and the magnitude of potential risk of low birthweight and PTD, it is not possible for the committee to quantify the degree of risk to Vietnam veterans of having pre-term deliveries or children with low birthweight.

CHILDHOOD CANCER

The American Cancer Society estimates that about 8,600 children under the age of 15 years will be diagnosed with cancer in the United States in 2001. Nearly half the cases will be in children 0–4 years old. Treatment and supportive care of children with cancer have greatly improved, and mortality rates have declined by 50% over the last 3 decades. Despite those advances, cancer remains the leading cause of death from disease in children under the age of 15 years, with 1,500 deaths projected in 2001.

Leukemia is the most common cancer in children. It accounts for about one-third of all childhood cancer cases; nearly 2,700 children are projected to be

diagnosed in 2001 (ACS, 2001). Of those, nearly 2,000 will be diagnosed with acute lymphocytic leukemia (ALL) and most of the rest with acute myelogenous leukemia (AML)[1]. ALL is most common in early childhood, peaking between the ages of 2 and 3 years, and AML is most common during the first 2 years of life. ALL incidence is consistently higher in boys than in girls, whereas AML has a similar incidence in boys and girls (NCI, 2001). Through early adulthood, ALL rates are about twice as high in whites as in blacks, whereas AML has no consistent pattern. Chapter 6 contains additional information on leukemia as part of the discussion of adult cancer.

The second-most common group of cancers in children are those of the central nervous system—the brain and the spinal cord. Other cancers in children include lymphomas, bone cancers, soft-tissue sarcomas, kidney cancers, eye cancers, and adrenal gland cancers. Compared with adult cancers, relatively little is known about the etiology of most childhood cancers, especially about potential environmental risk factors and the effect of parental exposures.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and childhood cancers. Additional information available to the committees responsible for *Update 1996* and *Update 1998* did not change that finding. The committee responsible for *Update 2000* reviewed the material in earlier *Veterans and Agent Orange* reports and newly available published literature and determined there was limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and AML. After the release of *Update 2000*, researchers from one of the studies discovered an error in their published data. The committee reconvened to evaluate the previously reviewed and new literature regarding that illness, and the *Acute Myelogenous Leukemia* (IOM, 2002) report was produced. It reclassified AML from "limited/suggestive evidence of an association" to "inadequate evidence to determine whether an association exists." Table 7-5 provides summaries of the results of the studies underlying that conclusion.

[1]Acute myelogenous leukemia (ICD-9 205) is also commonly referred to as "acute myeloid leukemia" and "acute nonlymphocytic leukemia." There are also numerous subtypes of the disease. For consistency, this report uses *acute myelogenous leukemia*, or the abbreviation AML, regardless of usage in the source materials.

TABLE 7-5 Selected Epidemiologic Studies—Childhood Cancers

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL STUDIES			
Heacock et al., 2000	Cohort of sawmill workers' offspring—exposure via fungicides contaminated with PCDDs and PCDFs		
	Leukemia all workers (paternal exposure)	11	SIR = 1.0 (0.5–1.8)
	Brain cancer all workers (paternal exposure)	9	SIR = 1.3 (0.6–2.5)
	Leukemia, high chlorophenate exposure (paternal exposure)	5	OR = 0.8 (0.2–3.6)
	Brain cancer, high chlorophenate exposure (paternal exposure)	5	OR = 1.5 (0.4–6.9)
Buckley et al., 1989	Children's Cancer Study Group—case–control study of children of parents exposed to pesticides or weed killers		
	AML in children with any paternal exposure	27	OR = 2.3 (p = 0.05)
	AML in children with paternal exposure >1,000 days	17	OR = 2.7 (1.0–7.0)
	AML in children with maternal exposure >1,000 days	7	OR undefined (no cases in controls)
ENVIRONMENTAL STUDIES			
New Studies			
Buckley et al., 2000	Cases of NHL diagnosed at the age of ≥ 20 years in 1986–1990	*	(*)
Daniels et al., 2001	Case–control study of neuroblastoma in children whose:		
	Parents reported using pesticides in the home	*	1.6 (1.0–2.3)
	Parents reported using herbicides in the garden	*	1.9 (1.1–3.2)
	Mothers reported applying herbicides in the garden	*	2.2 (1.3–3.8)
Kerr et al., 2000	Neuroblastoma risk in children		
	Mothers whose occupation involves handling insecticides	40	2.3 (1.4–3.7)
	Fathers exposed to dioxin	7	6.9 (1.3–68.4)
Studies Reviewed in *Update 2000*			
Meinert et al., 2000	Population-based case-control study of childhood cancer		
	Leukemia, paternal exposure, year before pregnancy	62	1.5 (1.1–2.2)
	Leukemia, paternal exposure, during pregnancy	57	1.6 (1.1–2.3)

continues

TABLE 7-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	Lymphoma, paternal exposure, year before pregnancy	11	1.5 (0.7–3.1)
	Lymphoma, paternal exposure, during pregnancy	10	1.6 (0.7–3.6)
	Leukemia, maternal exposure, year before pregnancy	19	2.1 (1.1–4.2)
	Leukemia, maternal exposure, during pregnancy	15	3.6 (1.5–8.8)
	Lymphoma, maternal exposure, year before pregnancy	3	2.9 (0.7–13)
	Lymphoma, maternal exposure, during pregnancy	4	11.8 (2.2–64)
Pearce and Parker, 2000	Cohort study examining paternal occupation on death certificate of children who died of kidney cancer	(total cases =	0.9 (0.2–3.8)
	Paternal agricultural occupation	21)	
Infante-Rivard et al., 1999	Population-based case–control study of childhood ALL and household herbicide use during pregnancy, in utero exposure, others not excluded	118	1.8 (1.3–2.6)
Studies Reviewed in *Update 1996*			
Pesatori et al., 1993	Seveso residents 0–19 years old—10-year follow-up, morbidity, all exposures		
	All cancer	17	1.2 (0.7–2.1)
	Ovary and uterine adnexa	2	— (0 expected)
	Brain	3	1.1 (0.3–4.1)
	Thyroid	2	4.6 (0.6–32.7)
	Hodgkin's lymphoma	3	2.0 (0.5–7.6)
	Lymphatic leukemia	2	1.3 (0.3–6.2)
	Myeloid leukemia	3	2.7 (0.7–11.4)
Bertazzi et al., 1992	Seveso residents 0–19 years old—10-year follow-up, mortality, all exposures		
	All cancer	10	7.9 (3.8–13.6)
	Leukemia	5	3.9 (1.2–1.8)
	Lymphatic leukemia	2	1.6 (0.1–4.5)
	Myeloid leukemia	1	0.8 (0.0–3.1)
	Leukemia, others	2	1.6 (0.1–4.6)
	Central nervous system tumors	2	1.6 (0.1–4.6)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AIHW, 2000	Australian Vietnam veterans' children—Validation Study		
	AML	13 (estimated)	3 expected (0–6)

TABLE 7-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Wen et al., 2000	Case–control study of children's leukemia		
	AML and ALL		
	Father ever served in Vietnam or Cambodia	117	1.2 (0.9–1.6)
	<1 year in Vietnam or Cambodia	61	1.4 (0.9–2.0)
	>1 year in Vietnam or Cambodia	49	1.2 (0.8–1.7)
	AML only		
	Father ever served in Vietnam or Cambodia	40	1.7 (1.0–2.9)
	<1 year in Vietnam or Cambodia	13	2.4 (1.1–5.4)
	>1 year in Vietnam or Cambodia	16	1.5 (0.7–3.2)
Studies Reviewed in *VAO*			
CDC, 1989	Vietnam Experience Study		
	Cancer in children of veterans (paternal exposure)	25	1.5 (0.7–2.8)
	Leukemia in children of veterans (paternal exposure)	12	1.6 (0.6–4.0)
Field and Kerr, 1988	Cancer in children of Australian Vietnam veterans (paternal exposure)	4	(*)
Erikson et al, 1984b	CDC Birth Defects Study		
	"Other" neoplasms—children of Vietnam veterans (paternal exposure)	87	1.8 (1.0–3.3)

[a] Given when available.

* Information not provided by study authors.

—When information was denoted by a dash in the original study.

ABBREVIATIONS: AIHW, Australian Institute of Health and Welfare; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CDC, Centers for Disease Control and Prevention; CI, confidence interval; NHL, non-Hodgkin's lymphoma; OR, odds ratio; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofurons; SIR, standardized incidence ratio.

Update of Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2000* (IOM, 2001).

Environmental Studies

Two recent papers address risk factors for neuroblastoma in children (Daniels et al., 2001; Kerr et al., 2000). Children in New York state, excluding New York

City, who developed this form of brain cancer were significantly more likely than community-matched controls to have mothers whose occupation involved handling insecticides (OR = 2.3, 1.4–3.7) or fathers who were exposed to dioxins (6.9, 1.3–68.4). Similarly increased risks were observed in association with mother's employment in retail trade and maternal exposure to lead (Kerr et al., 2000).

Daniels et al. (2001) found that children enrolled in treatment for neuroblastoma in one of two major clinical-trial groups were significantly more likely than community-matched controls to have both parents report having ever used pesticides in the home (1.6, 1.0–2.3); estimated risks did not achieve statistical significance for pesticide use during specific periods (preconception, pregnancy, or during childhood) and for use of pesticides by one of the parents. Among people who used pesticides in the garden, the association was strongest for reports of herbicide use (1.9, 1.1–3.2); when they were asked who had applied the chemicals in the garden, the association was strongest in the subset of mothers who reported applying the pesticide (2.2, 1.3–3.8).

Buckley et al. (2000) examined childhood non-Hodgkin's lymphoma (NHL) in relation to a wide array of exposures during gestation and childhood. The authors participate with a consortium of hospitals that conduct epidemiologic studies of rare childhood cancers. Cases of NHL diagnosed at the age of 20 years or less in 1986-1990 were eligible. The case definition also included those with " 'lymphomatous leukemia,' defined as leukemia with bulk disease in the mediastinum, peripheral lymph nodes, liver, spleen or other abdominal site and at least 25% lymphoblasts in the bone marrow." The final analysis included 268 cases and an equal number of controls who were matched, to the extent feasible, on date of birth, race, and sex. The questionnaire included a broad array of characteristics, including childhood infections, allergies, vaccinations and immune-related disorders, family history of malignancies, and other lifestyle and exposure-related factors.

Five main questions addressed pesticide exposure: household insecticide use by the mother, garden sprays by the mother, exterminations around the home, herbicide or pesticide exposure of the child, and occupational pesticide exposure of either parent. Statistical analyses evaluated NHL combined with the lymphomatous leukemias and in immunologic, histologic, and age-at-diagnosis categories. Cases were similar to controls with respect to age, sex, race, and education. For each of the five questions, cases were more likely to have been exposed than controls. No question addressed herbicides alone. Subtype analyses revealed similar risks for B-cell and T-cell lineage of the lymphoma. Some variation was seen in histologic subtypes for associations with large ORs, for example, household insecticide use with lymphoblastic histology, insect extermination with large-cell and Burkitt lymphoma, child's exposure with large-cell and Burkitt lymphoma, and occupational exposure with Burkitt lymphoma. Overall, the primary

focus of the study was childhood infections, so the questions regarding pesticide exposure were few. Most important for the purposes of this update, no information on herbicides as a class, distinct from insecticides or other pesticides, was available. In addition, exposures before conception were not singled out, nor was a distinction between maternal and paternal exposure made. Another point of concern is the omission of response rates for cases and controls.

Vietnam-Veteran Studies

Pham and Lannigan (2001) reported a single case of carcinoma of the larynx in a 7-year old child born in Australia, whose father, of Vietnamese origin, was exposed to herbicides during the Vietnam conflict. Cancer of the larynx is rare but not unknown in children, and most cases are of squamous-cell origin. Historically, such cases were associated with radiation. The authors do not itemize the risk factors that they believe were ruled out but state "further inquiries with regards to family history, social and demographic factors and environmental exposure to cigarette smoke or toxic substances were negative." The conclusion that there is limited or suggestive evidence of an association between laryngeal cancer and the exposure of concern in adults is presented elsewhere in this volume. Adult risk factors may not apply to children in this case as in other malignancies. However, one case report of a young adolescent (cited in Pham and Lannigan, 2001) suggests at least one shared risk factor in cigarette-smoking. There is insufficient evidence to assess the risk posed by paternal exposure to Agent Orange or arsenic compounds on the basis of a single case.

Synthesis

A single case study of a malignancy, even a rare cancer, among the many children of Vietnam-era veterans does not constitute evidence of an association. It suggests only that such an event is possible. Whether such an event may occur by chance alone or there is an underlying association cannot be determined from a single case report. Such case reports are generally regarded as hints that it would be prudent to look for more cases or to conduct a study. In this situation, however, the medical community is alert, public interest is high and the outcome is very unusual. There are many incentives to identify and report such cases, but the absence of other reported cases in such situations is usually taken as evidence that the event is unlikely to be frequent.

The evidence of an association between pesticides, possibly including herbicides or TCDD exposure, and neuroblastoma in children conceived around the time of exposure is suggestive. The evidence of such an effect among offspring of Vietnam veterans conceived months or years later is inadequate and insufficient.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and childhood cancers.

Biologic Plausibility

Susceptibility to cancers in childhood following environmental exposures may be influenced by several factors, one of which is that the child may inherit a genetic susceptibility trait that would increase the likelihood of developing cancer after exposure to a carcinogen. The mother or father would have to transmit an acquired genetic defect that predisposed the child to cancer, and the child could be exposed to a carcinogen in utero or by exposure to a potent carcinogen in infancy or early childhood either directly or by exposure in breast milk. TCDD and dioxin-like compounds cross the placenta and are present in breast milk, so a pathway of exposure is demonstrated. Women who were breastfed as infants appear to have a lower incidence of endometriosis, a finding that has led to speculation that the effects of TCDD and dioxin-like compounds with estrogen-like activity may persist beyond childhood, even into adult life (Tsutsumi et al., 2000). However, laryngeal carcinoma has not been considered an estrogen-responsive malignancy.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the exposure of Vietnam veterans and the magnitude of risk, if any, of childhood cancers, it is not possible for the committee to quantify the risk likely to be experienced by the offspring of Vietnam veterans because of exposure to herbicides in Vietnam.

SEX RATIO

Sex ratio (ratio of males to females at birth)—about 106 males per 100 females (Pyeritz, 1998), or about 0.51 percent males among all births—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 before birth will alter the sex ratio at birth. For instance, a lethal mutation on the paternal X chromosome would differentially affect female conceptuses. Investigators have evaluated the sex ratio among various species in relation to such exposures as radiation for

some years. More recently, it has been suggested that the sex ratio is controlled by parental hormones at conception and that changes in gonadotropin and steroid concentrations may affect it (James, 1996). The specific mechanisms involved (such as zygote formation, implantation, regulation of sex-determining factors, and selective fetal loss) are uncertain, and direct experimental evidence supporting or refuting the hypothesis is lacking. James (1997) has suggested that a reduction in testosterone and high gonadotropin after TCDD exposure would result in an excess of female offspring. Potential confounding factors for this altered sex ratio are uncertain, but parental age, social class, illness, race, smoking, and stress have been considered.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The potential association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and altered sex ratio was not explored in the *VAO* and *Update 1996* reports. The committees responsible for *Update 1998* and *Update 2000* reviewed papers addressing altered sex ratio as part of their examination of literature on fertility. There was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and sex ratio.

Update of Scientific Literature

Occupational Studies

Okubo et al. (2000) reported results of an examination of the offspring of 15 male industrial workers. In 1980-1997, the workers were employed by a company in Japan that made plastic products and were also engaged in the recovery of dicyclopentadiene. The workers were exposed to several chemical materials—dicyclopentadiene, cyclopentadiene, epoxy resin, and such raw materials as bisphenol A epichlorohydrin. Using a binomial test, they reported a statistically significant ($p < 0.01$) excess number of female births (18 of 24) compared with the 1980 sex ratio of 0.5 in Japan. They also reported that there was no association between the sex ratio and several characteristics on the basis of information obtained from personal interviews. Those characteristics included year of birth of the offspring, paternal age at the birth of offspring, paternal age when joining the company (19–22 years), and the period of employment until the birth of offspring (mean = 9.5 years; SD = 3.7 years). This study, although interesting enough to warrant a more careful look at the study population, suffers from potential shortcomings. The results may not be specifically attributed to any of the chemicals to which the workers were exposed. Moreover, the results may be, at least partially,

due to the heat to which they were also exposed. The study results are also based on a small sample size, further limiting their usefulness.

In a letter to *Lancet*, Moshammer and Neuberger (2000) report on the effect of occupational exposure to TCDD on the sex ratio, on the basis of the Austrian chloracne cohort. After exposure to TCDD in the early 1970s, the number of live births showed a slight tendency toward having a lower sex ratio (that is, having fewer boys): 0.46 (26 boys in 56 births) compared with 0.61 (19 boys in 31 births). But no statistically significant trend was found with respect to TCDD load or the period between exposure and date of birth. Moreover, the large number of female births was mainly to fathers who were younger than 20 years old during the exposure. Those findings support findings from earlier studies (such as those of Seveso, Italy).

On the basis of data on participants in a cross-sectional medical study, Schnorr et al. (2001) investigated whether pregnancy outcomes (spontaneous abortions or altered sex ratio) were associated with paternal occupational exposure to TCDD. The study was conducted on a highly exposed population whose serum TCDD measurements were available. The medical study was conducted on 325 nonexposed controls that were matched for race, age (within 5 years), and neighborhood to 281 workers at two plants who were exposed to TCDD while engaged in the production of sodium trichlorophenol or any of its derivatives, most notably the derivative 2,4,5-T, which was one of the herbicides used in Vietnam. Health and risk-factor information were obtained from questionnaires and medical examination. For the reproductive study, additional information was obtained on reproductive outcomes and related characteristics from the fathers (via a brief questionnaire) and mothers (via telephone interview). A pharmacokinetic model was used to estimate serum TCDD at the time of conception for each pregnancy. The models used time-dependent BMI information to account for body burden. Paternal exposures were modeled as logarithms and also by categorizing into <20, 20–255, 255–1120, and ≥1120 ppt. Extrapolated TCDD concentrations at times of conception in this cohort were high (3–16,340 ppt) compared with those in other studies (the range in the Ranch Hand cohort was 0–1,424 ppt).

The analysis on sex ratios was limited to 1,191 live births (544 fathered by workers and 647 by nonexposed controls) from the 200 wives of the 259 male workers and 220 wives of the 243 controls with eligible pregnancies and whose serum TCDD measurements were available. Sex ratio was not significantly different between the two groups. On the basis of a logistic-regression paradigm that used a generalized-estimation-equations approach to account for multiple pregnancies, the probability of a male birth was modeled. In a model that adjusted for potential confounders (father's race and mother's education), there also was no significant trend with magnitude of paternal exposure to TCDD. In contrast with other prior studies (such as that of Seveso, Italy), there was no difference in sex ratio by age at first exposure. The sex ratios among those first exposed when

younger than 20 years old and those first exposed when older than 20 years were 0.6 (0.5–0.7) and 0.6 (0.5–0.6), respectively.

The strengths of that study include the availability of extensive questionnaire and interview information on potential risk factors and pregnancy outcomes and characteristics, the use of pharmacokinetic models to quantify fathers' exposure data, and confirmation of all pregnancies. One of its limitations is the modest rate of response (73% and 78% among controls' and workers' wives, respectively).

Savitz et al. (1997) reported results of the Ontario Farm Family Health Study. The study was conducted by using the 1986 Canadian census of agriculture as a sampling frame for selection of farms. Of the 2,693 farm couples that were eligible for the study (year-round occupants of the farm where the female was no older than 44 years at the time of interview), 1,898 couples provided questionnaire data; they had 3,984 eligible pregnancies (occurring on the study farm with known time intervals of pregnancy, and where questionnaire data were provided by the fathers). Of reported farm activities over the preceding 5 years, direct pesticide exposure was said to have occurred if activities involved mixing or applying crop herbicides, crop insecticides and fungicides, livestock chemicals, yard herbicides, or building pesticides. For exposure during the period from 3 months before conception to the time of conception (to account for sperm-mediated effects), men were grouped for each pregnancy into "chemical activity" if involved in activities with direct pesticide exposure for at least 1 month, "nonchemical activity" if involved in farm activities that were not associated with direct pesticide exposure (such as milking cows), or "no activity" if not involved in any farm-related activity during the period of interest. Exposure information was further refined by asking about use of protective equipment and about specific pesticides applied on the farm. On the basis of logistic-regression models that used the generalized-estimation-equations approach to account for within-woman across-pregnancies correlation, sex ratio was not found to be associated with farm chemical activities. There was a trend toward lower sex ratio for fathers who did not report using protective equipment, with an OR of 0.8 for all classifications of specific activities. But none of the trends was statistically significant. The models adjusted for mother's age, father's age, and father's off-farm job. The study has the strength of having a detailed exposure assessment, and the advantage of maternally reported pregnancy outcomes. The authors report that TCDD is not likely to be present to any substantial degree in the pesticides considered, but they also list the types of chemicals that were involved, including relevant exposures, such as to phenoxy herbicides.

Environmental Studies

Yoshimura et al. (2001) reported results of a study on the sex ratio of live births (during 1968–1977) to parents that were accidentally exposed to PCBs and

polychlorinated dibenzofurans (PCDFs) in 1968 in Yusho, Japan. The study focused on two regions of Japan that were affected by the exposure: Fukuoka and a region of Nagasaki. Using a two-sided binomial test and 85 live births to affected parents in the study area in February 1968 to December 1977, they showed that the sex ratio was not statistically different from the ratio of 0.5 in the general Japanese population. That result is different than the findings in Seveso, Italy, and Yucheng, Taiwan. But, as the authors rightly acknowledged, the exposure in this Japanese study and in the Yucheng study was to PCBs and PCDFs, not to TCDD, as in Seveso (Italy). The study seems to have used appropriate statistical methods for testing the main null hypotheses. It also appears that the study period was too brief to allow for possible effects on sex ratio in offspring of victims who were younger than 19 years old at the time of the incident.

Revich et al. (2001) studied the relationship between the relatively high dioxin concentrations in Chapaevsk, Russia (as detected in the air, the soil, drinking water, and cows' milk because of pollution from a chemical plant in the area), and its effects on the reproductive health of the study population. The sex ratio of births in Chapaevsk was examined with demographic data. The overall sex ratio for 1983–1997 was 0.5. The year-specific sex ratio ranged from 0.4 for 1989 to 0.6 for 1987 and 1995. The authors conclude that these results support the decline in sex ratio that has been shown in other industrial countries. However, the pattern is not clear. Moreover, the nonspecific nature of the exposure and the poor study design limit the usefulness of these results.

