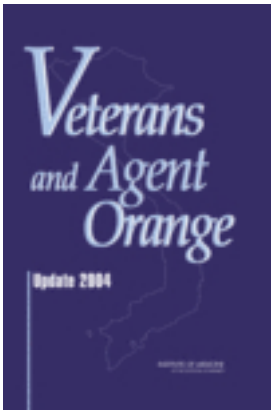

Veterans and Agent Orange: Update 2004

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

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Veterans and Agent Orange

Update 2004

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Vietnam Veterans of Exposure to Herbicides
(Fifth Biennial Update)

Board on Health Promotion and Disease Prevention

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Willing is not enough; we must do."
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COMMITTEE TO REVIEW THE HEALTH EFFECTS IN VIETNAM VETERANS OF EXPOSURE TO HERBICIDES (FIFTH BIENNIAL UPDATE)

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This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of 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 for their review of this report:

Hans Berkel, President and CEO, Cancer Prevention Institute, Dayton, Ohio
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Bailus Walker, Jr., Professor, Howard University Cancer Center, Washington DC

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 **Dan Blazer**, Duke University Medical Center, Durham, North Carolina. Appointed by the 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 author committee and the institution.

Preface

In 1991, because of continuing uncertainty about the long-term health effects on Vietnam veterans who where exposed to herbicides during their service in Vietnam (mixtures of 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), Congress passed Public Law 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 resulting report, *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (*VAO*) was published by the NAS Institute of Medicine (IOM) in 1994. The Secretary also asked 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. PL 107-103, The Veterans Education and Benefits Expansion Act of 2001, extended the updates until 2014.

The first report in the resulting series was *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (abbreviated as *VAO* in this report). It evaluated and integrated the scientific evidence regarding statistical associations between health outcomes and exposure to TCDD or other compounds in these herbicides that had accumulated prior to 1994. Public Law 102-4 also required the NAS to conduct biennial updates that would review newly published scientific literature regarding such associations. 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 pub-

lished in 1999. The third, *Veterans and Agent Orange: Update 2000* (*Update 2000*) was published in 2001. The fourth, *Veterans and Agent Orange: Update 2002* (*Update 2002*) was published in 2003.

The focus of this fifth updated review is on scientific studies published since the release of *Update 2002*. To conduct the review, the IOM established a committee of 11 members representing a wide range of expertise to take a fresh look at the studies reviewed in *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*, along with the newest scientific evidence. 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 2002*. 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 D.

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 NAS procedures, the committee met in a series of closed sessions in which members could freely examine, characterize, and weigh the strengths and limitations of the evidence. It also convened open meetings in May and July 2004 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 listed in Appendix B. The committee thanks the individuals who provided valuable insights into the health problems experienced by Vietnam veterans.

The committee is grateful to Michelle Catlin and Mary Paxton, who skillfully served as study directors for this project. The committee would also like to acknowledge the excellent work of IOM staff members Jennifer Cohen, Joe Esparza, Peter James, Sonia Cheruvillil, and David Butler. Thanks are also extended to Jim Banihashemi and Christie Bell, who handled the finances for the project; Kate Kelly, who provided editorial skills; and William McLeod, who conducted database searches.

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.

John Stegeman, *Chair*

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APPENDIXES

Veterans and Agent Orange

Update 2004

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 main chemical mixture 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 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. PL 107-103, the Veterans Education and Benefits Expansion Act of 2001, extended the updates until 2014.

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*, *Update 2000*, and *Update 2002*) and in focused reports on the scientific evidence regarding type 2 diabetes (*Type 2 Diabetes*), acute myelogenous leukemia in children (*Acute Myelogenous Leukemia*), and the latency period for respiratory cancer (*Respiratory Cancer*). This report is the fifth review of recently published scientific evidence regarding associations between health outcomes and exposure to TCDD and other chemical compounds in herbicides used in Vietnam.