Karmaus et al. (2002) examined the relationship between environmental parental exposure to PCBs and dichlorophenyl dichloroethene (DDE) in Michigan fish-eaters and sex ratio in their offspring. The study was based on a cohort of 1,177 people who were recruited as a result of three surveys (1973–1974, 1979–1982, and 1989–1991) that assessed total serum PCB concentrations in Michigan anglers. Notably, dioxin-like activity was not assessed in this investigation. A telephone interview was conducted in 2000 to collect data on their children's birth characteristics, such as birth date, sex, birthweight, and gestational age. Exposure data were obtained from analyses of serum PCB and DDE in samples obtained in each of the three surveys. For each birth of a child, the paternal and maternal exposures (dichotomized at the median) that were closest to the birth were used as the most relevant exposures. Logistic-regression models that used the generalized-estimation-equations approach to account for multiple births in a family were fitted to estimate the OR for sex ratio after adjustment for calendar period of the child's birth, age of the mother at the child's birth, and whether there was an older brother in the family. The models were based on 101 families, which had 208 offspring born after 1963 and paternal measurements of PCB and DDE. The results indicate that a significantly higher OR (with a higher sex ratio) was associated with paternal PCB concentrations over 8.1 μg/L serum (OR = 2.3, 1.1–4.7). There was no significant association with maternal PCB concentration, but the estimated OR was in the opposite direction (OR = 0.7, 0.4–1.5). Some of

the strengths of the study are the attempt to assess the reliability of questionnaire information on a sample of 30 parents (yielding a kappa statistic of 91% and complete agreement on the sex of the children) and the use of appropriate statistical techniques to account for multiple births in a family. But PCB concentrations were determined only at the times of the three surveys, and it was not possible to study the effects of PCB congeners, such as those that display dioxin-like activity.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

Of the four occupational studies evaluated in this section, the two larger studies (Savitz et al., 1997; Schnorr et al. 2001) are of sufficient size to yield results that may be reliable and hence amenable to serious interpretation. Savitz et al. (1997) is a well-designed and well-analyzed case-control study. Schnorr et al. (2001) dealt with a study population with high TCDD exposures, and hence this study is relevant to the charge of the committee. In any case, both studies give evidence of no association of the exposures with sex ratio.

The intriguing finding of excess number of female births in the relatively small study of Okubo et al. (2000) suffers from the shortcomings of the study, one of which is the results may not be specifically attributed to any of the chemicals involved. Moshammer and Neuberger (2000) reported that excess female births were observed after exposures to TCDD in the 1970s, but the results were significantly linked to TCDD load or the interval between exposure and date of birth.

The results from Yoshimura et al. (2001) show lack of association of exposures to sex ratio, contradicting previous results from Seveso, Italy, and Yucheng, Taiwan, but the exposures were to PCBs and PCDFs, not to TCDD. The results from Revich et al. (2001) support previous findings of declining sex ratios in other industrial countries, but this study suffers from poor design. The results from Karmaus et al. (2002) indicate higher sex ORs (more male births) after exposure to PCB and DDE, but exposure data were based on data from three surveys, and it was not possible to study whether the effects were the result of PCB congeners that may have dioxin-like activity.

There is not enough data to determine whether an association exists between exposure to the chemicals of interest and altered sex ratio, but regardless the committee does not necessarily consider this an adverse health outcome. Although a large change in the sex ratio would have adverse effects on the population as a whole, a higher-than-expected number of females may not in itself be an

adverse event in terms of social or personal capacity on an individual veteran basis. Altered sex ratio might indicate an underlying functional abnormality in one or both parents that might be adverse, such as loss of male fetuses, or alteration of motility of sperm, but altered sex ratio is not necessarily associated with functional deficit in affected persons. Therefore, the committee has reviewed the data but does not treat sex ratio as a health outcome but not an adverse one.

SUMMARY

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and altered hormone concentrations, semen quality, or infertility; spontaneous abortion; late-fetal, neonatal, or infant death; low birthweight or preterm delivery; birth defects other than spina bifida; and childhood cancers.

Biologic Plausibility

This section summarizes the general biologic plausibility of a connection between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and reproductive and developmental effects on the basis of data from animal and cellular studies. Details of the committee's evaluation of data from the recent studies are presented in Chapter 3.

TCDD is reported to cause a number of reproductive and developmental effects in laboratory animals. In males, sperm count and production and seminal vesicle weight have been affected by TCDD. Effects on female reproductive organs have also been seen. The mechanisms of these effects are not known, but one hypothesis is that they are mediated through effects on hormones. Effects on male and female reproductive organs are not always accompanied by effects on reproductive outcomes. On the basis of animal data, there is a biologically plausible mechanism of male and female reproductive effects in humans. In animal studies, offspring of female hamsters given TCDD orally on gestation day 15 had reduced body weight. Although body weight is not consistently reduced in mice and rats exposed to TCDD in utero, those data are suggestive that exposure to TCDD in utero could affect the body weight of newborn humans.

Experiments have examined the effects of TCDD on the adult female reproductive system. TCDD exposure did not increase egg mortality or affect time to hatching of newly fertilized zebrafish eggs, but pericardial edema and craniofacial malformations were observed in zebrafish larvae. 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. Immature

female rats treated with TCDD have been shown to produce significantly fewer ova; the reduction might have a number of pathways, including direct effects on the ovaries and effects on the ovaries that are secondary to effects on other hormone-producing tissues.

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. Effects on the prostate have been seen after TCDD exposure. TCDD decreased the concentrations of hormones, such as gonadotropin and testosterone, in rats. High doses of TCDD, however, are required to elicit many of those effects.

TCDD is teratogenic in mice, inducing cleft palate and hydronephrosis. Research indicates that coexposure with either of two other chemicals, hydrocortisone or retinoic acid, synergistically enhances expression of cleft palate. The synergy suggests that the pathways controlled by these agents converge at one or more points in cells of the developing palate. Several reports 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; cytochrome P450 1A1 induction in the endothelium might mediate the early lesions that result in TCDD-related vascular derangements. That antioxidant treatment provides substantial protection against TCDD-induced embryotoxicity suggests that reactive oxygen species might be involved in the teratogenic effects of TCDD.

Studies in female rats show that a single dose of TCDD results in malformations of the external genitalia and in functional reproductive alterations in female progeny, such as decreased fertility rate, reduced fecundity, cystic endometrial hyperplasia, and increased incidence of constant estrus. Those effects depend on the timing of exposure.

Little research has been conducted on the offspring of male animals exposed to herbicides. A study of male mice fed various concentrations of simulated Agent Orange mixtures concluded that there were no adverse effects in offspring. A statistically significant excess of fused sternebrae in the offspring of the two most highly exposed groups was attributed to an anomalously low rate of this defect in the controls.

The effects of in utero and lactational exposure on the male reproductive system have been investigated. In utero and lactational exposure to TCDD led to decreased daily sperm production and cauda epididymal sperm number in male rat and hamster offspring. Research suggests that in utero and lactational TCDD exposure selectively impairs rat prostatic growth and development without inhibiting testicular androgen production or consistently decreasing prostatic dihydrotestosterone concentrations. In utero exposure to TCDD also caused decreased seminal vesicle weight and branching and decreased sperm production and increased sperm transit time in male offspring.

Studies in female animals are few but demonstrate that in utero and lacta-

tional exposure reduced fertility, decreased the ability to carry pregnancy to term, decreased litter size, increased fetal death, impaired ovarian function, and decreased concentrations of hormones, such as estradiol and progesterone. Most of those 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. TCDD also induced changes in serum concentrations of reproductive hormones in immature female rats given TCDD by gastric intubation, partially because of the action of TCDD on the pituitary gland.

The mechanism by which TCDD could exert reproductive and developmental effects is not established. Extrapolating results to humans is not straightforward, because the factors that determine susceptibility to reproductive and developmental effects vary among species. TCDD has a wide array of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells; these effects in turn could lead to reproductive or developmental toxicity.

Most studies are consistent with the hypothesis that the effects of TCDD are mediated by the aryl hydrocarbon receptor (AhR), a protein in animal and human cells to which TCDD can bind. The TCDD–AhR complex has been shown to bind DNA and lead to changes in transcription; that is, genes are differentially regulated. Modulation of those genes may alter cell function.

Although structural differences in the AhR have been identified among species, it operates in a similar manner in animals and humans. Therefore, a common mechanism is likely to underlie the toxic effects of TCDD in humans and animals, and data in animals support a biologic basis of TCDD's toxic effects. Because of the many species and strain differences in TCDD responses, however, controversy remains regarding the TCDD exposure that causes reproductive or developmental effects.

Little information is available on reproductive and developmental effects of the herbicides discussed in this report. Studies indicate that 2,4-D does not affect male or female fertility and does not produce fetal abnormalities. However, when pregnant rats or mice are exposed to 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), of which 2,4-D is a major metabolite, the rate of growth of offspring is reduced, and their mortality increased (Charles et al., 1999); very high doses of 2,4-D and 2,4-DB were required to elicit these effects. 2,4-D has also been shown to alter the concentration and function of reproductive hormones and prostaglandins. One study reported an increased incidence of malformed offspring of male mice exposed to a mixture of 2,4-D and picloram in drinking water. However, paternal toxicity was observed in the high-dose group, and there was no clear dose–response relationship; both findings were a concern in that study. Data have suggested that picloram alone may produce fetal abnormalities in rabbits at doses that are also toxic to the pregnant animals, but that effect has not been seen in many studies. 2,4,5-T was toxic to fetuses when administered to pregnant rats,

mice, and hamsters. Its ability to interfere with calcium homeostasis in vitro has been documented and linked to its teratogenic effects 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.

The foregoing suggests that a connection between TCDD exposure and human reproductive and developmental effects is, in general, biologically plausible. However, more-definitive conclusions about the presence or absence of a mechanism for the induction of such toxicity by TCDD in humans is complicated by differences in sensitivity and susceptibility among individual animals, strains, and species; the lack of strong evidence of organ-specific effects among species; and differences in route, dose, duration, and timing of exposure. Experiments with 2,4-D and 2,4,5-T indicate that they can have effects on cells at the subcellular level that could provide a biologically plausible mechanism for reproductive and developmental effects. Evidence in animals, however, indicates that they do not have reproductive effects and that they have developmental effects only at very high doses. There is insufficient information on picloram and cacodylic acid to assess the biologic plausibility of these compounds' reproductive or developmental effects.

Considerable uncertainty remains about how to apply this information to the evaluation of potential health effects of herbicide or TCDD exposure in Vietnam veterans. Scientists disagree over the extent to which information derived from animal and cellular studies predicts human health outcomes and the extent to which the health effects resulting from high-dose exposure can be extrapolated to low-dose exposure. The biologic mechanisms underlying TCDD's toxic effects continues to be an active field of research, and future updates of this report might have more and better information on which to base conclusions, at least for TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the large uncertainties that remain about the magnitude of potential risk of reproductive and developmental outcomes associated with exposure to herbicides in the studies that have been reviewed, it is not possible for the committee to quantify the degree of risk likely to be experienced by Vietnam veterans because of their exposure to herbicides in Vietnam.

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8

Neurobehavioral Disorders

Neurologic problems in clinical medicine cover a wide variety of disorders. The nervous system actually consists anatomically and functionally of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes the brain and spinal cord, and CNS dysfunction can be divided into two general categories: neurobehavioral dysfunction and motor or sensory dysfunction. Neurobehavioral difficulties involve cognitive decline, including memory problems and dementia; and neuropsychiatric disorders, including neurasthenia (a collection of such symptoms as difficulty in concentrating, headache, insomnia, and fatigue), depression, posttraumatic stress disorder (PTSD), and suicide. Motor dysfunction is characterized by such problems as weakness, tremors, involuntary movements, incoordination, and walking abnormalities; these are usually associated with subcortical or cerebellar disorders. The anatomic elements of the PNS include the spinal rootlets that leave the spinal cord, the brachial and lumbar plexus, and the peripheral nerves that innervate muscles. PNS dysfunctions, involving either the somatic nerves or the autonomic system, are known as peripheral neuropathies.

Neurologic dysfunction can be further classified, on the basis of anatomic distribution as either global or focal; on the basis of temporal onset as acute, subacute, or chronic; or on the basis of temporal course as transient or persistent. For example, global cerebral dysfunction may lead to altered levels of consciousness, whereas focal lesions may cause isolated signs of cortical dysfunction, such as aphasia. Acute onset of motor or coordination disturbances leads to symptoms that develop over minutes or hours, whereas subacute onset occurs over days or weeks, and chronic onset over months or years. Transient peripheral neuropathies

resolve spontaneously, whereas persistent ones may lead to chronic deficits. In the original *VAO* report, attention was deliberately focused on persistent neurobehavioral dysfunction. Later reports, including this one, review all new data pertinent to clinical neurobehavioral dysfunction and peripheral neuropathy.

Case identification in neurology is often difficult. Despite advances in neuroimaging, many types of neurologic alterations are biochemical and show no abnormalities on scanning tests. The nervous system is not usually accessible for biopsy, so pathologic confirmation is not feasible for many neurologic disorders. Behavioral and neurophysiologic changes can be partly or largely subjective and, even when objectively documented, are often reversible. Timing is important in assessing the effect of chemical exposure on neurologic function. Some symptoms of neurologic importance appear acutely but are short-lived, whereas others appear slowly and are detectable for extended periods. These caveats must be considered in the design and critique of epidemiologic studies aimed at evaluating an association between exposure to a chemical agent and neurologic or neurobehavioral dysfunction.

Many reports have addressed the possible contribution of herbicides and pesticides to nervous system dysfunction, and reported abnormalities have ranged from mild and transient to severe and persistent. Those assessments have been conducted in three general settings: in relation to occupational, environmental, and Vietnam-veteran exposures. This chapter reviews reports of the following neurologic alterations associated with human exposure to the chemicals of interest (2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), and cacodylic acid (dimenthylarsenic acid, DMA): cognitive and neuropsychiatric effects, motor or coordination dysfunction, chronic persistent peripheral neuropathy, and acute and subacute transient peripheral neuropathy. The potential neurotoxicity of those chemicals in recent animal studies is discussed in Chapter 3. The categories of association and the committee's approach to categorizing the health outcomes are discussed in Chapters 1 and 2.

COGNITIVE AND NEUROPSYCHIATRIC EFFECTS

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

On the basis of the data available at the time, it was concluded in *Veterans and Agent Orange* (hereafter referred to as *VAO*; IOM, 1994), *Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996*; IOM, 1996), and *Veterans and Agent Orange: Update 1998* (hereafter, *Update 1998*; IOM, 1999) that there was inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and cognitive or neuropsychiatric disorders. The majority of the data that formed the basis for those conclusions

came from the Air Force Health Studies (AFHS, 1991, 1995). The 1987 AFHS (AFHS, 1991), originally reviewed in *VAO*, found no association between serum TCDD (both baseline and current concentrations) and such variables as anxiety, depression, and hostility on the Symptom Checklist-90–Revised (SCL-90-R) or between TCDD and the presence of sleep problems. In contrast, some scales on the Millon Clinical Multiaxial Inventory (MCMI) had significant associations with TCDD in a variety of analyses. The belief that the findings from the SCL-90-R and the MCMI and the reported medical information were inconsistent led to the conclusion of inadequate or insufficient evidence of an association between exposure and cognitive or neuropsychiatric disorders (IOM, 1994).

In the 1992 AFHS (AFHS, 1995) some checklist variables (anxiety, hostility, obsessive–compulsive behavior, paranoid ideation, somatization, global severity index, and other neuroses) were significantly increased across all occupations in Ranch Hands, but the association was not significant for some after adjustment for covariates. In a later follow-up examination, the 1997 AFHS (AFHS, 2000), described in *Update 2000* (IOM, 2001), a repeat psychologic assessment was performed with SCL-90-R and reported psychologic disorders verified through medical record review. The verified psychologic disorders were combined with those obtained on previous examinations—baseline, 1985, 1987, and 1992. Of the five psychologic diagnoses—psychoses, alcohol dependence, drug dependence, anxiety, and other neuroses—a dose–response pattern was found only for 1987 TCDD concentrations and prevalence of "other neuroses" in the enlisted ground crew. When the relationship between the 1987 lipid-adjusted serum TCDD concentrations from all Ranch Hands and the psychologic end points were examined, however, no significant results were found. The checklist results were not different across Ranch Hand occupational groups and were not associated with TCDD exposure. Both *VAO* and *Update 2000* (IOM, 2001) had noted inconsistencies in the methods used to establish psychologic diagnoses in the 1987 and 1997 AFHS examinations (AFHS, 1991, 2000). Therefore, the conclusion of inadequate or insufficient evidence of an association between exposure and cognitive or neuropsychiatric disorders remained unchanged (IOM, 2001).

Update of the Scientific Literature

Since *Update 2000* (IOM, 2001), three relevant studies of cognitive and neuropsychiatric effects have been published: an update of the AFHS (Barrett et al., 2001), an occupational study in Czechoslovakia (Pazderova-Vejlupkova et al., 1981), and a study of Alzeimer's disease after environmental exposure to herbicides and insecticides (Gauthier et al., 2001).

Results of cognitive functioning from the AFHS examination in 1982 were published (Barrett et al., 2001). Neuropsychologic performance was measured in 937 Ranch Hand veterans (388 exposed to TCDD at background concentrations, 274 at low concentrations, and 275 at high concentrations) and 1,052 comparison

veterans who served in Southeast Asia but were not involved in spraying herbicides (all of whom had a serum TCDD concentration below 10 ppt). Cognitive functioning was assessed with the Halsted Reitan (HR) neuropsychologic test battery (16 measures), the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (11 measures), the Wechsler Memory Scale Form 1 (WMS) (five measures), and the reading subtest of the Wide Range Achievement Test (WRAT). Comparison veterans had been matched to Ranch Hand veterans on age, race, and military occupation. For all tests of cognitive functioning, mean scores for the three TCDD-exposed veteran groups were contrasted with the comparison group after adjustment for military occupation, age, race, drinking history, marital status, combat-exposure quartile, four psychiatric-diagnosis indicators (see *Update 2000*, page 442, for detailed description), and a psychotropic-medication use indicator. Finger tapping (HR) with the dominant and nondominant hands was significantly lower (poorer) in the Ranch Hand low-TCDD group than in the comparison group. Nondominant grip strength (HR) was significantly lower (weaker) in the Ranch Hand background-TCDD group than in the comparison group. Veterans in the Ranch Hand low-TCDD group were 3 times as likely to be rated severely impaired on the HR impairment index as all other veterans.

When Vietnam veterans were separated into quintiles on the basis of TCDD concentration, and the second, third, fourth, and fifth TCDD-concentration quintiles were contrasted with veterans in the first quintile, the mean dominant-hand grip strength for veterans in the fourth quintile and the mean nondominant-hand grip strength for veterans in the third and fourth quintiles were significantly increased. WAIS-R information score was significantly decreased for veterans in the third quintile, and WAIS-R similarities score was significantly increased (better) for veterans in the fourth quintile. Contrasts between the fifth and first quintiles were not significant for any of the subtests on the WAIS-R and HR. The Ranch Hand veterans had significantly lower mean scores in immediate and delayed recall of Logical Memory (WMS) than the comparison veterans. Also, veterans in the fifth quintile had significantly lower Logical Memory scores than veterans in the first quintile. Enlisted Ranch Hand personnel who reported greater skin exposure than enlisted comparison veterans had significant decrements in immediate and delayed recall WMS Logical Memory and HR Tactual Memory. Associate Learning (VMS), another test of verbal memory, had no meaningful change in any Ranch Hand TCDD category.

VAO reviewed a 10-year follow-up study of 55 men in Czechoslovakia with TCDD exposure during the production of 2,4,5-T (Pazderova-Vejlupkova et al., 1981). Initially, 7% of the workers had features of encephalopathy, and 75% had neurasthenia. Over time, the number of workers with neurasthenia decreased. *VAO* concluded that there were methodologic problems, including use of self-reported symptoms, lack of an objective measure of exposure, and selection bias. In a 30-year follow-up (Pelclova et al., 2001), 13 of the workers were re-examined. They had a mean plasma TCDD concentration of 256 ± 139 pg/g of lipid

(range = 14–760 pg/g of lipid) that was extrapolated to an estimated concentration of 5,000 pg/g of plasma fat at the time of initial exposure. All subjects had chloracne on the earlier examinations; two workers with TCDD of 760 and 420 pg/g of fat still had the condition. TCDD was correlated significantly with the memory quotient from WMS, the verbal IQ from WAIS-R, and the Benton test of visual memory. Age-corrected norms were used to determine abnormal performance. Surprisingly, education did not affect the results, but no demographic data on education were presented. Ten of 13 subjects drank alcohol every day, but this was not taken into account in the analyses. The low-voltage electroencephalogram with increased beta activity (seven subjects) could be related to the daily alcohol consumption. It is not possible to determine the relationship between TCDD and cognitive functioning without attention to confounding. The age-corrected norms used for test interpretation were not generated in a population similar to those workers. In the 1970s, five of the 13 subjects had abnormal tibial nerve studies compared to one in 1996. Because no data are presented, the underlying pathologic condition cannot be evaluated. As a general rule, toxic neuropathies are expected to improve once exposure has ceased or diminished, but because of selection bias of subjects the association of neuropathy with TCDD exposure cannot be determined.

Gauthier et al. (2001) found that long-term exposure to herbicides and insecticides was not significantly related to the development of Alzheimer's disease (AD). Sixty-seven cases diagnosed with NINCDS-ADRDA criteria of probable and possible AD were matched for age and sex with nondemented controls. Exposure data on each municipality were examined to establish the area sprayed with herbicides and insecticides in 1971, 1976, 1981, 1986, and 1991. The results were combined with the subjects' residential histories to establish potential environmental pesticide exposure. Logistic regression with adjustment for confounders found that long-term exposure to herbicides and insecticides did not have a significant effect on the development of AD. Occupational exposure to neurotoxic substances, including pesticides, was also not significantly related to AD.

Synthesis

Cognitive functioning in the Ranch Hand veterans evaluated with about 33 measures from HR, WAIS-R, WMS, and WRAT found eight significant group differences that did not support a dose-effect relationship with TCDD, that is, worse performance was seen in the background or low-TCDD groups. Ranch Hand veterans with the highest TCDD exposure had significantly lower scores on Logical Memory (WMS). That finding could be attributed to chance alone and was not in agreement with other administered tests of verbal memory—Associate Learning (WMS) and Information and Vocabulary (WAIS-R). Each test of verbal memory measures memory in a different way. When performance on one test of verbal memory is mildly depressed and performance on other tests of verbal

memory is normal, the conclusion that verbal memory is reduced is not warranted.

Military occupation served as the surrogate for education and training. A better indicator than occupation or formal years of education is the WRAT-R Reading Test, it is a measure of educational achievement and educational experience that is believed to assess premorbid intelligence. Reading tests are considered to be "hold" tests—in other words, resistant to change—when cognitive functioning declines, whether because of neurotoxic exposure or age. WRAT-R was administered in this study but was not used as a measure of educational achievement; it would have been a more robust covariate for education than military occupation and might have accounted for the significant findings with Logical Memory. As noted by Barrett et al. (2001), "differences [on Logical Memory] were relatively small and of uncertain clinical significance."

As discussed in *VAO*, inconsistencies in the methods used to establish psychologic diagnoses in the 1987 AFHS (1991) examination brought the diagnoses into question; and an association between TCDD exposure and numerous dissimilar neuropsychiatric diagnoses is improbable (IOM, 2001). Therefore, the use of that information as covariates does not appear justified. It is also unclear how marital status and combat exposure 20 years after cessation are related to neuropsychologic test performance.

Overall, the weaknesses in the study design, analyses, and interpretation of the results in the examination of serum TCDD and cognitive functioning in the Ranch Hand veterans prevent establishment of an association between exposure and neuropsychologic performance.

Conclusion

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is still inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and cognitive or neuropsychiatric disorders.

MOTOR OR COORDINATION DYSFUNCTION

This section summarizes the data from previous *Veterans and Agent Orange* reports and updates the scientific literature on Parkinson's disease and on amyotrophic lateral sclerosis.

Parkinson's Disease and Parkinsonism

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

Because of the increasing concern about a possible link between Parkinson's disease (PD) and various chemicals used as herbicides and pesticides, *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* suggested that attention be paid to the frequency and character of new cases in exposed versus nonexposed persons as Vietnam veterans age and are in the decades when PD is more prevalent.

Table 8-1 summarizes studies (reviewed in *Update 1996*, *Update 1998*, *Update 2000,* and this report) from numerous countries that examine the association between PD and pesticide (herbicide and insecticide) exposure. In those studies, cases of PD were identified with strict guidelines, either neurologic examination or review of medical data that required the presence of signs of PD (resting tremor, bradykinesia, cogwheel rigidity, and postural reflex impairment). Routine clinical diagnosis of PD has an accuracy of 75% by neuropathologic criteria that can be improved to 80–90% when stricter diagnostic criteria are applied (Langston, 1998). Clinical features were not verified in the large population studies that relied on death certificates or hospital admission diagnoses (Chaturvedi et al., 1995; Ritz and Yu, 2000; Schulte et al., 1996; Tuschen and Jensen, 2000). Exclusion criteria included the presence of atypical features—such as cerebellar involvement, gaze impairment, or pronounced autonomic dysfunction—or on all other causes of secondary parkinsonism, such as drugs, infections, or toxins. In the studies reviewed, for subjects to be included in the study pesticide exposure was usually required to occur before disease onset, but knowledge of when it occurred in relation to disease onset was not presented.

In *Update 1998*, emphasis on the detection of early-onset parkinsonism was considered vital to test the hypothesis that the disease is related to a toxic exposure because aging is currently the only known definitive risk factor for PD. PD becomes clinically apparent when about 60–70% of the neurons in the substantia nigra have deteriorated. One possible reason for the early onset of PD is that neuronal loss is accelerated in people with pesticide exposure and causes expression of the disease at an earlier age than is usual in the general population (see Weiss, 2000 for review).

In *Update 2000*, of the 30 epidemiologic studies of pesticide exposure and PD summarized in Table 8-1, only eight provided an estimate of relative risk posed by herbicides; of these studies, five had a positive significant association (Butterfield et al., 1993; Gorrell et al., 1998; Liou et al., 1997; Seidler et al., 1996; Semchuk et al., 1992), one had no association (Taylor et al., 1999), and the remaining two had a negative association (Kuopio et al., 1999; Stern et al., 1991). When a specific herbicide, paraquat, was examined in Taiwan (Liou et al., 1997), the OR was 3.2 (2.4–4.3).

TABLE 8-1 Epidemiologic Studies of Pesticide Exposure and Parkinson's Disease[a]

Reference and Country	Study Group	Comparison Group	Exposure Assessment	Significant Association with Pesticides	OR (95% CI)	Neurologic Dysfunction Diagnosis
Butterfield et al., 1993; US[b,c]	63 young onset, (age < 50 years)	68	Questionnaire—pesticide or insecticide use 10 times in any year	+	Insecticides 5.8 Herbicides 3.2 (2.5–4.1) Past dwelling fumigated 5.3	Standard criteria of PD by history
Chan et al., 1998; Hong Kong[c]	215	313	Interview—exposure to pesticides during farming (years)	+	Pesticides in women 6.8 (1.9–24.7) Pesticides in men 0.7 (0.3–1.8)	Neurologic examination
Chaturvedi et al., 1995; Canada[c]	87 (age > 64 years)	2,070	Survey—exposure positive if frequently used		Pesticides 1.8 (0.9–3.4)	History of PD
Engel et al., 2001; US	238	72	Self-administered questionnaire for occupational exposure	+	Pesticides 0.8 (0.5–1.2) Herbicide 0.9 (0.6–1.3) Highest tertile pesticide 2.0 (1.0–4.2)	Neurologic examination by trained nurse
Fall et al., 1999; Sweden[c]	113	263	Questionnaire—any job handling pesticides		Pesticides 2.8 (0.9–8.7)	Neurologic examination
Golbe et al., 1990; US[b,c]	106	106	Telephone survey—sprayed pesticides or insect spray once a year for a total of 5 years	+	Sprayed pesticide 7.0 (5.8–8.5)	Neurologic examination

Gorrell et al., 1998; US[c]	144 (age > 50 years)	464	Interview—herbicide and insecticide use while working on a farm or gardening	+	Occupational herbicides 4.1 (1.4–12.2) Occupational insecticides 3.6 (1.8–7.2)	Standard criteria of PD by history
Hertzman et al., 1990; Canada	57	122	Questionnaire—ever worked in an orchard	+	Working in orchards 3.7 (1.3–10.3)	Neurologic examination
Hertzman et al., 1994; Canada[c]	127	245	Interview—occupation with probable pesticide exposure	+	Pesticides in men 2.3 (l.1–4.9)	Neurologic examination
Ho et al., 1989; Hong Kong[c]	35 (age >60 years)	105	Interview—use of insecticides or herbicides (Y/N), farming, eating raw vegetables	+	Herbicides and pesticides 3.6 (1.0–12.9)	Neurologic examination
Hubble et al., 1993; US[c]	63	76	Questionnaire—pesticide or herbicide use 20 days per year for >5 years	+	Pesticide or herbicide 3.4 (1.3–7.3)	Neurologic examination
Hubble et al., 1998; US	43 PD with dementia	51 PD without dementia	Interviews—pesticide exposure >20 days in any year and presence of allele for poor drug metabolism	+	Pesticide exposure and genetic trait 3.17 (1.1–9.1)	Neurologic examination
Jimenez-Jimenez et al., 1992; Spain[c]	128	256	Interview—exposure: applied pesticides, or lived and ate vegetables where pesticides used		Pesticide 1.3 (0.9–2.1)	Standard criteria of PD by history

continued

TABLE 8-1 *Continued*

Reference and Country	Study Group	Comparison Group	Exposure Assessment	Significant Association with Pesticides	OR (95 % CI)	Neurologic Dysfunction Diagnosis
Koller et al., 1990; US[c]	150	150	Interview—acre-years= acres multiplied by years of herbicide or pesticide use		Herbicide or pesticide use 1.1 (0.9–1.3)	Neurologic examination
Kuopio et al., 1999; Finland	123 (onset of PD before 1984)	279	Interview—pesticides or herbicides regularly or occasionally used		Regular use herbicides of 0.7 (0.3-1.3)	Neurologic examination
Liou et al., 1997; Taiwan[b,c]	120	240	Interview—occupational exposures to herbicides or pesticides	+	Herbicides or pesticides, no paraquat 2.2 (0.9–5.6) Paraquat use 3.2 (2.4–4.3)	Neurologic examination
McCann et al., 1998; Australia[c]	224	310	Questionnaire—daily or weekly exposure to industrial herbicides and pesticides >6 months		Herbicides or pesticides 1.2 (0.8–1.5)	Neurologic examination
Menegon et al., 1998; Australia	96	95	Interview—pesticide exposure more than once weekly for >6 months before onset of PD	+	Pesticide 2.3 (1.2–4.4)	Standard criteria of PD by history

Morano et al., 1994; Spain[c]	74	148	Interview—direct and indirect—exposure to pesticides		Pesticide 1.73 (1.0–3.0)	Neurologic examination
Petrovitch et al., 2002; US	2,623	5,363	Total years plantation work and years of pesticide exposure	+	Plantation work >20 years 1.9 (1.0–3.5)	Medical records and neurologic examination
Ritz and Yu, 2000; US	7,516 (PD cause of death 1984–1994)	498,461 (ischemic heart disease cause of death 1984–1994)	Counties ranked by pesticide use from pesticide registry and agricultural census data	+	Prevalence OR: Moderate pesticide 1.36 (1.3–1.5) High insecticide 1.45 (1.3–1.6)	ICD-9 332
Schulte et al., 1996; US[b]	43,425 PD cause of death in 27 states 1982–1991		Occupational exposure	+	PMR excess in male pesticide appliers, horticultural farmers, farm workers, and graders and sorters of agricultural products	ICD-9 332
Seidler et al., 1996; Germany[b,c]	380 (<66 years with PD after 1987)	755	Interview—dose-years = years of application weighted by use	+	Neighborhood controls for herbicide 1.7 (1.0–2.7) Regional controls for herbicide 1.7 (1.0–2.6)	Neurologic examination
Semchuk et al., 1992; Canada[b,c]	130	260	Interview—occupational exposure for each job held >1 month	+	Pesticide 2.25 (1.3–4.0) Herbicide 3.06 (1.3–7.0) Insecticide 2.05 (1.0–4.1)	Neurologic examination

continued

TABLE 8-1 *Continued*

Reference and Country	Study Group	Comparison Group	Exposure Assessment	Significant Association with Pesticides	OR (95 % CI)	Neurologic Dysfunction Diagnosis
Stern et al., 1991; US[c]	69 (onset before age 40 years) 80 (onset after age 59 years)	149	Interview—insecticides and pesticides measured by self-report of home or garden use		Herbicide—young onset 0.9 (0.5–1.7) Herbicide—old onset 1.3 (0.7–2.4) Insecticide—young onset 0.6 (0.2–1.7) Insecticide—old onset 0.8 (0.3–2.1)	Standard criteria of PD by history
Smargiassi et al., 1998; Italy[c]	86	86	Interview—occupational exposure for at least 10 consecutive years		Pesticides or herbicides 1.15 (0.6–2.4)	Standard criteria of PD by history
Tanner et al., 1989; China	100	200	Interview—exposure for at least 1 year before onset of PD		Fruit growing 1.00 (1.0–1.0) Corn growing 0.54 (0.3–1.1) Rice growing 1.29 (0.7–2.3)	Neurologic examination
Taylor et al., 1999; US	140	147	Interview—exposure recorded as total days for lifetime		Pesticide 1.02 (0.9–1.2) Herbicide 1.06 (0.7–1.7)	Neurologic examination
Tuchsen and Jensen, 2000; Denmark	134	128,935 expected cases 101.5	Occupations in farming, horticulture, and landscape expected to have exposure to pesticides	+	Age-standardized hospitalization ratio for all men in agriculture and horticulture 134 (109–162)	First-time hospitalization for PD

Wechsler et al., 1991; US	34 (age >39 years)	22	Questionnaire—duration of occupational and home pesticide use	Home pesticides used more frequently by cases	Standard criteria of PD by history
Wong et al., 1991; US[c]	38 (19 sibling pairs with PD)	38 age and sex matched and 19 sibling pairs with essential tremor	Interview—acre-years = number of years exposed multiplied by number of acres applied herbicides or pesticides	Herbicides or pesticides 1.0 (0.7–1.4)	Neurologic examination

[a]Modified from Le Couteur et al. (1999). [b]Previously quoted in *Update 1996* or *Update 1998*. [c]Studies used in meta-analysis (Priyadarshi et al., 2000).
ABBREVIATIONS: PMR, proportionate mortality ratio.

As described in *Update 2000*, a meta-analysis of 19 of the studies (see Table 8-1) examined the association between PD and exposure to pesticides (Priyadarshi et al., 2000). All were case–control studies, so the parameter calculated to estimate relative risk is an OR. Of the 19 studies, 17 had a positive association between PD and exposure to pesticide and eight had an estimated OR that was significant. Of the remaining two studies, one showed a negative association (Stern et al., 1991) and the other no association (Wong et al., 1991) between PD and exposure to pesticides. Heterogeneity was significant among the studies ($p < 0.001$); the random-effect model used generated a combined estimate for the 19 studies of 1.9 (1.5–2.5). The combined estimates for those studies by geographic location were as follows: United States, 2.1 (1.1–4.1); Asia, 2.5 (1.6–4.1); Europe, 1.8 (1.4–2.2); and Canada, 1.9 (1.4–2.8). In six studies, no increased incidence of PD was found with increasing dose as measured by duration of exposure (Chan et al., 1998; Gorell et al., 1998; Morano et al., 1994; Seidler et al., 1996; Semchuk et al., 1992; Smargiassi et al., 1998). Only one (Gorrell et al., 1998) showed an increased risk of PD with longer exposure to pesticide (>10 years), with an OR of 5.8 (2.0–17.0).

Update of the Scientific Literature

In a recent study (Engel et al., 2001), 238 subjects exposed to pesticides in an occupational setting and 72 nonexposed controls were examined for the presence of parkinsonism by a trained nurse using the Unified Parkinson's Disease Rating Scale (UPDRS). The signs rated for parkinsonism were rest tremor, rigidity, bradykinesia, and impairment of postural reflexes. A self-administered questionnaire on use of pesticides included detailed information on the use of specific insecticides, herbicides, and fungicides. Information on the category of chlorophenoxy herbicides or specific compounds in this category was not included in the exposure assessment. Pesticide exposure occurred in the setting of orchardists, professional pesticide appliers, pesticide-formulation plant workers, and other farm or agricultural workers. Parkinsonism was more prevalent with more years of exposure but was similar between the nonexposed and the most-exposed group. Prevalence ratio (PR) (95% CI) for parkinsonism, adjusted for age and pack-years of smoking, for any exposure to pesticides was 0.8 (0.5–1.2) and for any exposure to herbicides was 0.9 (0.6–1.3). General exposure to pesticides analyzed by tertile of years of exposure found a significantly increased adjusted PR (95% CI) for the highest tertile (2.0, 1.0–4.2). With different cut scores for rigidity on the UPDRS, only the highest tertile of years of exposure to herbicides was significantly associated with parkinsonism; the adjusted PR was 2.5 (95% CI 1.0–6.0). The overall results are similar to those of many other studies reviewed in *Update 2000* in which an association with many years of occupational exposure is associated with parkinsonism but no association is found with any individual pesticide or class of pesticides.

Petrovich et al. (2002) conducted a prospective cohort study with 30 years of follow-up on 7,986 Japanese-American men (Honolulu Heart Program) who worked on sugar cane or pineapple plantations in Hawaii to determine whether working on a plantation or exposure to pesticides is associated with an increased risk of PD. Before 1991, incident cases of PD were identified through hospital records, death certificates, and medical records from offices of local neurologists. The entire cohort was re-examined in 1991 and 1993, and those with PD or parkinsonism were referred to a neurologist who used the UPDRS. After 1993, cases were added through record review. Exposure analysis used total plantation work, plantation type, and exposure to pesticides. Years of pesticide exposure summed days of exposure per year across years worked. Covariates adjusted for in the analyses included age, pack-years of cigarette-smoking, and caffeine intake. During the 30-year follow-up, 116 incident cases of PD were identified. As duration of work increased, pesticide exposure increased significantly. Even though age-adjusted incidence of PD increased with increasing pesticide exposure, the trend was not significant ($p = 0.101$). Those with over 20 years of plantation work had twice the risk of PD (10.3/10,000 vs 5.8/10,000 person-years) of those with no plantation work. With 10 years of plantation work or less, there was no increase in risk of PD, but a significant trend ($p = 0.006$) of increased risk occurred with further years of exposure.

Although the results are intriguing, an association of PD with exposure to 2,4-D, 2,4,5-T, or TCDD is not reported in any of the studies.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease with adult onset that presents with muscle atrophy, weaknesses, and fasciculations. Most cases of ALS are sporadic; only 5–10% are familial in origin. The annual incidence of sporadic ALS is 1–2 per 100,000 person-years and the incidence of ALS peaks between the ages of 55 and 75 (Brooks, 1996). Of familial-ALS patients, 20% have mutations in the gene encoding superoxide dismutase 1 (Rosen et al., 1993); the remaining patients with familial ALS have mutations in other genes. A specific diagnostic test does not exist, but it is believed that clinical diagnosis has a high degree of accuracy (Rowland, 1998; Rowland and Shneider et al., 2001). For the sporadic cases of ALS, interest in the role of occupational or environmental exposure originated in cases of motor neuron disease associated with exposure to heavy metals (Roelofs-Iverson et al., 1984, McGuire et al., 1997), chemical plants (Deapen and Henderson, 1986; McGuire et al., 1997), animal carcasses (Hanisch et al., 1976), heavy manual labor (Breland and Currier, 1967), work with electricity (Deapen and Henderson, 1986; Savettieri et al., 1991), pneumatic tools (Gallagher and Sanders, 1987; Savettieri et al., 1991), work in the plastic industry (Deapen and Henderson, 1986), and work as a

truck driver (Kurtzke and Beebe, 1980). During the period 1970–1983 in Sweden all 1961 cases of ALS were examined for association with occupations. The male cases of ALS were associated with a variety of occupations, including some that were protective. One county had a cluster of 25 male cases in agricultural work, 3 times the incidence expected (OR 3.4, 95% CI 1.2–9.3); but no specific exposures were examined (Gunnarsson et al., 1991).

Table 8-2 summarizes epidemiologic studies that examine the association of pesticide exposure and ALS.

A case–control study of 518 ALS cases (65% male) and 518 matched controls from all over the United States examined roles of physical trauma, prior neurologic diseases, or infection (Deapen and Henderson, 1986). Part of the exposure history included long-term occupational exposure to a variety of metals, plastic manufacturing, pesticides, and animal hides. The association between pesticides and ALS was not statistically significant but was positive (OR 2.0, 95% CI 0.8–5.4).

A case–control study of ALS in Palermo, Italy, that included 46 patients (25 men and 21 women) and 92 matched controls examined numerous risk factors—trauma, exposure to domestic animals, agricultural chemicals, organic solvents, and electric shock (Savettieri et al., 1991). No statistically significant associations were found between ALS and those exposures, although there was a positive association between ALS and agricultural chemicals (OR 3.0, 95% CI 0.4–20.3).

A case–control study in Scotland of 103 ALS cases (61 men) from a Scottish motor neuron disease register and 103 age- and sex-matched controls identified risk factors for development of the disease (Chancellor et al., 1993). Significant differences with increased exposure in cases were found for occupational exposure to lead (OR = 5.7, 95% CI 1.6–30) and "solvent/chemicals" (OR = 3.3, 95% CI 1.3–10). Occupational pesticide exposure was not significantly different but did have a positive association (OR = 1.4, 95% CI 0.6–3.1).

The results of a mortality study of male employees of the Dow Chemical Company who worked in the manufacturing or formulation of 2,4-D in 1945–1994 (Burns et al., 2001) are discussed in Chapter 6. In this cohort, death-certificate examination of the six employees in the ICDA-8 category "Diseases of the Nervous and Sensory Organs" revealed three with ALS. Analyses of their deaths found a significantly increased RR of death due to ALS (RR 3.45, 95% CI 1.1–11.1). All three died more than 20 years after their first exposure; duration of employment was 1.3, 1.8, and 12.5 years.

McGuire et al. (1997) conducted a population-based case–control study to examine the relationship between ALS and occupational exposures to metals, solvents, and agricultural chemicals. Cases (95 men and 79 women) were newly diagnosed in 1990–1994, and two controls for each case matched for sex and age were obtained from the same geographic area. Cumulative exposure index was

TABLE 8-2 Epidemiologic Studies of Pesticide Exposure and Amyotrophic Lateral Sclerosis

Reference; Country	Study Group	Comparison Group	Exposure Assessment	Significant Association Pesticides	OR with (95% CI)	Neurologic Dysfunction Diagnosis
Burns et al., 2001; US	1,567	40,600	Industrial hygienist ranked job exposure. Cumulative exposure, years, or each job times weighted exposure	+	3.45 (1.1–11.1)	Death certificates
Chancellor et al., 1993; Scotland	103	103	Required regular occupational exposure to pesticides for 12 months or more		1.4 (0.6–3.1)	Scottish Motor Neuron Register
Deapen and Henderson, 1986; US	518	518	Ever worked in presence of pesticides		2.0 (0.8–5.4)	ALS Society of America
McGuire et al., 1997; US	174	348	Self-reported lifetime job history and workplace exposure reviewed by panel of four industrial hygienists	+	2.4 (1.2–4.8); significant trend analysis for dose-effect relationship $p = 0.03$	Newly diagnosed with ALS 1990–1994 in western Washington state
Savettieri et al., 1991; Italy	46	92	Continual exposure to agricultural chemicals		3.0 (0.4–20.3)	Cases reviewed by neurologists

created by lifetime job history and workplace exposure to specific chemical agents collected by self-report and by a panel of four industrial hygienists blinded to the disease status of the participants. Exposure 10 years before diagnosis was not included. Exposure to metals and solvents was not associated with ALS. Association between exposure to agricultural chemicals and ALS was observed in men (OR = 2.4, 95% CI 1.2–4.8); the OR for low exposure compared with no exposure was 1.5 (95% CI 0.4–5.3) and for high exposure 2.8 (95% CI 1.3–6.1) (p trend = 0.03). The same trend was found with less than 3 years of exposure to agricultural chemicals (OR 1.2, 95% CI 0.3–4.1) compared with exposure greater than 3 years (OR 2.7, 95% CI 1.3–5.5) (p for trend = 0.03). Exposure to specific agricultural chemicals, such as herbicides, did not pose a significantly increased risk of ALS (OR 3.0, CI 95% 0.9–9.6). Excess exposure to agricultural chemicals from accidents or spills was associated with ALS (OR = 4.4, 95% CI 1.1–17.7), but this accounted for six cases and only three controls.