CHARGE TO THE COMMITTEE

In accordance with PL 102-4, the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Fifth Biennial Update) was asked to "determine (to the extent that available scientific 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 was not charged to focus on broader issues, such as the potential costs of compensation for veterans or policies regarding such compensation.

COMMITTEE'S APPROACH TO ITS CHARGE

To fulfill its charge of assessing whether specific human health effects are associated with exposure to at least one of the herbicides or TCDD, the committee concentrated its review on epidemiologic studies. The committee also consid-

ered controlled laboratory investigations that provide information on whether it is biologically plausible to suppose that the compounds of interest might be related to a given effect. 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 reviewing studies of Vietnam veterans, reviewed studies of other groups potentially exposed to the constituents of the herbicides used in Vietnam (2,4-D, 2,4,5-T, TCDD, cacodylic acid, and picloram). 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 unintended release or 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 databases covering biologic, medical, toxicologic, chemical, historical, and regulatory information. Literature identification continued through June 1, 2004. More than 3,000 potentially relevant studies were identified in those searches, and more than 550 were reviewed. Additional information came from veterans and other interested persons who testified at public hearings and offered written submissions.

To determine whether there is an association between exposure and health outcome, epidemiologists estimate the magnitude of an appropriate measure (such as the relative risk or the odds ratio) that describes the relationship between exposure and disease in a defined population or group. In evaluating the strength of the evidence linking herbicide exposure with a particular outcome, the committee considered whether such estimates of risk might be incorrect (the result of confounding, chance, or bias from errors in selection and measurement) or might accurately represent true associations. It has been the practice of all VAO committees to evaluate all studies according to the same criteria whether or not their subjects are Vietnam veterans and then to weight findings of similar strength and validity equivalently when drawing conclusions. The committee recognizes that an absolute conclusion about the absence of association might never be attained, because, as is generally the case in science, studies of health outcomes after herbicide exposure cannot demonstrate that a purported effect is impossible, only that it is statistically improbable.

EVIDENCE REVIEWED BY THE COMMITTEE

Toxicology

Since *Update 2002*, several experimental studies have been published on the chemical compounds of interest. Some examine particular disease outcomes in animals after exposure to the compounds; others focus more on the mechanism or mode of action by which effects are elicited in cells, tissues, or whole animals. Despite extensive study, the exact mechanisms by which the compounds exert their effects are still unclear. Toxicologic information on disease outcomes in animals can, however, support an association seen in an epidemiologic study by providing evidence that an effect is biologically plausible.

Many health effects have been seen in animals after exposure to TCDD or to the herbicides used in Vietnam. Although animal experiments demonstrate that some of the compounds (alone or in conjunction with other treatments) can cause specific cancers, none of the compounds of interest has been shown to act directly by mutating DNA. TCDD is thought to be the most toxic of the compounds, and recent experimental research has shown a great deal about the cellular effects of TCDD. All of the data are consistent with the hypothesis that those effects are mediated by the ability of TCDD to bind a cellular protein, the aryl hydrocarbon receptor, to modulate cell-signaling pathways. However, the exact mechanisms by which those events cause the various effects seen in humans and animals remains unknown.

Relevant effects observed in experimental animals, and their relevance to human health outcomes, are discussed as part of the biologic plausibility section for each outcome.

Exposure Assessment

Assessment of exposure to a toxic substance is an important element in determining whether specific health outcomes are linked to that substance. Under ideal circumstances exposure assessment would quantify the concentration of a substance at its site of action; in human studies that is rarely possible. Exposure estimates, therefore, should be viewed as surrogates for the actual dose.

Recent studies of Vietnam veterans, including those of the Ranch Hand and Army Chemical Corps cohorts, have used the measurement of serum blood levels of TCDD as the best available estimator of historical exposures to Agent Orange. This approach has also been used to study health effects in the Seveso population in Italy. Since *Update 2002*, a study of Korean Vietnam veterans developed an exposure index based on duration of service, troop location relative to herbicide spraying, and individual activity data from questionnaires. The investigators attempted to validate this exposure index with serum blood levels of TCDD, but samples were pooled within presumed exposure categories and only a narrow

range of TCCD concentrations was measured across these categories. All concentrations were less than one picogram TCDD per gram of serum (lipid-adjusted).