Synthesis

Epidemiologic studies continue to support an increased risk of PD with pesticide exposure, but specific pesticides or specific classes are lacking. As studies in the future collect exposure data that more closely reflect the dose at the critical receptor level and as underlying genetic susceptibilities to PD are identified, the relationship of PD and herbicide exposure may be clarified.

Continuing support for the biologic plausibility of PD and pesticide exposure is found in new studies of the rotenone model of PD that used in vivo and in vitro approaches to demonstrate how this pesticide produces a systemic defect in mitochondrial complex I, resulting in nigrostriatal dopaminergic degeneration that is expressed as hypokinesia and rigidity, features of parkinsonism (Greenamyre, 2001). Cytoplasmic inclusions that resemble Lewy bodies (abnormal protein collections) contained ubiquitin and α-synuclein. With chronic exposure to rotenone, oxidative damage to proteins and DNA was measured. Mitochondrial depolarization was observed with release of cytochrome c and activation of caspase-3. The rotenone model of PD reproduces many of the pathologic features of PD related to oxidative mechanisms.

Another in vitro study examined brain cell cultures (neurons and glia) from the rat mesencephalon after 2 days of 20 nM and 8 days of 1 nM rotenone and found significant neurodegeneration (Gao et al., 2002). The changes not present in the neuron-enriched cultures were attributed to rotenone's ability to stimulate release of superoxide from microglia. The neurotoxicity of rotenone was significantly diminished by blocking the release of superoxide from microglia. This study helps to explain the mechanism by which pesticides are associated with PD.

According to the results of epidemiologic studies, the association between pesticide exposure and PD is much stronger than the association between pesticide exposure and ALS. Known risk factors for ALS are age and a family history

of ALS. The careful attention to exposure assessment in the study by McGuire et al. (1997) makes the association between agricultural chemicals and ALS intriguing, but there are few exposed subjects, and further studies are needed.

Conclusions

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there remains inadequate or insufficient evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and motor or coordination dysfunction or Parkinson's disease. In the future, as diagnostic accuracy for PD improves, herbicide exposure assessment is quantified with specific biomarkers, and further research confirms the gene–toxicant interaction in larger prospective studies of PD, the evidence of an association may change; this underscores the importance of a prospective study of Vietnam veterans for the development of PD.

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and motor neuron disease or amyotrophic lateral sclerosis. More epidemiologic studies in the future might clarify the realtionship between exposure to herbicides and ALS. As with PD, prospective studies of Vietnam veterans for the development of ALS should be conducted.

CHRONIC PERSISTENT PERIPHERAL NEUROPATHY

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

On the basis of data available at the time, it was concluded in *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* that there was inadequate or insufficient evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chronic persistent peripheral neuropathy. Data from the Air Force Health Studies constituted a large part of the basis of the conclusions. In 1982, a baseline study of 1,208 Air Force Ranch Hands and a comparison group of 1,238 Air Force personnel found no differences between the groups in measures of peripheral nerve function, including neurologic symptom evaluation, physical examination, and nerve-conduction velocity tests (AFHS, 1984). A follow-up study conducted in 1985 used the same protocol except that nerve-conduction velocity was not assessed; once again, no differences were seen between groups (AFHS, 1987). In a 1987 follow-up, Ranch Hands had significantly more hereditary and degenerative diseases, such as benign essential tremor (not found to be associated with TCDD), but their

peripheral nerve status was not remarkable (AFHS, 1991). In 1992, the neurologic assessment was comparable between the two groups, and there was no consistent evidence of a dose–response relationship for either estimated initial TCDD or current TCDD. In 1997 (AFHS, 2000), the peripheral nerve examination was based on physical examinations and verified vibrotactile measurement. The percentage of participants with a confirmed polyneuropathy index was consistently higher in Ranch Hands than in the comparison group. After adjustment for the covariates, the results of TCDD exposure were marginally significant for the enlisted ground crew. The development of neuropathy 30 years after exposure is highly unusual and not compatible with TCDD exposure. It was concluded that evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chronic persistent peripheral neuropathy was still inadequate or insufficient.

Update of the Scientific Literature

A publication relating serum TCDD and peripheral neuropathy from the 1982, 1985, 1987, 1992, and 1997 examinations of the Ranch Hand study (Michalek et al., 2001) found significantly increased risk of peripheral neuropathy among Ranch Hand veterans in the high-exposure category in 1997. Exposure categories and numbers of veterans in the "comparison," "background," "low," and "high" categories are described in Chapter 5. As part of the protocol, veterans received the diagnosis of "diabetic" if diagnosed by a physician as noted in the medical record or if a 2-hour postprandial glucose-tolerance test result was over 200 mg/dL. A neurologic examination recorded tremor, cranial nerve function, sensation, motor strength and coordination, and reflexes. In 1982, nerve-conduction studies of ulnar, peroneal, and sural nerves were performed. In 1992 and 1997, vibrotactile thresholds of the great toe were measured. The diagnosis of possible peripheral neuropathy required one of three physical signs: absent ankle jerk, abnormal vibration at the ankle, or abnormal pinprick in the foot bilaterally. For probable peripheral neuropathy, at least two of the three abnormalities had to be present. For a diagnosis of peripheral neuropathy, a diagnosis of probable peripheral neuropathy and bilateral abnormal vibrotactile measures were required.

Nerve-conduction studies (1982) and vibrotactile abnormalities (1992 and 1997) did not support any peripheral nerve differences between low and high exposure to TCDD. In the high-TCDD category in 1997, the odds of possible peripheral neuropathy (OR = 1.8, 95% CI 1.2–2.7) or probable peripheral neuropathy (OR = 5.0, 95% CI 2.2–11.2) were significantly increased with a significant trend with increasing exposure ($p < 0.001$). To determine whether the OR was different in veterans with and without diabetes, the groups were analyzed separately. In nondiabetic veterans, the odds of probable peripheral neuropathy were significantly increased (OR = 8.7, 95% CI 1.9–39.3); and in diabetic veter-

ans, the odds were also significantly increased (OR = 3.5, 95% CI 1.3–9.4). In the 1992 examination, six veterans in the high-TCDD category had diagnosed neuropathy (OR = 4.9, 95% CI 1.5–15.3). In 1997, three of the six veterans had diagnosed neuropathy, one had normal measurements, one had missing data, and one did not attend. The number of nondiabetic veterans with diagnosed neuropathy in 1997 was too small for analysis, but the risk of diagnosed peripheral neuropathy in diabetic veterans in the high-TCDD category was significantly increased (OR = 5.8, 95% CI 1.6–20.3). When a secondary analysis was attempted and veterans were excluded if they had disease, disorders, exposures, or medications known to produce symptoms suggestive of neuropathy or had neurologic diseases unrelated to TCDD, the numbers were too small for analysis. In 1997, nine of the 14 veterans with probable peripheral neuropathy in the high-TCDD category had diabetes, and four veterans had preclinical diabetes. In the low-TCDD category, eight veterans had probable neuropathy, and seven were diabetic. Of the eight veterans with diagnosed peripheral neuropathy in the high-TCDD category, seven had diabetes, and one had preclinical diabetes. Of the five veterans with diagnosed peripheral neuropathy in the low-TCDD category, four had diabetes. That suggests a major problem in the interpretation of TCDD effects on the peripheral nerves in light of the presence of diabetes and preclinical diabetes, a major risk factor for peripheral neuropathy. That these cases of probable and possible peripheral neuropathy were identified for the first time in 1992 and 1997, when prior examinations were normal, weakens the ability to implicate TCDD exposure as the etiologic agent given that the peripheral nerve is known to repair itself after cessation of exposure or after diminution of the body burden of the responsible toxicant.

Synthesis

One of the classic features of a toxic neuropathy is improvement in peripheral nerve function after removal from exposure. The degree of recovery depends on the severity of the initial injury. A toxic neuropathy can begin days to weeks after high exposure or not until months or a few years after low exposure. Ranch Hand veterans exposed to Agent Orange 26–36 years previously showed no evidence of peripheral neuropathy associated with TCDD exposure at the time of the first examination in 1982. The finding of no association persisted in repeat examinations in 1985 and 1987. Only in 1992 and 1997, 10–15 years after the initial examination and 36–51 years after TCDD exposure, were odds ratios for the diagnosed neuropathy in the high-TCDD category significant. It is not plausible that peripheral nerve function was affected by TCDD in 1992 and 1997 if during the previous examinations when serum TCDD concentrations were higher no association with TCDD was found.

The case definitions of probable and diagnosed neuropathy are confusing when results are closely examined. A toxic neuropathy usually begins distally (in

the toes) and moves proximally (to the ankle). That is the basis of the term *dying-back neuropathy*. In 1997, some veterans with probable neuropathy had abnormal vibration at the ankle while vibrotactile measurements at the big toe were intact, the reverse of what the dying-back process would predict. Abnormal vibration at the ankle is found only after the neuropathy that began in the toes has progressed to the ankle. Therefore, vibrotactile score at the big toe should have been abnormal in the nine veterans with a probable neuropathy if vibration at the ankle was truly abnormal. The difficulty with agreement between different measures of peripheral nerve function may be an issue of sensitivity and specificity. Peripheral neuropathy was associated with TCDD exposure only after vibrotactile measures were added to the protocol in 1992, but odds of an abnormal vibrotactile score were not increased in the high-TCDD group. The small number of cases is also a problem, especially when some cases have normal results in examination at follow-up or for various reasons are not re-examined.

A greater problem was the confounding created by the high prevalence of diabetes or preclinical diabetes in veterans with probable or diagnosed neuropathy in the high-TCDD category. Diabetic neuropathy is the most common cause of peripheral neuropathy, occurring in about 50% of people with type 2 non-insulin-dependent diabetes over time but present in less than 10% when the diagnosis of diabetes is first made (Pirart, 1978). A study of outpatients with type 2 diabetes (mean age, 70.6 years; mean duration, 11.7 years) found polyneuropathy in 49% of them according to the criteria of lower-limb sensory and motor nerve-conduction velocity or latency more than 2 standard deviations above or below the age-matched controls (de Wytt et al., 1999). When a case definition of neuropathy in a diabetic population included symptoms, signs, electrodiagnostic studies, quantitative sensory testing, and autonomic testing, the prevalence increased to 66% (Dyck et al., 1993). In contrast, prevalence of a diabetic neuropathy was 28.5% in a large multicenter United Kingdom study (Young et al., 1993). Differences in case definitions of diabetic neuropathy probably account for the large range in its prevalence. As duration of diabetes progresses, the prevalence of peripheral neuropathy increases (Simmons and Feldman, 2002). In mild diabetic neuropathy, a median mononeuropathy was found in 23% of patients at a time when the lower extremities did not differ significantly from controls in electrodiagnostic studies (Albers et al., 1996)

The common neuropathy associated with type 2 diabetes is a distal symmetric sensorimotor polyneuropathy that affects primarily the sensory nerves. Type 2 diabetes can also affect other parts of the peripheral nervous system and produce autonomic neuropathy, polyradiculopathy, cranial mononeuropathies, limb mononeuropathy, and mononeuropathy multiplex.

Intensive glycemic control (careful attention to blood sugar concentration) appears to slow the progression of diabetic polyneuropathy. Persistent glycemia indirectly leads to increased release of free radicals and oxidative damage to the

nervous system. Those oxidative stressors are believed to lead to mitochondrial dysfunction and programmed cell death. That theory is supported by the finding that administration of antioxidants prevents the neuropathy (Feldman et al., 1999). Vascular factors in diabetes also account for damage to peripheral nerve fibers because of ischemic changes in the endoneurial capillaries (Simmons and Feldman, 2002).

A diabetic neuropathy may be difficult to differentiate clinically from neuropathy secondary to toxic exposure except by the presence of other features in the clinical history and presentation, such as gastrointestinal symptoms after lead or arsenic exposure or alopecia after thallium exposure. In addition, if caused by a toxic exposure, the neuropathy should improve after cessation of exposure, but diabetic neuropathy will usually progress unless a dramatic change is made in glycemic control. Complaints of peripheral nerve disorders, however, often occur in isolation and are monotonously similar. In the clinical setting, about 30% of cases of peripheral neuropathy are left with no etiology after a complete evaluation (McLeod, 1995). Examination of family members for evidence of mild or subclinical neuropathy can provide a hereditary etiology for a subset of this group (Dyck et al., 1981). Also, the PNS undergoes constant age-related changes that may increase its susceptibility to other metabolic and toxic exposures.

Conclusion

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that the evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chronic persistent peripheral neuropathy remains inadequate or insufficient. It should be noted, however, that the committee categorizes diabetes as having limited or suggestive evidence of an association.

ACUTE AND SUBACUTE TRANSIENT PERIPHERAL NEUROPATHY

Update of the Scientific Literature

The committee is aware of no new publications that investigate the association between exposure to the compounds of interest and acute and subacute transient peripheral neuropathy. 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 of acute or subacute transient peripheral neuropathy that develop long after service in Vietnam are associated with herbicide exposure.

SUMMARY

Strength of Evidence from Epidemiologic Studies

As in the earlier reports, on the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and disorders involving cognitive and neuropsychiatric dysfunction, motor or coordination deficits, or 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.

In *Update 1996*, the committee found that there was limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and acute or subacute transient peripheral neuropathy. The evidence regarding association was drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components. Information available to the committees responsible for *Update 1998*, *Update 2000*, and this report continues to support the conclusion.

Biologic Plausibility

This section summarizes the biologic plausibility of a connection between exposure to TCDD or herbicides and various neurobehavioral disorders on the basis of data from animal and cellular studies. Chapter 3 presents the details of the committee's evaluation of recent data from animal and cellular studies. Some of the preceding discussions of neurobehavioral outcomes include references to papers relevant to specific neurobehavioral effects.

Some information exists on the development of neurobehavioral disorders and TCDD exposure in laboratory animals. In vivo experiments have demonstrated that TCDD can affect biochemical processes, including having effects on calcium uptake and neurotransmission. Acute doses of TCDD administered to rats affect the metabolism of serotonin, a brain neurotransmitter that is able to modulate food intake. The biochemical change is consistent with observations of progressive weight loss and anorexia in experimental animals exposed to TCDD. A study in adult male Wistar rats suggests that a single low-dose intraperitoneal injection of TCDD could cause a toxic polyneuropathy (Grahmann et al., 1993; Grehl et al., 1993); no other studies in animals have reported such an effect. TCDD treatment has also been demonstrated to affect learning and memory in rats.

The mechanism by which TCDD could exert neurotoxic effects is not established. TCDD has a wide array of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells; these effects could in turn influence nerve cells. Furthermore, 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.

Most studies are consistent with the hypothesis that the effects of TCDD are mediated by the aryl hydrocarbon receptor (AhR), a protein in animal and human cells to which TCDD can bind. The TCDD–AhR complex is known to bind DNA and to lead to changes in transcription (that is, genes are differentially regulated). Modulation of genes could cause altered cell function.

Although structural differences in the AhR have been identified among different species, it operates in a similar manner in animals and humans. Therefore, a common mechanism is likely to underlie the neurotoxic effects of TCDD in humans and animals, and data in animals can support a biologic basis of TCDD's neurotoxicity. Because of the many species and strain differences in TCDD responses, however, controversy remains regarding the magnitude of TCDD exposure that is neurotoxic.

Little information is available on neurotoxic effects of exposure to the herbicides discussed in this report. At the cellular level, 2,4-D inhibited neurite extension. That effect was accompanied by a decrease in intracellular microtubules, inhibition of the polymerization of tubulin, disorganization of the Golgi apparatus, and inhibition of ganglioside synthesis. Studies in rats indicate an impairment of motor function, CNS depression, and inhibition of myelination in the brain. Behavioral alterations have also been seen after treatment of rats with 2,4-D. Results of in vitro mechanistic studies suggest that 2,4,5-T may acutely affect neuronal and muscular function by altering cellular metabolism and cholinergic transmission.

There is evidence that other chemicals can induce a Parkinson-like syndrome in humans, possibly by generating free radicals in the target tissue. Such results might be biologically relevant in that it is suspected that TCDD and some of the herbicides used in Vietnam could indirectly generate free radicals or sensitize cells to free-radical injury; the exact relevance, however, has not been established.

The foregoing evidence suggests that a connection between TCDD exposure and human neurotoxic effects is, in general, biologically plausible. However, definitive conclusions about the presence or absence of a mechanism of induction of neurotoxicity by TCDD in humans are complicated by differences in sensitivity and susceptibility among 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. Experiments with 2,4-D and 2,4,5-T indicate that they can have effects on brain cells at the subcellular level that could

provide a biologically plausible mechanism of neurotoxicity if such toxicity is seen in animals or humans, but alone they do not provide a basis for concluding that they are neurotoxic. The observation of behavioral alterations in rats after exposure to 2,4-D also would support the neurotoxicity of this compound, but the species, strain, and dose specificities of the effects remain unknown.

Considerable uncertainty remains about how to apply this information to the evaluation of potential health effects of herbicides or TCDD 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 with those resulting from low-dose exposure. Investigating the biologic mechanisms underlying TCDD's toxic effects continues to be the subject of active research, and future updates of this report might have more and better information on which to base conclusions, at least for this compound.

Increased Risk of Disease Among Vietnam Veterans

The most recent Air Force Health Study publications (Michalek et al., 2001; Barrett et al., 2001) reported differences in prevalence of chronic peripheral neuropathy and verbal memory performance between the Ranch Hand and comparison groups, but the clinical relevance is not clear. The available data do not support the notion that the differences are associated with exposure to herbicides or TCDD.

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9

Other Health Effects

This chapter discusses data on the possible association of the herbicides used in Vietnam (2,4-dichlorophenoxyacetic acid, 2,4-D; 2,4,5-trichlorophenoxyacetic acid, 2,4,5-T; picloram; and cacodylic acid) and the contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) with the following noncancer health outcomes: chloracne, porphyria cutanea tarda, respiratory disorders, immune system disorders, diabetes, lipid and lipoprotein disorders, gastrointestinal and digestive disease (including liver toxicity), circulatory disorders, amyloidosis, endometriosis, and adverse effects on thyroid homeostasis. Background information about each outcome is followed by a brief summary of the findings described in earlier *Veterans and Agent Orange* reports, a discussion of the most recent scientific literature, and a synthesis of the material reviewed. When appropriate, the literature is discussed by exposure type (occupational, environmental, or Vietnam veteran). Each section ends with the committee's conclusion regarding the strength of the evidence from epidemiologic studies, biologic plausibility, and evidence regarding Vietnam veterans. The categories of association and the committee's approach to categorizing the health outcomes are discussed in Chapters 1 and 2.

CHLORACNE

The skin disease chloracne is characteristic of exposure to TCDD and other cyclic organochlorine compounds. It shares some pathologic processes (for example, the occlusion of the orifice of the sebaceous follicle) with more common forms of acne (such as acne vulgaris), but it can be differentiated by the presence

of epidermoid inclusion cysts, which are caused by proliferation and hyperkeratinization (horn-like cornification) of the epidermis. Although chloracne is typically distributed over the eyes, ears, and neck, patterns of chloracne among chemical-industry workers exposed to TCDD have also included the trunk, genitalia, and buttocks (Neuberger et al., 1998).

Chloracne has been extensively studied and is used as a marker of exposure in studies of populations exposed to TCDD and other organochlorine compounds, such as polychlorinated biphenyls (PCBs) and pentachlorophenol. It is one of the few findings consistently associated with such exposure and is a well-validated indicator of high exposure to those compounds, particularly TCDD (Sweeney et al., 1997/98). If chloracne occurs, however, it appears shortly after the chemical exposure, not after a long latency. Although it is refractory to acne treatments, it usually regresses over time. Therefore, new cases of chloracne would not be the result of exposures during Vietnam and are not a concern for this report.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (hereafter referred to as *VAO*; IOM, 1994) found there to be sufficient information of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chloracne. Additional information available to the committees responsible for *Veterans and Agent Orange: Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), and *Update 2000* (IOM, 2001) did not change that finding. Reviews of the studies underlying the finding may be found in the earlier reports.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

Chloracne is clearly associated with high exposure to cyclic organochlorine compounds, but it appears shortly after exposure, not after a long latency.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there

is sufficient evidence that an association exists between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chloracne.

Biologic Plausibility

As noted in previous reports, chloracne has been reported in response to exposure to TCDD but not to purified phenoxyacetic herbicides.

Increased Risk of Disease Among Vietnam Veterans

There is sufficient evidence that chloracne is associated with TCDD exposure. However, given the lack of ability to extrapolate from exposure in studies of TCDD and chloracne to individual Vietnam veterans, it is not possible to quantify the risk of chloracne in Vietnam veterans. Furthermore, because TCDD-associated chloracne is evident shortly after exposure, there is no risk of new cases long after service in Vietnam.

PORPHYRIA CUTANEA TARDA

Porphyria cutanea tarda (PCT) is an uncommon disorder of porphyrin metabolism which causes thinning and blistering of the skin in sun-exposed areas, hyperpigmentation (excess pigment in skin), and hypertrichosis (excess hair growth) (Muhlbauer and Pathak, 1979; Grossman and Poh-Fitzpatrick, 1986). The condition is not completely understood, but evidence indicates that people with particular mutations associated with hemochromatosis and people with an iron-overload condition are predisposed to PCT. Known risk factors are high alcohol intake, estrogen intake (as in oral contraceptives), liver disease, hemodialysis, HIV infection, and diabetes. In addition, data suggest poor diet might be a risk factor.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found there to be sufficient information to determine that an association existed between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and PCT in genetically susceptible people. The available data, however, indicated that PCT manifests shortly after exposure to TCDD. Therefore, new cases of PCT due to exposures during the Vietnam war will not occur. Additional information available to the committee responsible for *Update 1996* led it to conclude that there was only limited or suggestive evidence of an association, and *Update 1998* and *Update 2000* did not change that conclusion. Reviews of the studies underlying those findings may be found in the earlier reports.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

If PCT was caused by exposure to TCDD, it would appear soon after the exposure and recovery would occur after the exposure ceased. In any case, manifestation of PCT following exposure to TCDD would have been rare. Although it has been seen after exposure to TCDD in industrial settings, Vietnam veterans enrolled in the Ranch Hand study have not been found to have symptoms suggestive of the disorder.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and PCT.

Biologic Plausibility

PCT has not been replicated in animal studies in response to TCDD, although other porphyrin abnormalities have been reported after exposure to TCDD.

Increased Risk of Disease Among Vietnam Veterans

Given the available data on individual exposures in both Vietnam veterans and study subjects, it is not possible to estimate the risk of PCT in individual Vietnam veterans. However, because PCT is an early response to TCDD, no new cases of PCT due to wartime exposures are expected among Vietnam veterans.

RESPIRATORY DISORDERS

Nonmalignant respiratory disorders comprise acute and chronic lung diseases other than cancer. Acute lung diseases include pneumonia and other lung infections and could be increased in frequency and severity when the normal defense mechanisms of the lower respiratory tract are compromised. Chronic nonmalignant respiratory disorders generally take two forms: airways disease

and parenchymal disease. *Airways disease* is a general term for disorders characterized by obstruction of the flow of air out of the lungs and includes chronic obstructive pulmonary disease (COPD), emphysema, asthma, and chronic bronchitis. *Parenchymal disease*, or *interstitial disease*, is a general term for numerous disorders that cause inflammation and scarring of the deep lung tissue, including air sacs and supporting structures. Those disorders are less common than airways disease and are characterized by reductions in lung capacity, but they often include a component of airways obstruction. In addition, some severe chronic lung disorders, such as cystic fibrosis, are hereditary. Because Vietnam veterans underwent health screenings, no severe hereditary chronic lung disorders are expected in this population.

The major risk factor for both acute and chronic respiratory disorders is cigarette-smoking. Although cigarette-smoking is not associated with every disease of the lungs, it is the major cause of airways disorders, contributes to some interstitial disease, and compromises host defenses in such a way that people who smoke are generally more susceptible to some types of pneumonia. Cigarette-smoking also makes almost every respiratory disorder more severe and symptomatic than would otherwise be the case. The frequency of cigarette-smoking as a habit varies with occupation, socioeconomic status, and generation. For those reasons, cigarette-smoking is a major confounding factor in interpreting the literature on risk factors for respiratory disease other than smoking. Vietnam veterans are reported to smoke more heavily than non-Vietnam veterans (McKinney et al., 1997).

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found there to be inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and the respiratory disorders specified above. Additional information available to the committees responsible for *Update 1996* and *Update 1998* did not change that finding. *Update 2000* drew attention to findings from the Seveso cohort that suggested a higher mortality from nonmalignant respiratory disorders among those, particularly males, more heavily exposed to TCDD. Those findings were not replicated in several other relevant studies, although one showed an increase that did not attain statistical significance. The committee for *Update 2000* concluded that although new evidence suggested an increased risk of nonmalignant respiratory disorders, particularly COPD, among people exposed to TCDD, the observation is tentative and the information insufficient to determine whether an association exists between the exposures of interest and respiratory disorders.

Update of the Scientific Literature

In the one occupational study published since *Update 2000*, a cohort of 1,517 male employees of the Dow Chemical Company who were involved in the manufacture or formulation of 2,4-D at some time in 1945–1994 demonstrated no excess mortality from nonmalignant respiratory disorders (Burns et al., 2001). That fewer deaths were observed in all exposure categories compared to the national rates and latency groups studied than expected suggests a strong healthy-worker effect.

No relevant environmental or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

No new studies provide evidence of a direct risk of nonmalignant respiratory disorders in adults since those reviewed in *Update 2000* (IOM, 2001).

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and nonmalignant acute or chronic respiratory disorders.

Biologic Plausibility

Lung tissue has been found to have high concentrations of the aryl hydrocarbon receptor (AhR), which mediates the effects of TCDD, and recent data have shown that both cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1A2 (CYP1A2) are expressed in lung biopsy specimens from human subjects. It is biologically plausible that exposure to TCDD may result in acute and chronic lung disorders. Furthermore, it is noted that a major risk factor for these disorders is cigarette-smoking. These cytochrome P450 (CYP) enzymes are responsible, in part, for the activation of such chemicals as those found in tobacco smoke (which also contains AhR ligands) to more-toxic intermediates, so it is also biologically plausible that exposure to TCDD may synergize the toxic effects of a variety of other chemicals to which human lung tissue is exposed.

Increased Risk of Disease Among Vietnam Veterans

There are insufficient data on nonmalignant respiratory disorders in Vietnam veterans to draw a specific conclusion as to whether Vietnam veterans are at increased risk for those disorders.

IMMUNE SYSTEM DISORDERS

The immune system is responsible for protecting the body against invasion by infectious microorganisms and the development of cancer. The two major immune responses are the innate and the adaptive responses. The innate response is more general; the adaptive response is specific and confers immunologic memory. The principle reactive cells of the immune system are the leukocytes (white blood cells), which include neutrophils, eosinophils, lymphocytes, blood monocytes, and macrophages, which are widely distributed in tissues and include histiocytes, dendritic cells, and antigen-presenting cells. The major lymphatic organs are the lymph nodes, spleen, thymus, palatine tonsil, and Peyer's patches. The immune response consists of a complex and sophisticated network of events involving cells and their secretory products, and optimal function of the immune system results from a delicate balance in cellular interactions and responses. Disruption of those events can result in a compromised immune response, with the response either suppressed or enhanced.

A suppressed immune response can result in reduced resistance to infections or neoplasia. The immune system has considerable reserve, so the degree of suppression necessary for increased susceptibility to disease can depend heavily on factors involving the invading microorganism, the host response to the invasion, and the ability of the microorganism or cancer cells to escape immunosurveillance. If they are sufficiently weakened, impairment of host defenses can result in severe and recurrent infections with opportunistic microorganisms or can predispose the host to neoplasia.

The immune response can also be hyperstimulated or unable to curtail a normal immune reaction, and this can result in contact hypersensitivity, allergy (atopy and asthma), and autoimmune disease. Many of those conditions are not life-threatening, but asthma and autoimmune disease can result in death. Asthma usually occurs when foreign antigen-specific immunoglobulins bind to the surface of mast cells releasing histamine which causes constriction of pulmonary airways. Autoimmune disease is the pathologic consequence of an immune response to autologous (self) antigens in which the immune system attacks its own body's cells, tissues, or organs.

This section presents information on the effects of herbicide and TCDD exposure on the human immune system and susceptibility to disease other than neoplasia. Information regarding herbicide exposure and neoplasia in humans is presented in Chapter 6.

Summary of *VAO* and *Update 1996*, *Update 1998*, and *Update 2000*

The committees responsible for *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*, found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and immune system disorders. Reviews of the studies underlying those findings are in the previous reports (IOM, 1994, 1996, 1999, 2001).

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

TCDD is a known immunosuppressant in laboratory animals. In fact, it is one of the most potent immunotoxicants known to exist in the environment. Therefore, it would be expected to be immunosuppressive in humans. To date, however, the immune effects described in humans exposed to TCDD have been marginal and highly inconsistent, ranging from increasing the immune response to decreasing the immune response to having no effect. Furthermore, no pattern of increased infectious disease has developed in people exposed to high concentrations of TCDD or other herbicides that were used in Vietnam. Investigations in humans in the last several years, including several recent studies reviewed in *Update 2000* that looked at numerous immune measures in workers exposed to TCDD and in veterans of Operation Ranch Hand, have failed to demonstrate a consistent positive association between TCDD exposure (in utero, perinatally, or postnatally) and immune effects. No studies available in humans during the last 2 years change those findings. As mentioned in *Update 2000*, many of the immune measures assessed in humans are related to nonfunctional end points often considered biomarkers. The consistent immunosuppressive effects observed in laboratory animals that have been exposed to TCDD have not been confirmed in humans.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists

between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and immune suppression or autoimmunity.

Biologic Plausibility

A large number of studies in animals indicate that one of the organ systems most sensitive to TCDD toxicity is the immune system; the effects are species-specific and strain-specific. TCDD can alter the number and function of immune cells in some animals. Studies of the effect of exposure to TCDD on immune response in the mouse after infection with influenza A demonstrate that the humoral and cell-mediated response is suppressed and cytolytic activity is preserved. Chapter 3 discusses recent toxicologic studies that make up the biologic basis of an association between exposure to TCDD or herbicides and toxic end points.

Increased Risk of Disease Among Vietnam Veterans

No evidence is available to associate defects in the immune response with exposure to the herbicides or TCDD.

DIABETES

Primary diabetes (that is, diabetes that is not secondary to another known disease or condition, such as pancreatitis or pancreatic surgery) is a heterogeneous metabolic disorder characterized by hyperglycemia and quantitative or qualitative deficiency of insulin action (Orchard et al., 1992). Two main types have been recognized: 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 1 and NIDDM termed type 2. This new terminology is used in the remainder of this review except when the older diagnostic criteria are appropriate.

Type 2 diabetes accounts for about 90% of cases of primary diabetes. Onset rarely occurs before 30 years of age, but incidence increases steadily with age thereafter. It is generally accepted that the main factors for increased risk of type 2 diabetes include age (older people are at higher risk), obesity, central fat deposition, a history of gestational diabetes (if female), physical inactivity, ethnicity (for example, prevalence is greater in blacks and Hispanics), and, perhaps most important, a family history of type 2 diabetes. The relative contributions of those features, however, are controversial.

The etiology of type 2 diabetes is unclear, but three major components have been proposed: peripheral insulin resistance (thought by many to be primary) in target tissues (such as muscle, adipose tissue, and liver); a defect in beta-cell

insulin secretion, and hepatic glucose overproduction. Defects at many intracellular sites could account for the impaired insulin action and secretion in type 2 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 activity, TNF-α, rad (ras associated with diabetes), and prohormone convertase 1 (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, such as maturity-onset diabetes of youth, which results from a variety of mutations of the beta-cell glucokinase gene.

Pathogenetic diversity and diagnostic uncertainty are two of the more important problems associated with the epidemiologic study of diabetes. 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 (such as viruses, nutrition, and activity)—it is probable that many agents or behaviors contribute to diabetes risk, especially in genetically susceptible people. The multiple mechanisms may also lead to heterogeneous responses to various exposures. Because up to half the affected diabetic population is undiagnosed, the potential for ascertainment bias is high (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 nondiagnosed cases) is great. Furthermore, it may be difficult to differentiate cases that develop during early to middle age (20–44 years) into type 1 or type 2.

Summary of Previous IOM Reports

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and diabetes. Additional information available to the committees responsible for *Update 1996* and *Update 1998* did not change that finding.

In 1999, in response to a request from the Department of Veterans Affairs (VA), IOM called together a committee to conduct an interim review of the scientific evidence regarding type 2 diabetes. That review, which focused on information published since the deliberations of the *Update 1998* committee, resulted in the report *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (IOM, 2000). The committee responsible for that report found that there was limited or suggestive evidence of an association between type 2 diabetes and exposure to at least one of the chemicals of interest (2,4-D,

2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid). The committee responsible for *Update 2000* upheld the finding of the *Herbicide/Dioxin Exposure and Type 2 Diabetes* committee. Reviews of the studies underlying those findings can be found in the earlier reports (see Table 9-1 for a summary).

Update of the Scientific Literature

Relatively little new information has been reported on the association between TCDD and the risk of diabetes. The potential link between TCDD and diabetes was mentioned briefly in two general review articles on the overall health effects of TCDD (Kogevinas, 2001; Sweeney and Mocarelli, 2000). A more specific review on environmental risk factors for diabetes included a detailed overview of the literature on 2,3,7,8-TCDD (Longnecker and Daniels, 2001); this review shared the conclusion of *Update 2000* that the findings on TCDD and the etiology of diabetes were somewhat suggestive but still inconclusive.

Occupational Studies

A cross-sectional study was conducted in 1998 to assess the health status of workers exposed to high concentrations of polychlorinated dibenzo-*p*-dioxins (PCDD) at the Bika Center municipal waste incinerator in Japan (Kitamura et al., 2000). Data were collected on 92 of the 146 people employed at Bika Center since it was opened in 1988. Eight workers reported a history of diabetes, and a logistic-regression model (including age and body mass index) was used to assess the association between quartiles of PCDD and the prevalence of diabetes. The coefficients for the model were not shown; the authors indicated in the text that none of the ORs for diabetes in relation to PCDD were statistically significant.

In another study, the association between TCDD and diabetes was re-examined in a combined analysis of data from the Ranch Hand study of Vietnam veterans and a National Institute for Occupational Safety and Health (NIOSH) study of TCDD-exposed workers at chemical plants in New Jersey and Missouri (Steenland et al, 2001). The results of those studies had been reported separately in *Update 1998* (Henriksen et al., 1997) and *Update 2000* (Calvert et al., 1999), respectively. The combined analysis was conducted in an attempt to improve the precision of the estimates and to address differences between the studies in definition of exposure and outcome and in adjustment for potential confounders.

The NIOSH study sample of 541 was reduced by 55 subjects (31 women, 16 men from the neighborhood comparison group with TCDD above 10 ppt, and eight exposed men with missing TCDD data) to match the criteria in the Ranch Hand sample. The fasting-glucose component of the definition of diabetes in the NIOSH sample was also modified (from fasting glucose of 140 mg/dL to 126 mg/dL) to approximate the Ranch Hand definition more closely. The Ranch Hand

TABLE 9-1 Selected Epidemiologic Studies—Diabetes

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
OCCUPATIONAL			
New Studies			
Kitamura et al., 2000	Workers exposed to PCDD at a municipal waste incinerator	8	Not statistically significant*
Steenland et al., 2001	Highly exposed industrial cohorts (N = 5,132)		
	Exposed vs nonexposed for Ranch Hands	147	1.2 (0.9–1.5)
	Exposed vs nonexposed for NIOSH	28	1.2 (0.7–2.3)
Studies Reviewed in Update 2000			
Calvert et al., 1999	Workers exposed to 2,4,5-T and derivatives		
	All workers	26	1.5 (0.8–2.9)
	Serum TCDD <20 pg/g (ng/kg) of lipid	7	2.1 (0.8–5.8)
	Serum 20 <TCDD <75 pg/g (ng/kg) of lipid	6	1.5 (0.5–4.3)
	Serum 75 < TCDD <238 pg/g (ng/kg) of lipid	3	0.7 (0.2–2.6)
	Serum 238 <TCDD <3,400 pg/g (ng/kg) of lipid	10	2.0 (0.8–4.9)
Steenland et al., 1999	Highly exposed industrial cohorts (N = 5,132)		
	Diabetes as underlying cause	26	1.2 (0.8–1.7)
	Diabetes among multiple causes	89	1.1 (0.9–1.3)
	Chloracne subcohort (N = 608)	4	1.1 (0.3–2.7)
Vena et al., 1998	Exposed production workers and sprayers in 12 countries[a]	33	2.3 (0.5–9.5)
Steenland et al., 1992[b]	Dioxin-exposed workers—mortality rates		
	Diabetes as underlying cause	16	1.1 (0.6–1.8)
	Diabetes among multiple causes	58	1.1 (0.8–1.4)
Studies Reviewed in *Update 1998*			
Sweeney et al., 1997/98	NIOSH production workers		
Ramlow et al., 1996	Pentachlorophenol production workers	4	SMR = 1.2 (0.3–3.0)
Studies Reviewed in *Update 1996*			
Ott et al., 1994	Trichlorophenol production workers		p = 0.06
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	SMR = 0.7 (0.2–1.9)
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	*

TABLE 9-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Pazderova-Vejlupkova et al., 1981	2,4,5-T and TCP production workers	11	*
ENVIRONMENTAL			
New Study			
Masley et al., 2000	Population based survey in Saskatchewan	28	*
Studies Reviewed in *Update 2000*			
Bertazzi et al., 2001	Seveso residents—20-year follow-up		
	Zone A females	2	1.3 (0.3–5.1)
	Zone B males	6	0.9 (0.4–2.0)
	Zone B females	18	1.8 (1.1–2.9)
Cranmer et al., 2000	Non-diabetic residents near the Vertac/Hercules Superfund site OR are for high insulin subjects with TCDD >15ppt (7 subjects) compared to persons with TCDD <15ppt (62 subjects)		
	Fasting (high insulin level, >4.5 μIU/ml)	3	8.5 (1.5–49.4)
	30-minute insulin (high insulin level, >177 μIU/ml)	3	7 (1.3–39.0)
	60-minute insulin (high insulin level, >228 μIU/ml)	4	12 (2.2–70.1)
	120-minute insulin (high insulin level, >97.7 μIU/ml)	6	56 (5.7–556)
Bertazzi et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	2	1.8 (0.4–7.0)
	Zone B males	6	1.2 (0.5–2.7)
	Zone B females	13	1.8 (1.0–3.0)
Pesatori et al., 1998	Seveso residents—15-year follow-up		
	Zone A females	2	1.8 (0.4–7.3)
	Zone B males	6	1.3 (0.6–2.9)
	Zone B females	13	1.9 (1.1–3.2)
	Zone R males	37	1.1 (0.8–1.6)
	Zone R females	74	1.2 (1.0–1.6)
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans and comparisons		(Numerous analyses discussed in text)
Longnecker and Michalek, 2000	Ranch Hand unexposed referents only, OR by quartile and serum dioxin concentration		
	Quartile 1: <2.8 ng/kg (pg/g)	26	1.00—referent
	Quartile 2: 2.8–<4.0 ng/kg	25	0.9 (0.5–1.7)[c]
	Quartile 3: 4.0–<5.2 ng/kg	57	1.8 (1.0–3.0)[c]
	Quartile 4: ≥5.2 ng/kg	61	1.6 (0.9–2.7)[c]

continues

TABLE 9-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
CDVA, 1998a	Australian Vietnam veterans—male	2,391 reported[b] (6% of respondents)	1,780 expected (1,558–2,003)
CDVA, 1998b	Australian Vietnam veterans—female	5 reported[d] (2% of respondents)	10 expected (9–11)
Henriksen et al., 1997	Ranch Hands—high-exposure group		
	Glucose abnormalities	60	1.4 (1.1–1.8)
	Diabetes prevalence	57	1.5 (1.2–2.0)
	Use of oral medications for diabetes	19	2.3 (1.3–3.9)
	Serum insulin abnormalities	18	3.4 (1.9–6.1)
Studies reviewed in *Update 1998*			
Henriksen et al., 1997	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)[d]
Studies Reviewed in *VAO*			
AFHS, 1991	Air Force Ranch Hand veterans	85	$p = 0.001$, $p = 0.028$
AFHS, 1984	Air Force Ranch Hand veterans	158	$p = 0.234$

[a] May include some of the same subjects covered in the NIOSH cohorts addressed in the other references cited in the occupational-cohorts category.
[b] Not discussed in this report, but discussed as new studies in *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (IOM, 2000).
[c] Adjusted for age, race, body mass index, waist size, family history of diabetes, body mass index at time dioxin was measured, serum triglycerides, and military occupation.
[d] Self-reported medical history; answer to question, "Since your first day of service in Vietnam, have you been told by a doctor that you have diabetes?"
ABBREVIATIONS: N/A, not applicable; TCP, trichlorophenol, HDL, high-density lipoprotein; OR, odds ratio; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.

sample and definition of outcome were essentially unchanged. Accordingly, there was little change in the association between the dichotomous definition of exposure and diabetes in the Ranch Hand sample (OR = 1.18 in the new analysis and 1.11 in the original analysis). The OR for the sample association in the NIOSH data, however, was reduced from 1.49 to 1.22. The later analysis of a dose-response relationship between diabetes and TCDD at the time of examination was based on quartiles from the distribution of TCDD for both samples combined. The highest quartile (TCDD concentration ≥ 78 ppt) was associated with a markedly increased risk of diabetes in Ranch Hand subjects (OR = 3.2, 95% CI 1.8–5.7). There was no evidence of a dose-response relationship in the data from

the NIOSH sample and no excess risk for the highest quartile (OR = 0.84, 95% CI: 0.4–1.8) in this analysis. A similar pattern of findings was obtained in the analysis of back-extrapolated TCDD concentrations.

Environmental Studies

Masley et al. (2000) administered a population-based survey in 1995 in a rural area of southern Saskatchewan to investigate the effects of environmental and agricultural exposure on the health of rural populations. Data were collected from 369 farming households (727 adult respondents) and 163 nonfarming households (262 adult respondents). Health concerns in the survey included self-reported diagnosis of diabetes. A history of diabetes was reported by only 28 people and was not related to farm vs nonfarm status. No information was presented on the validity of this measure of diabetes.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001) other than the combined NIOSH and Ranch Hand analysis (Steenland et al., 2001) discussed above.

Synthesis

New publications on diabetes since *Update 2000* include two cross-sectional studies and a joint analysis of two previously published studies. The cross-sectional studies by Kitamura et al. (2000) and Masley et al. (2000) include weak and indirect tests of the association between herbicide exposure and diabetes. The study by Kitamura et al. (2000) is based on 92 workers at a municipal waste incinerator. There is no comparison group from a nonexposed worksite. The exposure in this study is PCDD concentration in the blood, with no separate analysis of TCDD in relation to diabetes or other health outcomes. Both cross-sectional studies rely on self-report for the measurement of diabetes, and neither study presents data on the validation of the self-reported diagnosis. The study sample of Masley et al. (2000) is larger (989 adult respondents), but the small number of cases (28) suggests an insensitive measure of diabetes. In addition, the exposure in this study is a crude comparison of living in a farming vs a nonfarming household. Given the limitations of measurement in these studies, the null findings with respect to diabetes cannot be interpreted as strong evidence of no association.

The modest relationship between plasma TCDD and diabetes in the Ranch Hand veterans and the chemical-plant workers of New Jersey and Missouri has already been summarized in *Update 1998* and *Update 2000*, respectively. In the new joint analysis of the data, the association between diabetes and TCDD in the

chemical-plant workers is essentially eliminated, precluding a pooled analysis of the two study populations. Steenland et al. (2001) do not formally analyze the reasons for the change in the results in the chemical-plant workers. The explanation may include the change in the definition of diabetes (suggesting that borderline cases were more common in the nonexposed) or the selective impact of removal of the 55 subjects from the analysis (perhaps a disproportionate number of exposed cases) to match the eligibility criteria of the Ranch Hand study. It should be noted that the initial analysis of the chemical-plant workers (Calvert et al., 1999) is not invalid, but the joint analysis reveals that the results were not robust.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and diabetes.