In 1997, a committee convened by IOM on behalf of the VA requested research proposals designed to reconstruct exposures of US veterans who served as ground troops in Vietnam. This request resulted in a project called "Characterizing Exposure of Veterans to Agent Orange and Other Herbicides in Vietnam". Since *Update 2002*, a final report of this work has been published by IOM. This report presents an "exposure-opportunity index" based on records of herbicide spraying and troop movements. The IOM committee's review of the report concluded that the exposure-opportunity index holds promise for epidemiologic investigations of this population.

Epidemiology

The health outcomes reviewed by the committee are categorized as cancer, reproductive and developmental effects, neurologic disorders, and other health effects. This section briefly summarizes the relevant epidemiologic studies published on those health outcomes since *Update 2002*. In the health outcomes chapters, this new literature was evaluated and considered in the context of the previous reviews to derive comprehensive updated conclusions integrating the entire body of information.

Cancer

Since *Update 2002*, several cohort studies were published that investigated multiple cancer outcomes. Cancer incidence and mortality were compared between the Ranch Hand cohort and the Air Force control cohort of Vietnam veterans; an excess incidence of melanoma was observed in some subgroups of the Ranch Hand Air Force veterans, but analyses did not establish this outcome in the entire cohort, and an increase in the incidence of, but not mortality from, prostate cancer was observed. A pilot case-control study of prostate cancer among Vietnam-era veterans found an elevated, but non-significant increase associated with reported Agent Orange exposure. An update of a cohort of Dow Chemical Company workers and a study of pesticide appliers in Iowa and North Carolina both also found an increase in prostate cancer, while a cancer mortality study of Dutch herbicide appliers showed only an increase in "all skin cancers." An ecological study of Japanese municipalities with and without waste incinerators had negative findings for cancer mortality overall. Case-control studies of soft-tissue sarcoma in Italian and Finnish populations had opposing results concerning residential exposure to the chemicals of interest. No other increases in cancer incidence or mortality were reported in association with the chemicals of interest.

Reproductive and Developmental Effects

Two environmental studies addressed birth defects: One examined proximity to municipal solid waste incinerators in France, and the other reviewed county-wide use of agricultural pesticides in four US states. Neither presented additional information on the occurrence of spina bifida. The French study reported associations with facial clefts and renal abnormalities, and the US study presented results for circulatory and respiratory anomalies; the exposure assessment in each study was ecological. One relatively small study of time-to-conception presented results that suggested a connection with infertility, but the assessment of herbicide exposure was nonspecific. Two ecological studies addressed outcomes that are tangentially related to fertility (sperm quality and regularity of menstrual cycling). One study investigating spontaneous abortion among women exposed during the Seveso accident reported no relationship with serum TCDD concentrations. Studies of birth weight and gestational duration for the pregnancies of Polish farm women and of Seveso residents presented data suggestive of a connection, but compromised by design limitations. A single study of cancers among the offspring of pesticide appliers presented indeterminate results for this quite non-specific outcome category. One new study of pesticide production workers reported a suggestion of a reduced sex ratio (fewer-than-expected male offspring) for the children of male, but not female, workers.

Neurologic Disorders

Since *Update 2002*, cognitive outcomes have been investigated in a study of elderly residents of Bordeaux, France; in an updated cohort of Czech workers; in an ecological study of a community adjacent to a wood treatment plant; and in two studies of Vietnam veterans, one of US Ranch Hands and one of Korean veterans. Only the Bordeaux study reported an increase in cognitive disorders, but it did not implicate the compounds of interest to this report.

Three publications examined possible associations with Parkinson's disease. Although some associations were reported, the limitations of those studies (particularly the lack of information on the compounds of interest) do not permit conclusive interpretation.