Biologic Plausibility

Evaluating the potential of TCDD to induce clinical diabetes in animals has been impaired by the lack of an appropriate animal model of type 2 diabetes. However, TCDD effects on triglycerides and high-density lipoproteins, glucose transport, protein kinase C, and other lipoproteins in animals suggest that TCDD could stimulate development of diabetes. Several studies have demonstrated that TCDD treatment decreases glucose transport and alters lipoprotein degradation in adipose-tissue cell lines. That might constitute a physiologic mechanism for linking TCDD exposure to diabetes. Nevertheless, until appropriate animal models are developed to show the etiology and pathogenesis of diabetes, the ability of TCDD to induce diabetes in animals will remain elusive.

Increased Risk of Disease Among Vietnam Veterans

Available data allow for the possibility of an increased risk of type 2 diabetes in Vietnam veterans. It must be noted, however, that studies indicate that the increased risk, if any, posed by herbicide or TCDD exposure appears to be small. The known predictors of diabetes risk—family history, physical inactivity, and obesity—continue to greatly outweigh any suggested increased risk posed by wartime exposure to herbicides.

LIPID AND LIPOPROTEIN DISORDERS

Plasma lipid (notably cholesterol) concentrations have been shown to predict cardiovascular disease and are considered fundamental to the underlying atherosclerotic process (Kuller and Orchard, 1988). The two major types of lipids, cholesterol and triglycerides, are carried in the blood attached to proteins to form lipoproteins, which are classified according to their density. Very-low-density lipoprotein (VLDL—the major "triglyceride" particle) is produced in the liver and is progressively catabolized (hydrolyzed) mainly by an insulin-mediated enzyme (lipoprotein lipase) to form intermediate-density lipoprotein (IDL) or VLDL remnants. Most of the VLDL remnants are rapidly cleared by the liver LDL receptors (types B and E); the rest form low-density lipoprotein (LDL), the major "bad" cholesterol particle. This LDL 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 the catabolism of VLDL. LDL is thought to be involved in the delivery of cholesterol to the tissues, whereas HDL 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 susceptibility to diabetes); and mixed hyperlipidemias in which both cholesterol and triglycerides are elevated. The mixed hyperlipidemias group includes familial combined hyperlipidemia, which is thought by many to result from hepatic overproduction of VLDL and apoprotein B, and type III dyslipidemia, which involves defective clearance of IDL–VLDL remnants and a buildup of those atherogenic particles. Although the bulk of blood lipid concentration is genetically determined, diet, activity, and other factors (concurrent illness, drugs, age, gender, hormones, and so on) do have major effects. In particular, the saturated-fat content of the diet might raise LDL concentrations via decreased LDL-receptor activity, whereas obesity and a high-carbohydrate diet may increase VLDL triglycerides and possibly are linked to insulin resistance and reduced lipoprotein lipase activity. Intercurrent illness may increase the triglyceride concentration and decrease the cholesterol concentration. Diabetes is also associated with increased triglycerides and decreased HDL cholesterol, whereas other diseases (such as thyroid and renal disorders) often result in hypercholesterolemia. It is evident, therefore, that multiple host and environmental factors influence lipid and lipoprotein concentrations and that those 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 triglyceride and glucose concentrations and lower HDL and LDL cholesterol must be recognized.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and lipid and lipoprotein disorders. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Reviews of the studies underlying the finding may be found in the earlier reports (see Table 9-2 for a summary).

Update of the Scientific Literature

An occupational study by Kitamura et al. (2000) of 92 workers at a municipal waste incinerator in Japan included measures of serum PCDD and self-reported history of a number of diseases and health-related conditions. Eight of the 92 subjects reported having been diagnosed with high cholesterol. The sample was divided into quartiles of PCDD concentration for the purpose of analysis, and a logistic-regression model (including terms for age and body mass index) was used to assess the association between PCDD and the prevalence of hyperlipidemia. The authors reported a statistically significant OR for this association (OR = 6.08, $p = 0.023$), but they did not indicate whether that OR was derived from the contrast of specific quartiles or from a single ordinal variable.

No relevant environmental or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

Only one new study on lipids is reviewed in this volume, and its contribution to the literature is limited for a number of reasons. The study is cross-sectional, so there is no opportunity to establish that the exposure clearly preceded the outcome. The sample is small, the exposure is an aggregate measure of PCDD (with TCDD as only one component), and the measure of high cholesterol is based on self-report with no additional information on its validity. Because of those limitations, the study does not change the inconclusive status of the epidemiologic evidence on the relationship between exposure to herbicides and high serum lipids.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there

TABLE 9-2 Selected Epidemiologic Studies—Lipid and Lipoprotein Disorders

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
OCCUPATIONAL			
New Studies			
Kitamura et al., 2000	Workers exposed to PCDD at a municipal waste incinerator—elevated cholesterol	8	6.1, $p = 0.02$
Studies Reviewed in *Update 1998*			
Calvert et al., 1996	Workers ($N = 273$) exposed to 2,4,5-T and derivatives vs. matched referents ($N = 259$)		
	OR for abnormal total cholesterol concentration		
	Overall	95	1.1 (0.8–1.6)
	High TCDD	18	1.0 (0.5–1.7)
	OR for abnormal HDL cholesterol concentration		
	Overall	46	1.2 (0.7–2.1)
	High TCDD	15	2.2 (1.1–4.7)
	OR for abnormal mean total to HDL cholesterol ratio		
	Overall	131	1.1 (0.8–1.6)
	High TCDD	36	1.5 (0.8–2.7)
	OR for abnormal mean triglyceride concentration		
	Overall	20	1.0 (0.5–2.0)
	High TCDD	7	1.7 (0.6–4.6)
Ott and Zober, 1996[a]	Production workers	42	
	Cholesterol		NSE
	Triglycerides		NSE
	HDL cholesterol		Increased; $p = 0.05$
Studies Reviewed in *VAO*			
Martin, 1984[a]	Production workers		
	Workers with some exposure	53	
	Cholesterol		Increased; $p < 0.005$
	Triglycerides		Increased; $p < 0.005$
	HDL cholesterol		NSE
	Workers with chloracne	39	
	Cholesterol		Increased; $p < 0.05$

continues

TABLE 9-2 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
	Triglycerides		Increased; $p < 0.01$
	HDL cholesterol		NSE
Moses et al., 1984[b]	TCP and 2,4,5-T production workers	118	
	Cholesterol		NSE
	Triglycerides		NSE
Suskind and Hertzberg, 1984[a]	TCP production workers	204	
	Cholesterol		NSE
	Triglycerides		NSE
	HDL cholesterol		NSE
May, 1982[a]	TCP production workers	94	
	Cholesterol		NSE
	Triglycerides		NSE
Pazderova-Vejlupkova et al., 1981[a]	TCP and 2,4,5-T production workers	55	
	Cholesterol		NSE
	Triglycerides		Increased VLDL; $p = 0.01$
ENVIRONMENTAL			
Studies Reviewed in *VAO*			
Assennato et al., 1989[a]	Adults exposed near Seveso Zone A subjects who developed chloracne	193	
	Cholesterol		NSE
	Triglycerides		NSE
Mocarelli et al., 1986[a]	Children exposed near Seveso	63	
	Cholesterol		NSE
	Triglycerides		NSE
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	858	
	Cholesterol		NSE
	Triglycerides		NSE
Studies reviewed in *Update 1998*			
AFHS, 1996[c]	Longitudinal analysis (1992 examination data)	884	
	Cholesterol		NSE (cholesterol: HDL ratio)

TABLE 9-2 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
	Triglycerides		NSE
	HDL cholesterol		NSE (cholesterol: HDL ratio)
O'Toole et al., 1996[d]	Australian Vietnam veterans	20	
	Cholesterol		3.0 (1.3–4.7)
Studies reviewed in *VAO*			
AFHS, 1991[e]	Serum dioxin analysis (1987 examination data)	283–304[f]	
	Cholesterol		$p = 0.175$
	Triglycerides		$p < 0.001$[g]
	HDL cholesterol		$p < 0.001$
AFHS, 1990[h]	Original exposure group analysis (1987 examination data)	8–142[f]	
	Cholesterol		1.2 (0.9–1.5)
	Triglycerides		1.3 (0.9–1.8)
	HDL Cholesterol		1.0 (0.4–2.4)
AFHS, 1984[i]; Wolfe et al., 1990[i]	Air Force Ranch Hand veterans to herbicide spraying (1982 data)	1,027	
	Cholesterol		NSE
	Triglycerides		NSE
	HDL cholesterol		NSE

[a] *p*-values comparing means with controls. Univariate analysis.
[b] *p*-values comparing means in production workers with later chloracne with those without.
[c] Comparing change over time between exposed and comparison groups.
[d] Compared with Australian population.
[e] Comparing mean dioxin across lipid groups.
[f] Number of exposed Ranch Hand veterans with "high" lipid values.
[g] Continuous analysis.
[h] Model 1, Ranch Hands vs comparisons–adjusted.
[i] Comparing means.
NOTE: Estimated risk and 95% CI reported unless *p*-values are specified.
ABBREVIATIONS: HDL, high-density lipoprotein; NSE, no significant effect; OR, odds ratio; PCDD, polychlorinated dibenzodioxin; TCP, trichlorophenol; VLDL, very-low density lipoprotein.

is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and lipid and lipoprotein disorders.

Biologic Plausibility

Although animal studies suggest potential mechanisms whereby TCDD may cause lipid disturbances, human data (such as those from lipoprotein kinetic studies) are still needed to determine whether and how TCDD-exposed subjects have altered lipoprotein metabolism. Chapter 3 discusses recent animal toxicity studies that could contribute to a biologic basis of an association between exposure to TCDD and herbicides and toxicity end points, and a general summary of the biologic basis of various end points is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

As discussed in *Update 2000* (IOM, 2001), the most recent Air Force Health Study (AFHS, 2000) reports an inconsistent association between TCDD exposure and lipid abnormalities in US veterans of Vietnam. The data are insufficient to permit a conclusion about whether they are at increased risk for these disorders.

GASTROINTESTINAL AND DIGESTIVE DISEASE, INCLUDING LIVER TOXICITY

This section covers a variety of conditions encompassed by ICD-9 codes 520–579. Conditions in this category include diseases of the esophagus, stomach, intestines, rectum, liver, and pancreas. Additional details on peptic ulcer and liver disease—the two conditions most often discussed in the literature reviewed—are provided below. The symptoms and signs of gastrointestinal disease and liver toxicity are highly varied and often vague.

The essential function of the gastrointestinal tract is to absorb nutrients and eliminate waste products. This complex task involves numerous chemical and molecular interactions on 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 up to 15% of adults. The most convenient way of categorizing diseases that affect the gastrointestinal system is by the affected anatomic segment. The conditions include esophageal disorders that predominantly affect swallowing, gastric disorders related to acid secretion, and conditions affecting the small and large intestine and reflected in alterations in nutrition, mucosal integrity, and motility. Some systemic disorders can also affect the gastrointestinal system (for example 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 that protect against ulceration. About 10% of the population has clinical evidence of duodenal ulcer during their lifetimes, and a similar percentage are affected by gastric ulcer. The peak incidence for duodenal ulcer occurs in the fifth decade of life, and the peak for gastric ulcer occurs about 10 years later. The natural history of duodenal ulcer is one of spontaneous remission (healing) and recurrences. It is estimated that 60% of healed duodenal ulcers recur in the first year and 80–90% within 2 years.

Increasing evidence indicates that the bacterium *Helicobacter pylori* (*H. pylori*) may be closely linked to peptic ulcer disease (both duodenal and gastric). This bacterium colonizes the gastric mucosa in 95–100% of patients with duodenal ulcer and 75–80% of patients with gastric ulcer. Healthy subjects in the United States under 30 years old have gastric colonization rates of about 10%. Over the age of 60 years, colonization rates exceed 60%. Colonization alone, however, is not sufficient for the development of ulcer disease; only 15–20% 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 about 3 times the general population's risk of developing duodenal ulcer. Some blood groups are associated with increased risk of 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, psychologic factors, particularly chronic anxiety and psychologic stress, may act to exacerbate duodenal ulcer disease.

Liver Disease

Blood tests reflecting liver function are the mainstay of diagnosis of liver disease. Increases in serum bilirubin and in the serum activity of some hepatic enzymes—including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase (GGT)—are commonly noted in liver disorders. The relative sensitivity and specificity of these enzymes for diagnosing 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 increase in GGT. Estimated serum activity of this enzyme constitutes 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. Increases are noted after many chemical and drug exposures that are not followed by evidence of liver injury. The confounding effects of alcohol ingestion (often associated with increased GGT) make interpretation of changes in GGT in exposed people difficult (Calvert et al., 1992). Moreover, an increase 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 in epidemiologic studies of herbicide or TCDD exposure. Pathologically, cirrhosis reflects irreversible chronic injury of the liver, with extensive scarring and resulting loss of liver function. Clinical symptoms and signs include jaundice, edema, abnormalities in blood clotting, and metabolic disturbances. 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 pathologic 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 infection (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*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and gastrointestinal and digestive disease, including liver toxicity. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Reviews of the studies underlying the finding can be found in the earlier reports.

Update of the Scientific Literature

Occupational Studies

Geusau et al. (2001) describe two cases of heavy TCDD intoxication and present a 2-year follow-up, including clinical, biochemical, hematologic, endocrine, and immunologic measures. Patient 1, a 30-year-old woman, presented with chloracne and had the highest TCDD concentration ever recorded in a human (144,000 pg/g of blood fat); and Patient 2, a 27-year-old woman who worked in the same room as Patient 1, had 26,000 pg/g of blood fat. Both women experienced gastrointestinal symptoms, including nausea, vomiting, epigastric pain, and loss of appetite. Those gastrointestinal symptoms lasted about 4 months.

Liver-function studies during this time were within the normal limits except for one value in Patient 1 of alkaline phosphatase at 1.5 times the upper limit of normal. Apart from the chloracne and gastrointestinal symptoms, few clinical signs or symptoms were observed in the patients in the acute phase of the intoxication.

Environmental Studies

No relevant environmental studies have been published since *Update 2000* (IOM, 2001).

Vietnam-Veteran Studies

Michalek et al. (2001) published a report evaluating hepatic abnormalities in Vietnam veterans of Operation Ranch Hand. The authors examined exposure to TCDD and the prevalence of liver disease and hepatomegaly through March 1993 in relation to tests of liver function at the 1992 physical examination. Hepatomegaly among veterans in the high-exposure category was slightly higher than that in nonexposed veterans in the comparison category (adjusted OR = 1.4; 95% CI 0.7–3.1). The prevalence of nonspecific liver disorders (coded as ICD-9 573.0–573.9) increased across categories of TCDD exposure and among Ranch Hands in the high-exposure category; this prevalence was about 60% greater than in the nonexposed group. The association between GGT and TCDD category is puzzling in that the mean GGT in the high-exposure group was significantly increased among veterans with history of light to moderate drinking. The authors conclude that evidence of clinically significant liver disease was limited to the increase in hepatomegaly. The increased GGT, however, could have been due to confounding.

Synthesis

Evaluation of the effect of herbicide and TCDD exposure on noncancer gastrointestinal ailments is more difficult than evaluation of the effect on some of the other outcomes examined in this report. Clinical experience suggests that medical history and physical examination are undependable diagnostic tools for some of these ailments, so incidence data are more problematic. The strong interdependence between characteristics of a given person (weight and laboratory indexes of hepatic function and health) and body burden of TCDD complicate the already difficult task of assessing association.

The latest AFHS report (Michalek et al., 2001) found an increased risk of other liver disorders among veterans with the highest TCDD concentrations: primarily increased transaminase and other nonspecific liver abnormalities. It is unclear whether the observed association is related to TCDD exposure.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and gastrointestinal and digestive diseases.

Biologic Plausibility

The liver is a primary target organ of TCDD in animals. Therefore TCDD would be expected to induce liver toxicity in humans at appropriate doses. Direct effects of TCDD and herbicides on other gastrointestinal and digestive diseases have not been seen. Chapter 3 discusses recent toxicologic studies that form the biologic basis of an association between exposure to TCDD or herbicides and toxicity end points.

Increased Risk of Disease Among Vietnam Veterans

The available data on Vietnam Ranch Hand veterans do not permit a conclusion about whether they are at increased risk for gastrointestinal and digestive diseases. There is no evidence that Vietnam veterans are at greatly increased risk for serious liver disease.

CIRCULATORY DISORDERS

This section covers 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, various methods were used to assess the circulatory system, including analysis of symptoms or history, physical examination of the heart and peripheral arteries, Doppler measurements of peripheral pulses, electrocardiography (ECG), and chest radiography. Doppler measurements and physical examination of pulses in the arms and legs are used to detect decreases in pulse strength, which can be caused by thickening and hardening of the arteries. ECG can be used to detect heart conditions and such abnormalities as arrhythmia (abnormal heart rhythm), heart enlargement, and previous heart attack. Chest radiography can be used to assess enlargement of the heart, which can result from heart failure and other heart conditions. Mortality studies attribute cause of death to circulatory disorders with various degrees of diagnostic confirmation.

There is growing evidence that exposure to inorganic arsenic is a risk factor for cardiovascular disease, and cacodylic acid (DMA) is a metabolite of inorganic arsenic. As discussed in Chapter 2, however, the data remain insufficient to conclude that studies of inorganic arsenic exposure are directly relevant to exposure to cacodylic acid. Therefore, the literature on inorganic arsenic is not considered in this section.

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The committee responsible for *VAO* found that there was inadequate or insufficient information to determine whether an association existed between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and circulatory disorders. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that finding. Reviews of the studies underlying the finding can be found in the earlier reports.

Update of the Scientific Literature

Occupational Studies

Burns et al. (2001) conducted a study of mortality in a cohort of 1,517 male workers involved in the manufacture or formulation of 2,4-D at a Dow chemical plant in 1945–1994. Mortality in the study cohort was compared with mortality in all white US males. Standardized mortality ratios (SMRs) were computed separately for assumed incubation periods of 0 and 20 years. For all diseases of the circulatory system (ICDA-8 390–458), the SMR in the absence of an incubation period was 95 (based on 158 deaths, 95% CI 80–111). The assumption of a 20-year incubation led to an SMR of 105 (130 deaths, 95% CI 87–124). The corresponding SMRs for all-cause mortality in this cohort were 90 and 94, respectively.

A cross-sectional study was conducted in 1998 to assess the association between serum PCDD and a variety of health conditions in a sample of workers employed at a municipal waste incinerator in Japan (Kitamura et al., 2000). Fourteen of the 92 workers participating in the study reported a history of hypertension. No information was provided on the date of this diagnosis relative to dates of employment at the plant. The sample was divided by quartile of PCDD concentration, and a logistic-regression model was fitted (with terms for age and body-mass index) to examine the association between PCDD and the prevalence of self-reported hypertension. The coefficients for the categories of PCDD were reported as not statistically significant, but the values of the coefficients and their standard errors were not given.

Environmental Studies

Mortality from cardiovascular diseases was examined by Revich et al. (2001) in their study of multiple health outcomes among residents of Chapaevsk, a Russian city with dioxin contamination of the air, soil, and water from a local chemical plant. The authors reported that mortality from cardiovascular diseases in men was 1.14 times greater than the mortality rate for Russia as a whole and that the difference in mortality was especially pronounced in men 30–49 years old. The rates were also elevated compared with the Samara region in general. However, they also noted that trends in mortality were directly related to trends in unemployment; this suggests the simultaneous effects of other aspects of the environment in the area.

A survey conducted in a rural area of southern Saskatchewan was administered to 727 adult residents of farming households and 262 residents of nonfarming households (Masley et al., 2000). The survey included questions about the use of pesticides and fertilizers and a number of health conditions and symptoms that might be associated with agricultural exposures. Physician-diagnosed hypertension and heart disease were reported by 154 and 44 survey respondents, respectively. Neither condition was associated with residing on a farm. No information was provided on the validity of measurement of the self-reported health conditions, and the report did not examine more-specific associations with pesticide or fertilizer use.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

The new studies that have examined hypertension as an outcome used self-reported history of a physician diagnosis for the definition of this variable. Neither study provided data on validation from medical records or direct measurement of blood pressure in a subset of the study population. Some misclassification is likely, with false negatives (nondiagnosed hypertension) being more common than false positives, especially among men. The studies by Kitamura et al. (2000) and Masley et al. (2000) also use nonspecific assessment of exposure. Masley et al. (2000) compare people from farming and nonfarming households, and give no information on the extent of herbicide use by the former. Kitamura et al. (2000) use PCDD exposure for the analysis of their study population of municipal-incinerator workers; this composite measure includes many exposures in addition to TCDD, and no comparisons are made with workers from another setting where such exposures are absent. It is possible that the null findings on hypertension in

these studies reflect the influence of misclassification that led to bias toward the null.

The occupational cohort study by Burns et al. (2001) found that mortality due to circulatory conditions among the workers was similar to that experienced by US white males in general. Mortality analyses of other occupational cohorts have tended to find cardiovascular effects among the more highly exposed workers, but a dose-specific analysis of the data on this outcome was not reported despite the availability of the data and of enough deaths for analysis. The ecologic finding of increased cardiovascular mortality in Chapaevsk compared with the Samara region in general and Russia in general is an interesting preliminary result, but Revich et al. (2001) concede that trends in cardiovascular mortality have also been associated with unemployment in Chapaevsk. A challenge of additional research in this setting will be to evaluate the etiologic role of TCDD exposure in the larger context of health-related social changes and in concert with the influence of individual-level risk factors, such as smoking, diet, and physical activity.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and specific circulatory disorders (such as coronary artery disease, myocardial infarction, stroke, and hypertension) or circulatory conditions in general. As noted in earlier reports, important sources of uncertainty include the quality of measurement of health outcomes, incomplete assessment of confounding, and inconsistency of findings among magnitudes of exposure.

Biologic Plausibility

There have been reports of developmental defects in the cardiovascular system of TCDD-treated birds and fish. Recently, a dose-dependent increase in myocardial fibrosis has been observed in marmosets acutely exposed to relatively low doses of TCDD. In addition, subchronic treatment of hyperlipidemic ApoE-deficient mice with TCDD caused a trend for earlier onset and greater severity of atherosclerotic lesions compared with vehicle-treated mice. Notably, ApoE-deficient mice have a lipoprotein profile similar to that of humans with type III hyperlipoproteinemia and develop extensive aortic and coronary atherosclerosis with lesions that are similar to those observed in humans. Therefore, there are

data that suggest some biologic plausibility of an association between TCDD exposure and increased risk of cardiovascular disease. However, it is clear that additional studies are needed to confirm the relationships and to determine the relevance to humans. Chapter 3 discusses recent animal toxicity studies that could contribute to a biologic basis of an association between exposure to TCDD and herbicides and toxicity end points, and a general summary of the biologic basis of various end points is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The available data on Vietnam veterans do not permit a conclusion about whether they are at an increased risk for circulatory disorders.

AL AMYLOIDOSIS

Amyloidosis (ICD-9 code 277.3) refers to a group of diseases in which insoluble fibrillar proteins (amyloid) accumulate in tissues to a point that causes organs to malfunction. There are several types of amyloidosis. The disease was formerly characterized by whether it was primary (occuring in the absence of a discernible preceding disease that led to it) or secondary to another disease. Currently, however, the disease is classified on the basis of the structure of the subunit fibril protein. In the type of amyloidosis reviewed here, light chain-associated (AL) amyloidosis (also sometimes referred to as primary amyloidosis), the light chain of immunoglobulin molecules is the aberrant protein (Gertz, 1999). AL amyloidosis is the most common form of systemic amyloidosis in the United States.

The study of systemic amyloidosis is difficult because it is also a complication that occurs in about 15–20% of patients with multiple myeloma (a disease of the bone marrow). Differentiation of the amyloid associated with myeloma from that of AL amyloidosis is artificial because the amyloid is of similar genesis and tissue distribution, and the conditions are more appropriately considered as parts of the spectrum of the same basic disease process.

Amyloidosis, like multiple myeloma, occurs mainly in people 50–70 years old and in more males than females. Annual incidence is estimated to be about one per 100,000, or over 2,000 new cases per year in the United States (Solomon, 1999).

Summary of *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*

The VA identified AL amyloidoisis as a concern after *Update 1998* and therefore it was specifically looked at by the committee responsible for *Update 2000*. That committee concluded that there was inadequate or insufficient evi-

dence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and AL amyloidosis.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2000* (IOM, 2001).

Synthesis

The association between TCDD exposure and AL amylodosis seen in one study in mice may or may not apply to human beings and cannot be readily interpreted with respect to the future risk for Vietnam veterans.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed in this and previous *Veterans and Agent Orange* reports, the committee finds that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and AL amyloidosis.

Biologic Plausibility

An association has been reported between AL amyloidosis and TCDD exposure in a single study in mice, but the TCDD exposure was relatively intense and may or may not be relevant to Vietnam veterans. Chapter 3 discusses recent animal toxicity studies that could contribute to a biologic basis of an association between exposure to TCDD and herbicides and toxicity end points, and a general summary of the biologic basis of various end points is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

There is no evidence to suggest that Vietnam veterans are at an increased risk of developing AL amyloidoisis. The disorder is rare. However, veterans of the Vietnam era and their generation are only now entering the age when AL amyloidosis is most likely to occur.

ENDOMETRIOSIS

Earlier volumes of *Veterans and Agent Orange* (IOM, 1994, 1996, 1999, 2001) did not address the association between the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and endometriosis. This section reviews the evidence on that health outcome.

Endometriosis (ICD-9 617) is a disease that affects over 5 1/2 million women in the United States and Canada (Endometriosis Association, 2002). Endometrium is the tissue that lines the inside of the uterus and is built up and shed each month during menstruation. In endometriosis, endometrium is found outside the uterus—usually in other parts of the reproductive system, the abdomen, or the tissues near the reproductive organs. That misplaced tissue develops into growths or lesions that continue to respond to hormonal changes in the body and break down and bleed each month in concert with a woman's menstrual cycle. Unlike blood released from endometrium in the uterus, blood released from the tissue in endometriosis has no way to leave the body, and this results in inflammation, internal bleeding, and degeneration of blood and tissue from the growth and can cause scarring, pain, infertility, adhesions, and intestinal problems.

Several theories exist as to why endometriosis occurs, including that the disease has a genetic component, but the cause remains unknown. It has been proposed that endometrium is distributed through the body via blood or the lymphatic system; that menstrual tissue backs up into the fallopian tubes, implants in the abdomen, and grows; and that all women experience some form of tissue backup during menstruation and that only those with immune system or hormonal problems experience the tissue growth associated with endometriosis. Despite numerous symptoms that can indicate endometriosis in the body, diagnosis of the disease can be determined only through laparoscopy or a more invasive surgical technique. Several treatments for endometriosis are available, but there is no cure.

Suspicion that TCDD is involved in the etiology of endometriosis started after the observation that the incidence of endometriosis was higher in monkeys treated with low doses of TCDD than in control monkeys. Experimental and epidemiologic studies have been conducted. A number of the epidemiologic studies have investigated non-dioxin-like PCBs, and some have also looked at TCDD or dioxin-like compounds.

Review of the Scientific Literature

Mayani et al. (1997) analyzed blood TCDD concentrations in 79 women who were being evaluated for infertility, 44 of whom were diagnosed with endometriosis using laparoscopy. All the women had resided in Jerusalem for at least 10 years and were of similar socioeconomic status. The authors found that eight of the 44 women with endometriosis were positive for TCDD (18%) compared with one of the 35 controls (3%), for an OR of 7.6 (95% CI 0.87–169.7).

The number of subjects in the study, however, is small, and the ethnic distribution differed among controls and patients with endometriosis. Triglycerides were measured and did not differ between groups, but TCDD measurements were not adjusted for blood lipids. Furthermore, the limit of detection—what was considered testing positive for TCDD—is not clear in the study, although a review article states that it was 2 ppt (Birnbaum and Cummings, 2002).

Pauwels et al. (2001) assessed whether TCDD toxic equivalents (TEQs) in serum are associated with endometriosis in an infertile population of women who enrolled in fertility treatment at one of the collaborating centers for reproductive medicine in Belgium in 1996–1998. The case–control study evaluated 42 women with endometriosis (cases) and 27 controls with infertility related to tubal disease, tuboperitoneal factors, cervical factors, or uterine factors but without endometriosis. The authors report no association between median total TEQs and endometriosis in infertile women. The lack of association was evident even in a subgroup of patients with very high exposures (TEQs at over 100 pg/g of serum lipids). Adjustments for potential confounders, such as body-mass index and alcohol consumption, did not change the overall results. Therefore, dioxin-like compounds do not appear to contribute to endometriosis among infertile women. Given the design of the study, it offers no information on the association between infertility and specific exposure to TCDD.

No relevant occupational or Vietnam-veteran studies have been published.

Synthesis

Of the two studies that investigate the association between TCDD or TCDD-like compounds and endometriosis, one (Mayani et al., 1997) shows an increased OR (7.6), but the confidence interval was very wide (0.87–169.7). The other (Pauwels et al., 2001) did not show a significant association between exposure to dioxins and endometriosis. Both studies, however, are relatively small.

Conclusions

Strength of Evidence from Epidemiologic Studies

There is inadequate or insufficient evidence to determine whether an association exists between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and endometriosis.

Biologic Plausibility

There is evidence from animal studies that TCDD can exacerbate or cause endometriosis, including a recent report that demonstrated increased endometrio-

sis in rhesus monkeys exposed to TCDD. One study, however, did not show any increase in surgically induced endometriosis with TCDD exposure. Other evidence does demonstrate that TCDD inhibits progesterone-associated transforming growth factor β_2 (TGFβ_2) expression and endometrial matrix metalloproteinase suppression; those effects have been suggested as mechanisms underlying an association between TCDD and endometriosis. The ability of TCDD to alter the expression of several growth factors, cytokines, and hormones may also mediate the promotion of endometriosis. Notably, the AhR and several AhR target genes are expressed in human endometriotic tissues. Because animal data and the sparse human data support the possible biologic plausibility of an association between TCDD exposure and endometriosis, that possible association should continue to be investigated.

Increased Risk of Disease Among Vietnam Veterans

There are insufficient data on endometriosis in Vietnam veterans to draw a specific conclusion as to whether Vietnam veterans are at increased risk for this disorder.

THYROID HOMEOSTASIS

Earlier volumes of *Veterans and Agent Orange* (IOM, 1994, 1996, 1999, 2001) did not address the thyrotoxic potential of TCDD and the herbicides used in Vietnam. This section reviews the evidence of that health outcome.

The thyroid gland secretes the hormones thyroxine (T4) and triiodothyronine (T3), which stimulate metabolic rate. The thyroid also secretes calcitonin, a hormone that controls calcium concentration in the blood and storage of calcium in bones. Secretion of T4 and T3 is under the control of thyroid-stimulating hormone (TSH), which is secreted by the anterior pituitary gland. Iodine plays a central role in thyroid physiology both as a constituent of thyroid hormones and as a regulator of glandular function. Control of circulating concentrations of these hormones is regulated primarily by a negative-feedback pathway that involves three organs: the thyroid, which produces thyroid hormones, and the pituitary and hypothalamus, which help to maintain optimal T3 and T4 concentrations. In the hypothalamus–pituitary–thyroid feedback scheme, the hypothalamus stimulates the pituitary through thyrotropin-releasing hormone (TRH) to produce TSH, which triggers the thyroid to produce T4 and T3. Cells in the hypothalamus and pituitary respond to concentrations of circulating T4 and T3. When T4 and T3 are low, the pituitary is stimulated to deliver more TSH to the thyroid to increase T4 and T3 output. When circulating T4 and T3 are high, they signal to reduce the output of TRH and TSH. This negative-feedback loop maintains hormone homeostasis. Chemical-induced alterations in thyroid homeostasis can adversely affect the development of many organ systems, including the nervous and repro-

ductive systems. Most adverse effects are caused by lack of thyroid hormone alone rather than by increases in TSH.

Effects on the thyroid can be stimulatory (hyperthyroidism) or suppressive (hypothyroidism). Graves' disease is an example of hyperthyroidism; cretinism is an extreme example of hypothyroidism. Insufficient iodine intake resulting in goiter is usually not associated with either hyperthyroidism or hypothyroidism.

TCDD affects the concentrations of thyroid hormones; the effects appear to be species-dependent and may reflect both the dose and the duration of exposure (IOM, 2001). TCDD influences the metabolism of thyroid hormones and TSH. However, contrasting results confuse interpretation of the effects of TCDD on the production and activity of the hormones.

Review of the Scientific Literature

Environmental Studies

Studies of environmental exposure have emphasized thyroid alterations in prenatal and early childhood development rather than in adults.

Pluim et al. (1992) evaluated 38 mother–infant pairs in Amsterdam, the Netherlands, selected for normal birthweight and no complications. Total T4 and thyroxine-binding globulin (TBG) were measured in all samples, which included plasma from maternal delivery blood, cord blood, and infant 1-week and 11-week samples. TSH was measured in cord blood and in many of the 1-week and 11-week samples; total T3, free T4, and reverse T3 were measured in cord; and total T3 was measured at 11 weeks. Concentrations of dioxins and dibenzofurans were measured in breast-milk samples taken 3 weeks after delivery and expressed in nanograms per kilogram of milk fat; these were multiplied by their toxic equivalency factors and summed to obtain a total TEQ. The mother–infant pairs were categorized into two groups, above and below the median TEQ, and these two groups were compared. In cord blood, the concentrations of total T4 and TBG were suggestively higher ($0.05 < p < 0.10$) in the high exposure group, but no other measurements approached significance. At 1 week, total T4 and the ratio of total T4 to TBG were significantly greater in the high-exposure than the low-exposure group, and the same was true at 11 weeks, when, in addition, TSH concentrations were also significantly higher. When only infants who were breastfed for the full 11 weeks were considered, only the ratio of total T4 to TBG remained significantly different between the two groups. Finally, in a subset of the births for which values were obtained in both the cord and 1-week samples, the increases in total T4 and in TBG were substantially higher in the high-exposure group. Concerns about those results are related to the size of the study sample and the loss of nearly one-fourth of the maternal-blood samples (nine) and five of the cord-blood samples for all thyroid measurements; several more samples were insufficient for some of the analyses.

In a larger series (105 mother–infant pairs) in Rotterdam, the Netherlands, Koopman-Esseboom and colleagues (1994) conducted similar analyses. Blood was collected from the mothers during the last month of pregnancy and from the cord after birth for measurement of PCBs; breast milk was collected in the second week after delivery for measurement of dioxins, dibenzofurans, and planar PCBs. TEQs were calculated from those measurements. Total T4, total T3, free T4, and TSH were measured in maternal plasma taken during the last month of pregnancy and 9–14 days after delivery, and in newborn infants' plasma taken at 2 weeks and 3 months. TEQ correlated negatively with maternal pregnancy total T3 and maternal postdelivery total T3 and total T4; similar associations were seen for planar PCB TEQ and total PCB and TEQ, and the associations with total T3 were also observed for nonplanar PCB TEQ. In addition, all four TEQ measurements correlated positively with infant 2-week TSH, and all except the nonplanar PCB TEQ were positively associated with the infant 3-month TSH.

Longnecker and colleagues (2000) examined PCB concentrations in breast-milk specimens, without adjustment for lipids, in relation to thyroid hormones in cord serum in a population with background exposure. They found little evidence of an association, although the direction of the coefficient for TSH in multiple-regression analysis was consistent with findings in other studies: increases in TSH with increases in PCBs. No congener-specific analysis was conducted, so no information on dioxin-like PCBs was available. Because non-dioxin-like PCBs are the most abundant, and PCBs are contaminated with furans, this study is not very informative for the effects of TCDD or the herbicides used in Vietnam.

Both studies with information on TEQs suggest some alterations in thyroid-hormone homeostatis in relation to TCDD and dioxin-like compounds, but the results are only partially consistent. Both studies observe changes in total T4, but in Koopman-Esseboom et al. (1994) this finding is in maternal plasma, not cord or newborn infant 2-week or 3-month plasma, whereas Pluim et al. (1992) found higher T4 at both 1 and 11 weeks in the infant. The studies are consistent with regard to increases in TSH, which are not observed at birth (both studies) or at 1 week (Koopman-Esseboom et al., 1994), but are found at 2 weeks (Pluim et al., 1992), 11 weeks (Koopman-Esseboom et al., 1994) and 3 months (Pluim et al., 1992). According to Kimbrough and Krouskas (2001), TSH concentrations undergo large changes shortly after birth.

Calvert et al. (1999) examined TCDD-exposed workers at two plants who were engaged in the production of 2,4,5-T or one of its derivatives. Referents were residents in the neighborhood of each worker, matched by age, race, and sex. Examinations were conducted in 1987–1988, and blood was collected. Serum specimens were analyzed for TCDD, total T4, TSH, and thyroid hormone binding resin, and the free T4 index was calculated. The mean TCDD concentration for the four categories of exposure were 11, 40, 135, and 729 pg/g of lipid, whereas the comparison group had a mean TCDD concentration of 7 pg/g of lipid. The results showed that workers had a significantly higher adjusted mean

free T4 index than referents ($p = 0.02$), and the highest index was among those with the highest half-life extrapolated TCDD ($p = 0.004$), but a clear dose-response relationship was not observed ($p = 0.02$). The mean total T4 was also suggestively higher in workers than in referents ($p = 0.07$). No association was observed with TSH.

Vietnam-Veteran studies

Pavuk et al. (in press) examined thyroid-hormone status in the AFHS cohort. At each of the 1982, 1985, 1987, 1992, and 1997 examinations, there was a trend toward an increasing concentration of TSH, which was not accompanied by changes in circulating T4 or in the percentage uptake of T3 (measured only in the earlier years). In a repeated-measures linear regression adjusted for age, race, and military occupation, the low-exposure and high-exposure Ranch Hands had TSH significantly higher than the comparison population, and the trend test showed a significant linear increase over the comparison and background-, low-, and high-exposure groups ($p = 0.002$). No changes in microsomal or antithyroid antibodies were observed, nor was there any evidence of changes in clinical thyroid disease. The percentage with abnormally high TSH was higher at each examination (ORs 1.4–1.9) in the high-exposure Ranch Hand group than in the comparison population, but these findings were not very precise.

Synthesis

Based on numerous animal experiments and several epidemiologic studies, TCDD and dioxin-like compounds exhibit an influence on thyroid homeostasis. These effects are hypothesized to provide a mechanism by which TCDD may affect early development of neurologic and sensory organs and motor function. Increases in TSH in three human studies without evidence of increases in T4 indicate that the infants (selected for uncomplicated gestation, labor, and delivery) and the Ranch Hand Air Force personnel studied were able to adapt to the changes that may have been induced by the higher body burdens of TCDD and TEQ. The possibility of neurodevelopmental effects secondary to mild hypothyroidism induced in early pregnancy by TCDD or dioxin-like compounds cannot be excluded (Vulsma, 2000).

Conclusions

Strength of Evidence from Epidemiologic Studies

There is inadequate or insufficient evidence of an association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and adverse effects on thyroid homeostasis. In humans,

some effects on thyroid homeostasis have been observed, mainly after exposure in the perinatal period, but the functional importance of those changes is unclear because adaptive capacity may be adequate to accommodate them.

Biologic Plausibility

TCDD is known to affect concentrations of T4, T3, and TSH. However, the effects have lacked consistency in demonstrating either a definite hyperthyroidism or hypothyroidism after exposure to TCDD. Nevertheless, long-term exposure of animals to TCDD usually results in suppressed T4 and T3 and stimulated TSH. Chapter 3 discusses recent toxicologic studies relevant to the biologic plausibility of the effects of TCDD and the herbicides on the thyroid gland.

Risk in Vietnam Veterans

The relevant studies conducted on thyroid alterations that focus primarily on perinatal effects mediated through the mother would be related primarily to the offspring of female Vietnam veterans. Those studies demonstrated biologic changes in TSH without accompanying effect on the health of the children. Similarly, the AFHS demonstrated biologic changes in TSH without accompanying effect on the health of the Ranch Hands.

SUMMARY

On the basis of the occupational, environmental, and veterans studies reviewed, the committee reached one of four conclusions about the strength of the evidence regarding association between exposure to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and each of the health effects discussed in this chapter. As explained in Chapter 2, the conclusions reflect the committee's judgment that if an association between exposure and an outcome exists, it would be found in a large, well-designed epidemiologic study in which exposure to herbicides or TCDD was sufficiently high, well characterized, and appropriately measured on an individual basis. To be consistent with the charge to the committee by the secretary of veterans affairs in Public Law 102-4 and with accepted standards of scientific reviews, the distinctions between the 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*, *Update 1996*, *Update 1998*, and *Update 2000*.

Health Outcomes with Sufficient Evidence of an Association

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 of bias and confounding and that show an association that is consistent in magnitude and direction as sufficient to conclude that there is an association.

In *VAO*, *Update 1996*, *Update 1998*, and *Update 2000*, the committee found sufficient evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and chloracne. The scientific literature continues to support the classification of chloracne in the category of sufficient evidence. On the basis of the literature, no additional health effects discussed in this chapter satisfy the criteria necessary for this category.

Health Outcomes with Limited or Suggestive Evidence of Association

For this category, the evidence must suggest 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 might be inconsistent.

In *Update 1996*, *Update 1998*, and *Update 2000*, the committee found limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and porphyria cutanea tarda. The scientific literature continues to support the classification of this disorder in the category of limited or suggestive evidence.

On the basis of its evaluation of available scientific evidence, the committee responsible for the *Type 2 Diabetes* report found that there was limited or suggestive evidence of an association between exposure to at least one of the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and type 2 diabetes. Evidence reviewed in the present report continues to support that finding.

No other changes have been made in the list of health outcomes in the category of limited or suggestive evidence.

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

The scientific data on many of the health effects reviewed by the committee were inadequate or insufficient to determine whether an association exists between exposure to the chemicals of interest and the health outcome. For the health effects 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 category includes nonmalignant respiratory disorders, such as asthma in isolation, pleurisy, pneumonia, and tuberculosis; immune system disorders (immune suppression and autoimmunity); lipid and lipoprotein disorders; gastrointestinal diseases; digestive diseases; liver toxicity; circulatory disorders; AL amyloidosis; endometriosis; and thyroid homeostasis disorders.

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

To classify outcomes in this category, several adequate studies covering the full range of exposure that human beings are known to encounter must be consistent in not showing a positive association between exposure to herbicides and the outcome at any magnitude of exposure. The studies must also have relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, magnitudes of exposure, and periods of observation covered by the available studies. In addition, the possibility of a very small increase in risk at the exposure studied can never be excluded.

The committees responsible for *VAO*, *Update 1996*, *Update 1998*, and *Update 2000* concluded that none of the health outcomes discussed in this chapter had limited or suggestive evidence of *no* association with the exposures of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid). The most recent scientific evidence continues to support that conclusion.

Biologic Plausibility

This section summarizes the biologic plausibility of a connection between exposure to TCDD or herbicides and various noncancer health effects on the basis of data from animal and cellular studies. The preceding discussions of individual health outcomes include a discussion of biologic plausibility for the specific effects. Details of the committee's evaluation of data from recent toxicologic studies are presented in Chapter 3.

TCDD has been shown to elicit a diverse spectrum of effects in animal and experimental studies, including immunotoxicity, hepatotoxicity, chloracne, loss of body weight, induction of phase I and phase II drug-metabolizing enzymes, modulation of hormone systems, and modulation of factors associated with the regulation of cellular differentiation and proliferation. Those effects depend on sex, strain, age, and species.

Effects of TCDD on the liver include modulation of the rate at which hepatocytes multiply, increasing the rate of death of other types of liver cells, increasing the fat content of liver cells, decreasing bile flow, and increasing proteins and substances that are precursors to heme synthesis. TCDD also increases the amount of some enzymes in the liver, but this effect is not necessarily considered toxic. Liver toxicity is species-specific; 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. TCDD has been shown to inhibit hepatocyte DNA synthesis, decrease hepatic plasma membrane epidermal growth factor receptor, inhibit hepatic pyruvate carboxylase activity, induce porphyrin accumulation in fish and chick embryo hepatocyte cultures, and alter liver enzyme concentrations and activity. Hepatomegaly has occurred after high subchronic doses. The mechanism of TCDD hepatoxicity is not established, but most studies are consistent with the hypothesis that the effects of TCDD are mediated by the AhR, a protein in animal and human cells to which TCDD can bind. The TCDD–AhR complex is thought to bind DNA and to lead to changes in transcription (genes are differentially regulated) that alter cell function. Although structural differences in the AhR have been identified in various species, this receptor operates in a similar manner in animals and humans. Animal data support a biologic basis of TCDD's toxic effects. Because of the many species and strain differences in TCDD responses, however, the extent to which animal data inform the evaluation of human health outcomes is controversial.

The myocardium has also been shown to be a target of TCDD toxicity. TCDD inhibits myocardium contraction possibly through effects on adenosine 3',5'-cyclic-monophosphate.