One study reported a higher rate of peripheral neuropathy among Korean Vietnam veterans than was found among veterans who did not serve in Vietnam, but the results were unrelated to estimated dioxin exposures. Although the committee carefully reviewed the available literature on peripheral neuropathy, neither the new report nor the previous epidemiologic literature convincingly indicates an association between exposure and persistent neuropathy in veterans who do not have diabetes. Peripheral neuropathy is seen secondary to diabetes, for which an association with the compounds of interest has been noted. It is not possible to determine from the available literature whether observed increases in peripheral neuropathy are primary effects from dioxin exposure or secondary to diabetes.

Other Health Effects

Several studies have investigated health effects other than those classified as cancer, reproductive and developmental effects, or neurological disorders. The incidence of chloracne; increases in uroporphyrins; changes in immune parameters; and increased hypertension, valvular heart disease, and ischemic heart disease were observed to be higher in Korean Vietnam veterans who served in Vietnam than in those who did not. The study's limitations, however, do not permit conclusions to be drawn based only on its data. Changes in immune parameters also were seen in studies of residents of Seveso. Some respiratory effects were suggested by a study of people living near a wood treatment plant, but there was no evidence that those effects were specifically related to the compounds of interest. Several other studies presented evidence of additional effects, but none was of high enough quality or sufficiently relevant to the compounds of interest to affect the conclusions of previous reviews.

COMMITTEE'S CONCLUSIONS

Health Outcomes

The present committee weighed the strengths and limitations of the epidemiologic evidence reviewed in this report and in previous *Veterans and Agent Orange* reports. Its conclusions were drawn from the new evidence in the context of the entire body of literature. It assigned each health outcome to one of four categories on the basis of the evidence. Table ES-1 defines these categories and gives criteria for assigning a health outcome to each of them. Based on the committee's evaluation of occupational, environmental, and veterans studies, this table also lists the relative weight of evidence for association between particular health outcomes and exposure to the herbicides that were used in Vietnam or to any of their components or contaminants (with no intention of specifying a particular chemical). After careful consideration, the present committee did not change the categorization of health outcomes in this report from *Update 2002*. It did, however, modify the previous terminology of "acute and subacute" transient peripheral neuropathy to "early-onset" transient peripheral neuropathy as being more reflective of the intended properties of the condition's symptoms, rather than suggesting the conditions of exposure.

As mandated by PL 102-4, the distinctions among categories are based on statistical association, not on causality. The committee was directed to review the scientific data, not to recommend VA policy; therefore, conclusions reported in Table ES-1 are not intended to imply or suggest policy decisions. The conclusions are related to associations between exposure and outcomes in human populations, not to the likelihood that any individual's health problem is associated with or caused by the herbicides in question.

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)
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 (lung and bronchus, larynx, and trachea)
Prostate cancer
Multiple myeloma
Early-onset transient peripheral neuropathy[b]
Porphyria cutanea tarda
Type 2 diabetes (mellitis)
Spina bifida in offspring of exposed individuals

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
Oral, nasal, and pharyngeal cancer
Bone and joint cancer
Skin cancers (melanoma, basal cell, and squamous cell)
Breast cancer
Female reproductive cancer (cervix, uterus, ovary)
Testicular cancer
Urinary bladder cancer
Renal cancer
Leukemia (other than CLL)
Abnormal sperm characteristics and infertility
Spontaneous abortion
Neonatal or infant death and stillbirth in offspring of exposed individuals

TABLE ES-1 *Continued*

Low birthweight in offspring of exposed individuals
Birth defects (other than spina bifida) in offspring of exposed individuals
Childhood cancer (including acute myelogenous leukemia) in offspring of exposed individuals
Neurobehavioral disorders (cognitive and neuropsychiatric)
Movement disorders, including Parkinson's disease and amyotrophic lateral sclerosis (ALS)
Chronic peripheral nervous system disorders
Respiratory disorders
Gastrointestinal, metabolic, and digestive disorders (changes in liver enzymes, lipid abnormalities, ulcers)
Immune system disorders (immune suppression, autoimmunity)
Circulatory disorders
AL 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 (esophagus, stomach, pancreas, colon, rectum)
Brain tumors

[a] *Herbicides* indicates the following chemicals of interest: 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 was drawn from occupational, environmental, and veterans studies in which individuals were exposed to the herbicides used in Vietnam, to their components, or to their contaminants.

[b] For clarity, this committee has changed the nomenclature from "acute and subacute" to "early-onset" transient peripheral neuropathy.

Risk in 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 the chemicals of interest and disease are based on studies of people exposed in various occupational and environmental settings rather than on studies of Vietnam veterans. The committee believes that there is sufficient evidence to reach general conclusions about asso-

ciations between herbicide exposure and the health outcomes, but the lack of adequate exposure data on Vietnam veterans themselves makes it difficult to estimate the degree of increased risk of disease in Vietnam veterans, as a group or individually. Thus, quantification of the actual risks experienced by veterans exposed to the compounds of interest during the Vietnam War is not possible.

Because of those limitations, only general assertions can be made about risks to Vietnam veterans, depending upon which category of the association has been attributed to a given health outcome. 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 is attributable to exposure to the compounds of interest, but that conclusion is limited to the conditions, exposures, and lengths of observation covered by the studies reviewed by the committee. Even qualitative estimates are not possible when there is "inadequate or insufficient" evidence of an association. For outcomes categorized as having "sufficient" or "limited or suggestive" evidence of an association with herbicide exposure, the lack of exposure information for Vietnam veterans prevents calculation of precise risk estimates.

RESEARCH RECOMMENDATIONS

IOM has 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 and their contaminants used in Vietnam.

Great strides have been made over the past several years in understanding the health effects of exposure to TCDD and to the herbicides used in Vietnam and in elucidating the mechanisms that underlie those 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.

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. Although the explicit purpose of the newly established exposure database was to determine exposures of US service personnel who spent time in Vietnam, the possibility of using it to identify study populations among Vietnamese residents should be considered.

1

Introduction

The Agent Orange Act of 1991 (Public Law [PL] 102-4 enacted February 6, 1991, and 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 herbicides used during military operations in Vietnam. The herbicides picloram and cacodylic acid were to be addressed, as well as the most well-known of the formulations, Agent Orange (a 50:50 mixture of the herbicides 2,4-dichlorophenoxyacetic acid [2,4-D] and 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], which contained a contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]). The legislation also called for biennial reviews of newly available information for a period of 10 years, which was extended until 2014 by the Veterans Education and Benefits Expansion Act of 2001. NAS also was asked to recommend, as appropriate, additional 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 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: Health Effects of Herbicides Used in Vietnam* (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), *Update 2000* (IOM, 2001), and *Update 2002* (IOM, 2003). In 1999, VA requested that IOM convene a com-

mittee 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, *Type 2 Diabetes*; IOM, 2000). In 2001, VA requested that IOM convene a committee to conduct an interim review of acute myelogenous leukemia (AML) in children associated with parental exposure. 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 2001, Congress (PL 107-103) directed the Secretary of Veterans Affairs to request that NAS review "available scientific literature on the effects of exposure to an herbicide agent containing dioxin on the development of respiratory cancers in humans," and to address "whether it is possible to identify a period of time after exposure to herbicides after which a presumption of service-connection" for the disease would not be warranted. *Veterans and Agent Orange: Length of Presumptive Period for Association Between Exposure and Respiratory Cancer* (hereafter, *Respiratory Cancer*; IOM, 2004) is the result of that effort.

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 scientific 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 the committee's approach to its charge and the methods it used in reaching conclusions are provided in Chapter 2.