The immune system is one of the most sensitive to TCDD toxicity. Studies in mice, rats, guinea pigs, and monkeys indicate that TCDD suppresses the function of some components of the immune system in a dose-related manner; that is, as the dose of TCDD increases, its ability to suppress immune function increases. TCDD suppresses cell-mediated immunity, primarily by affecting the T-cell arm of the immune response, including a decrease in the numbers and responses of some types of T cells. It is not known whether TCDD directly affects T cells. TCDD may indirectly affect T cells and cell-mediated immunity by altering thymus function or cytokine production. The generation of antibodies by B cells, an indication of humoral immunity, may also be affected by TCDD. Effects of arachidonic acid have been hypothesized to mediate TCDD's immunotoxicity, but recent evidence indicates that not all of TCDD's immunotoxic effects are mediated by arachidonic acid. As with other effects of TCDD, the immunotoxic effects are species-specific and strain-specific. Increased susceptibility to infectious disease has been reported after TCDD administration. In addition, TCDD increased the number of tumors that formed in mice after injection of tumor cells. It should be emphasized, however, that very little change in the overall immune competence of the intact animals (animals not challenged experimentally with a pathogen or tumor cells) has been reported. Despite considerable laboratory research, the mechanisms underlying the immunotoxic effects of TCDD are still unclear, but most studies are consistent with the hypothesis that the effects are mediated by the AhR. TCDD's wide range of effects on growth regulation, hormone systems, and other factors could also mediate its immunotoxicity. As with

other TCDD-mediated effects, the similarity in function of the AhR among animals and humans suggests a possible common mechanism of immunotoxicity. Nevertheless, the available data have not confirmed in humans the universal immunosuppressive effects observed in laboratory animals.

TCDD has been shown to induce differentiation in human keratinocytes. TCDD has been reported to decrease an acidic type I keratin involved in epidermal development and to lead to keratinocyte hyperproliferation and skin irritations, such as chloracne. The data provide a biologically plausible mechanism for the induction of chloracne by TCDD.

Although there is not extensive data on the health effects of the herbicides discussed in this report, effects have been seen in a number of organs in laboratory animals. The liver is a target organ for 2,4-D, 2,4,5-T, and picloram, with effects 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 heme and red 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 the interaction and its biologic consequences are unknown. 2,4,5-T has been shown to be a weak myelotoxin.

Few studies have been conducted on the potential immunotoxicity of the herbicides used in Vietnam. 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 (that is, to produce an allergic response on the skin) was tested, but other aspects of its immunotoxicity were not examined.

The foregoing suggests that a connection between TCDD or herbicide exposure and human toxic effects is, in general, biologically plausible. However, definitive conclusions about the presence or absence of a mechanism for the induction of specific toxicity by these compounds in humans are complicated by the differences in sensitivity and susceptibility among individual animals, strains, and species; the lack of strong evidence of organ-specific effects among species; and differences in route, dose, duration, and timing of exposure. Investigating the biologic mechanisms underlying TCDD's toxic effects continues to be the subject of active research, and future updates of this report might have more and better information on which to base conclusions, at least for that compound.

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 disease in veterans exposed to herbicides and TCDD during their service in Vietnam. Little is known about health risks for Vietnam veterans;

where specific information is available, it is discussed under the specific health outcome.

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10

Research Recommendations

As part of their charge, the committees responsible for producing the Agent Orange reports make recommendations concerning the need, if any, for additional scientific studies to resolve uncertainties concerning the health effects of the compounds sprayed in Vietnam—2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), picloram, and cacodylic acid. This chapter summarizes the present committee's research recommendations.

Although great strides have been made over the last several years in understanding the health effects of exposure to the chemicals sprayed in Vietnam and in elucidating the mechanisms underlying those effects, there are still important gaps in our knowledge. The scope of potential research on these chemical compounds is wide, but information from some kinds of research would be more informative for the committee's charge than information from others. Because of the importance of epidemiologic and other human studies to the committee's conclusions, the focus of these recommendations is on such studies. The lack of discussion of a particular kind of research should not be interpreted as a lack of value in it.

VIETNAM-VETERAN STUDIES

As did the previous committees in their reports (IOM, 1994, 1996, 1999, 2000, 2001, 2002), on the basis of its review of the epidemiologic evidence and consideration of the quality of available exposure information, especially from studies of Vietnam veterans, this committee concludes that continuation of epide-

miologic studies of veterans could yield valuable information. That is true especially because diseases of aging could emerge as the population grows older, and as a new exposure-reconstruction model is developed and validated.

Air Force Health Study

The Air Force Health Study (AFHS) is an epidemiologic study whose purpose is to determine whether exposure to the herbicides used in Vietnam might underlie any adverse health conditions observed in a cohort of Air Force personnel (termed the Ranch Hands) who conducted aerial spray missions (Operation Ranch Hand). A baseline morbidity study of them and a matched comparison cohort was conducted in 1982, and there were follow-up assessments in 1985, 1987, 1992, and 1997. In accordance with the study protocol, one additional assessment is under way and will be completed in April 2003. A final report will be issued in early 2005 (personal communication, Joel Michalek, Brooks Air Force Base, September 17, 2002).

The AFHS is one of the few primary sources of information on the health of Vietnam veterans known to be exposed to Agent Orange and other herbicides. The study is coming to its scheduled end as the cohorts are reaching the age at which several health outcomes of interest may be expected to manifest, such as cancers and diseases related to aging. The committee recommends continuing the study past its planned completion date to enable further study of those diseases. Given the increased incidence of such diseases as amyotrophic lateral sclerosis, Parkinson's disease, prostatic cancer, and brain cancer in aging populations and the increasing age of the Vietnam-veteran cohort, research should specifically examine those diseases in the Vietnam veterans. Such studies should be conducted with an appropriate control population. Similarly, continued study of other exposed cohorts (for example, the cohort studied by the National Institute for Occupational Safety and Health) could also provide information on diseases of aging.

The committee also recommends retaining and maintaining medical records and samples on the AFHS cohort so that—with proper respect for the privacy of the study participants—they can be available for future research. The federal government should examine how the various forms of data and specimens collected in the course of the AFHS might be maintained and what form of oversight should be established for their future use. Any extension of the research or future use of the records would, of course, have to have the full knowledge and consent of the AFHS population and respect for the privacy of the participants. The committee's judgment is that continued research on the health of the Ranch Hands and comparison veterans is likely to yield important information on the determinants of health and disease in those who served in Vietnam and perhaps in their offspring.

Army Chemical Corps Studies

Members of the Army Chemical Corps constitute the largest cohort of Vietnam veterans exposed directly to herbicides and TCDD. They were involved in the handling and distribution of the compounds in Vietnam. Preliminary studies of this cohort by scientists in the Department of Veterans Affairs (VA) have demonstrated increased TCDD concentrations in Chemical Corps veterans who reported spraying herbicides as part of their duties. Research on the health effects in this population has been and is being conducted. Continued careful and expanded long-term study of the cohort could be a valuable addition to current research on Vietnam veterans. As with all Vietnam-veteran research, the federal government should consider the form of oversight that best facilitates the research effort and ensures the scientific validity of such studies.

Exposure-Reconstruction Study

The committee is aware that an assessment of herbicide exposure of Vietnam veterans, being overseen by the Institute of Medicine Committee on the Assessment of Wartime Exposure to Herbicides in Vietnam, is nearing completion (see Chapter 5). The assessment should provide more accurate and precise data on the potential exposure of people to herbicides sprayed in Vietnam. The data could be used in epidemiologic studies, such as studies of ground troops, to examine possible associations between health effects and exposure to the herbicide mixtures used in Vietnam. Combining research into the health effects of the herbicides in Vietnam veterans and potential information from this database might provide better information on the health effects of the chemicals of interest.

Other Studies of Vietnam Veterans

Several other concerns have been raised by veterans that the committee considers worthy of further investigation. A case series of glioblastomas and possibly astrocytomas was brought to the attention of the committee at its public hearing in Seattle. Despite the fact that these tumors are currently classified in the *no* association category, the committee believes that these concerns should be further investigated. These are extremely rare tumors, and the likelihood of detected changes in their rates in occupational cohorts, the AFHS, or the Seveso population is low. Other methods, such as making use of or improving VA databases, might be appropriate first steps toward investigating these concerns. Although more-thorough studies of Vietnam veterans are needed if the actual health experience of the veterans is to be adequately understood, recording or monitoring of trends in diseases of aging Vietnam veterans and rare diseases could be especially useful for indicating which diseases might warrant futher study.

STUDIES OF THE VIETNAMESE

Another population that has been understudied is the Vietnamese, including those who served in the military during the war and civilians. Anecdotal evidence and studies published in non-English-language journals suggest an array of long-term health effects that could potentially be related to the chemicals used by US troops in Vietnam.

This population provides several opportunities that others do not. First, there is a high probability that the number of exposed persons is substantially larger than the number previously studied. Second, exposures not only were high at the time of application of herbicides, but, because of persistence in the environment, continued long beyond the conclusion of military activities; studies suggest that body burdens and environmental concentrations might still be high in some areas of Vietnam (Schecter et al., 2002; Verger et al., 1994). Third, the establishment of diplomatic relations between the United States and Vietnam and a recent initiative overseen by the National Institute of Environmental Health Sciences (NIEHS) have opened the door for significant scientific collaborations. In March 2002, a US–Vietnamese Workshop on Health and Environmental Effects of the war was held in Hanoi. After the workshop, during a one-day meeting organized by the NIEHS, US and Vietnamese scientists held intensive discussions regarding types of studies that were deemed useful. Although the development of collaborative research between scientists from the two countries presents challenges, the committee believes that the hurdles might be overcome. Careful planning and the strategic building of local capacity in Vietnam through investment in training and infrastructure can lay the foundation for high-quality research. It must be stressed, however, that efforts to conduct research will have to be accompanied by efforts to build trust. Because such research has the potential to close a number of gaps in our understanding of the long-term health consequences of exposure to TCDD and herbicides used in Vietnam, the committee supports any further steps that can be taken to develop collaborative programs of research.

The possibility of using the newly established exposure database to assess exposure of the Vietnamese is also worth consideration, although it should be recognized that the explicit purpose of the database was to determine exposures of US service personnel who spent time in Vietnam.

OTHER RESEARCH

As stated previously, the committee has focused its recommendations on studies of human populations. The committee believes, however, that experimental research in the mechanisms that might underlie the human health outcomes can provide information valuable for determining the risk of disease in Vietnam veterans and the interactions between various exposures that lead to disease. For example, experimental studies in animals could examine the interaction of smok-

ing and TCDD exposure with health outcomes of interest to provide better information on potential confounders in epidemiologic studies. The committee recognizes that although it might be difficult to make conclusive links to effects in humans on the basis of such research, those studies could provide information useful for interpreting the results of epidemiologic studies, especially studies in which there might be multiple exposures or other factors that complicate the drawing of conclusions.

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APPENDIX A

Agendas of Public Meetings Held by the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Fourth Biennial Update)

FIRST PUBLIC MEETING

Wednesday, April 3, 2002
Board Room, NAS Building
Washington, D.C.

Presentations

- **Welcome, opening remarks, and introductions**
  Irva Hertz-Picciott, PhD
  Committee Chair

- **Charge to the committee**
  Mark Brown, PhD
  Director, Environmental Agents Service
  Department of Veterans Affairs
  Washington, D.C.

- **Recent data from Ranch Hand Studies**
  Joel Michalek, PhD
  Principal Investigator, Air Force Research Laboratory
  Brooks Air Force Base, Texas

- **Husband's death and Agent Orange**
  Jennie LeFevre
  Shady Side, Maryland

- **Veterans' health problems, heart disease, diabetes, peripheral neuropathy, soft knots covering body, chloracne**
  Shelia Winsett (testimony presented by Jennie LeFevre)
  Jasper, Alabama

- **Testimony on the behalf of Vietnam Veterans of America**
  Rick Weidman
  Director of Government Relations
  Vietnam Veterans of American
  Silver Spring, Maryland

SECOND PUBLIC MEETING

Monday, September 9, 2002
Board Room, Hilton Hotel
Seattle, WA

Presentations

- **Glioblastomas in Vietnam Veterans**
  Danna Hughes (testimony presented by Darren Driggs)
  President, Vietnam Veterans Wives
  Republic, WA

- **Glioblastomas and Diabetes and Agent Orange**
  Michael Sallis
  President and Volunteer Service Officer
  Grant County Veterans Association
  Moses Lake, WA

- Written testimony was submitted by *Julie Cummings*, Silverthorne, Colorado

- Public input and discussion among session participants

APPENDIX B

ICD-9 Codes for Health Outcomes of Interest

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 health care data. Many of the studies reviewed by the committee use ICD-9 classifications. Table B-1 lists the codes for the various forms of cancer.

TABLE B-1 Surveillance, Epidemiology, and End Results (SEER) Program Site Groupings for ICD-9 National Center for Health Statistics (NCHS) Data

Site	ICD-9 codes
Cancer	
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

continues

TABLE B-1 *Continued*

Site	ICD-9 codes
Other buccal cavity and pharynx	149.0–149.9
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 nonepithelial skin	173.0–173.9
Breast	174.0–174.9, 175.0
Female genital system	
Cervix	180.0–180.9
Corpus	182.0–182.1, 182.8
Uterus, NOS	179.0
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	185.0
Testis	186.0–186.9
Penis	187.1–187.4
Other male genital organs	187.5–187.9
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

TABLE B-1 *Continued*

Site	ICD-9 codes
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	193.0
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: 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. Table A-4.

APPENDIX C

Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Fourth Biennial Update)

Irva Hertz-Picciotto, PhD *(Chair)*, is professor in the Department of Epidemiology, School of Public Health, at the University of North Carolina, Chapel Hill. She is also associate professor in the Department of Epidemiology and Preventive Medicine at the University of California, Davis. She has published extensively on risk assessment, occupational-related cancer, environmental exposure, reproductive outcomes, and methods for epidemiologic data analysis. Dr. Hertz-Picciotto serves on several editorial boards and is president of the International Society for Environmental Epidemiology. She was recently a delegate to the US–Vietnam Scientific Conference on the Environmental and Health Effects of the Vietnam War sponsored by National Institute of Environmental Health Sciences. Dr. Hertz-Picciotto also served as chair of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

Kiros T. Berhane, PhD, is an assistant professor in the Department of Preventive Medicine at the University of Southern California, Los Angeles. He has conducted research on longitudinal time-series analysis, flexible-modeling techniques, modeling time-to-event data, and latency. Dr. Berhane received his PhD in biostatistics from the University of Toronto (Canada) and completed a postdoctoral fellowship in biostatistics at Johns Hopkins University (1994–1995). He is also currently serving as a member on a scientific advisory panel for an air pollution study under the auspices of the Western Interprovincial Scientific Studies Association (WISSA) in Canada.

Margit L. Bleecker, MD, PhD, is director of the Center for Occupational and Environmental Neurology in Baltimore. Her research interests are in clinical

industrial neurotoxicology and occupational neurology. Dr. Bleecker recently served on the Institute of Medicine (IOM) Committee on the Safety of Silicone Breast Implants and has served on the IOM Committee on the Evaluation of the Department of Defense Comprehensive Clinical Evaluation Protocol and the IOM Committee on the Persian Gulf Syndrome Comprehensive Clinical Evaluation Program. She also served as a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

Paul F. Engstrom, MD, is senior vice president for population science, overseeing Fox Chase programs in behavioral research, human genetics, epidemiologic research, and cancer-prevention research. He is also medical director of the Fox Chase Network and the International Programs for Fox Chase Cancer Center. Dr. Engstrom is a medical oncologist specializing in gastrointestinal cancers. He is a member of the National Cancer Institute (NCI) Cancer Epidemiology, Prevention and Control Review Committee and the American Cancer Society Council for Extramural Grants. He is former chair of the board of scientific counselors for NCI's Division of Cancer Prevention and Control and former chair of NCI's committee on treatment strategies to cut cancer death rates in half by the year 2000. Dr. Engstrom is a member of the editorial board of *Cancer Epidemiology Biomarkers & Prevention*, *Journal of Cancer Prevention* and *Journal of Clinical Oncology*, and he is the author or coauthor of several texts and book chapters on cancer control and medical oncology.

Richard A. Fenske, PhD, is professor of environmental health in the Industrial Hygiene and Safety Program at the University of Washington School of Public Health and Community Medicine and is the director of the Pacific Northwest Agricultural Safety and Health Center. Dr. Fenske's work has focused on the evaluation of environmental health risks in special populations. His specialties include health risks posed by pesticide exposures, development of new exposure-assessment methods, and investigation of the role of skin exposure of workers and children. Dr. Fenske serves on the editorial review boards of *Applied Occupational and Environmental Hygiene* and the *Journal of Agricultural Safety and Health*.

Thomas A. Gasiewicz, PhD, is professor and chairman of the Department of Environmental Medicine and deputy director of the Environmental Health Sciences Center at the University of Rochester School of Medicine. He serves on the editorial board of *Biochemical Pharmacology* and the National Toxicology Program Board of Counselors and is the associate editor of *Toxicology and Applied Pharmacology*. He also is a peer reviewer for several other scientific journals, including *Cancer Research*, *Molecular Pharmacology*, *Carcinogenesis*, *Science*, *Toxicological Sciences*, and *Archives of Biochemistry and Biophysics*. Dr. Gasie-

wicz has published extensively on the toxicokinetics of dioxin, dioxin toxicity, and the role of the aryl hydrocarbon receptor in the molecular mechanism of dioxin toxicity. He also served as a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

Tee L. Guidotti, MD, MPH, is the chair of the Department of Environmental and Occupational Health in the School of Public Health and Health Services of the George Washington University. He is also director of the Division of Occupational Medicine and Toxicology in the Department of Medicine of George Washington University School of Medicine and Health Science and is cross-appointed as professor of pulmonary medicine, health policy, and epidemiology. Dr. Guidotti is certified as a specialist in internal medicine, lung diseases, and occupational medicine. His primary research interests are air quality, inhalation toxicology, and occupational and environmental lung diseases. Dr. Guidotti is past president of the Association of Occupational and Environmental Clinics and sits on the Board of Directors of the American College of Occupational and Environmental Medicine and of the International Commission on Occupational Health. He also served as a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

Loren D. Koller, DVM, PhD, served in academe for nearly 30 years, the last 16 as professor in the College of Veterinary Medicine of Oregon State University, Corvallis. For 10 of those years, he served as dean of the college. He operates a business in environmental health and toxicology. Dr. Koller pioneered the discipline now known as immunotoxicology with a research focus also in toxicology, pathology, carcinogenesis, and risk assessment. He is on the Institute of Medicine Committee on the Assessment of Wartime Exposure to Herbicides and served for 6 years as a member of the National Research Council Committee on Toxicology. He also served as a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

John J. Stegeman, PhD, is senior scientist and chair of the Biology Department at the Woods Hole Oceanographic Institution in Woods Hole, Massachusetts. He received his PhD in biochemistry, concentrating on enzymology, from Northwestern University. His research centers on metabolism of foreign chemicals in animals and humans and on the structure, function, and regulation of the enzymes that accomplish this metabolism. He also served as a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

David S. Strogatz, PhD, MSPH, is associate professor and chair of the Department of Epidemiology of the University at Albany, State University of New

York. He is also director of the Prevention Research Center of the School of Public Health of the University at Albany. Dr. Strogatz received his MSPH and PhD in epidemiology from the University of North Carolina, Chapel Hill. His research examines the epidemiology of cardiovascular disease and diabetes and the impact of socioeconomic status and race on health. He also served as a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Third Biennial Update).

Staff Biographies

Rose Marie Martinez, ScD, is director of the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. Before joining IOM, she was a senior health researcher at Mathematica Policy Research, where she conducted research on the impact of health-system change on the public-health infrastructure, access to care for vulnerable populations, managed care, and the health-care workforce. Dr. Martinez is a former assistant director for health financing and policy with the US General Accounting Office, where she directed evaluations and policy analysis in national and public-health issues. Dr. Martinez received her doctorate from the Johns Hopkins School of Hygiene and Public Health.

Michelle Catlin, PhD, is a program officer in the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. Before joining IOM, she served as a program officer with the Board on Environmental Studies and Toxicology of the National Research Council. She received her MSc in pharmacology and toxicology from Queen's University, Canada, and a PhD in environmental health (Toxicology Program) from the University of Washington. Dr. Catlin has worked on numerous National Academies reports, including *Copper in Drinking Water*, *Toxicological Effects of Methylmercury*, *Arsenic in Drinking Water: 2001 Update*, and *Veterans and Agent Orange: Update 2000*.

Jennifer A. Cohen is a research associate in the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. She received her undergraduate degree in art history from the University of Maryland. She has also been involved with the IOM committees that produced *Organ Procurement and Transplantation; Clearing the Air: Asthma and Indoor Air Exposures*; *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes; Veterans and Agent Orange: Update 2000*; and *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Acute Myelogenous Leukemia in the Children of Vietnam Veterans*.

Anna B. Staton, MPA, is a research assistant in the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. Ms. Staton joined IOM in

December 1999 and has worked with the committees that produced *No Time to Lose: Getting More from HIV Prevention* and *Veterans and Agent Orange: Update 2000*. Before joining IOM, she worked at the Baltimore Women's Health Study. Ms. Staton graduated from the University of Maryland Baltimore County with a BA in visual arts (major) and women's studies (minor). She earned her MPA in nonprofit management at the George Washington University School of Business and Public Management.

Elizabeth J. Albrigo is a project assistant in the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. She received her undergraduate degree in psychology from the Virginia Polytechnic Institute and State University. She is involved with the IOM Committee on the Review of the USDA *E. coli* O157: H7 Farm-to-Table Process Risk Assessment and Committee on the Assessment of Wartime Exposure to Herbicides in Vietnam.

Joe A. Esparza is a project assistant in the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. Joe attended Columbia University in the City of New York where he studied biochemistry. Before joining IOM, he worked with the Board on Agriculture and Natural Resources (BANR) of the National Research Council. While with BANR Joe was involved with the committees that produced *Frontiers in Agricultural Research: Food, Health, Environment, and Communities*; *Air Emissions from Animal Feeding Operations: Current Knowledge, Future Needs*; and *Publicly Funded Agricultural Research and the Changing Structure of US Agriculture*. *Veterans and Agent Orange: Update 2002*, is Joe's first report with IOM.

Index

Note to the reader: This index contains entries for each of the five 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), *Veterans and Agent Orange: Update 1998* (III), *Veterans and Agent Orange: Update 2000* (IV), *Veterans and Agent Orange: Update 2002* (V). 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; IV: 118" 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* and on page 118 of *Veterans and Agent Orange: Update 2000*.

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