CONCLUSIONS OF PREVIOUS *VETERANS AND AGENT ORANGE* REPORTS

Health Outcomes

VAO, *Update 1996*, *Update 1998*, *Update 2000*, *Update 2002*, *Type 2 Diabetes*, *Acute Myelogenous Leukemia*, and *Respiratory Cancer* 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 exposure by assigning each of the health outcomes under study to one of four categories on the basis of the epidemiologic evidence reviewed. Those categories were adapted from the ones used by the International Agency for Research on Cancer (IARC) in evaluating evidence of the carcinogenicity of various substances (IARC, 1977). Successor committees have adopted the same categories.

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 conclusions of *Update 2002* (IOM, 2003) for associations between health outcomes and exposure to the herbicides used in Vietnam or to any of their components or contaminants. This integration of the literature prior to 2002 served as the starting point for the current committee's deliberations. It should be noted that the categories of association concern the occurrence of health outcomes in human populations in relation to chemical exposures; they do not consider 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

In this category, a positive association between herbicides and the outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. The committee regarded evidence from several small studies that have satisfactorily addressed bias and confounding and that show an association that is consistent in magnitude and direction as sufficient evidence of an association.

The original committee found sufficient evidence of an association between exposure to 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

TABLE 1-1 Summary of Conclusions from *Update 2002* on Specific Health Outcomes and Exposure to Herbicides[a]

Sufficient Evidence of an Association	
	Chronic lymphocytic leukemia (CLL)
	Soft-tissue sarcoma
	Non-Hodgkin's lymphoma
	Hodgkin's disease
	Chloracne
Limited or Suggestive Evidence of an Association	
	Respiratory cancers (lung/bronchus, larynx, and trachea)
	Prostate cancer
	Multiple myeloma
	Acute and subacute (or early-onset) transient peripheral neuropathy
	Porphyria cutanea tarda
	Type 2 diabetes (mellitis)
	Spina bifida in offspring of exposed individuals
Inadequate or Insufficient Evidence to Determine Whether an Association Exists	
	Hepatobiliary cancers
	Nasal or nasopharyngeal cancer
	Bone cancer
	Breast cancer
	Female reproductive cancers (cervix, uterus, and ovary)
	Urinary bladder cancer
	Renal cancer
	Testicular cancer
	Leukemia (other than CLL)
	Skin cancers
	Abnormal sperm characteristics and infertility
	Spontaneous abortion
	Neonatal or infant death and stillbirth in offspring of exposed individuals
	Low birthweight in offspring of exposed individuals
	Birth defects (other than spina bifida) in offspring of exposed individuals
	Childhood cancer (including acute myelogenous leukemia) in offspring of exposed individuals
	Cognitive and neuropsychiatric disorders
	Motor or coordination dysfunction
	Chronic peripheral nervous system disorders
	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* indicates the following chemicals of interest: 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 was drawn from occupational, environmental, and veterans studies in which individuals were exposed the herbicides used in Vietnam, their components, or to their contaminants.

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*.

As the committee responsible for *Update 2002* began its work, VA requested that they evaluate whether chronic lymphocytic leukemia (CLL) should be considered separately from other leukemias. The committee concluded that CLL could be considered separately and, on the basis of the given epidemiology literature and the etiology of the disease, placed CLL in the sufficient category.

Limited or Suggestive Evidence of Association

In this category, the evidence must suggest an association between exposure to herbicides and the outcome considered, but the evidence can be limited by the inability to confidently rule out chance, bias, or confounding. 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 exposure to herbicides and three categories of cancer: respiratory cancers, prostatic cancer, and multiple myeloma. The *Update 1996* committee added three health outcomes to this list: PCT, acute and subacute transient peripheral neuropathy (henceforth called "early-onset transient peripheral neuropathy"), and spina bifida in children of veterans. Transient peripheral neuropathies had not been addressed in *VAO* because they are not amenable to epidemiologic study. In response to a VA request, however, the committee responsible for *Update 1996* reviewed those neuropathies and based its determination on case histories (Chapter 10, *Update 1996*). A 1995 analysis of birth defects among the offspring of veterans of operation Ranch Hand, combined 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 reproductive outcomes and classify it in the limited or suggestive-evidence category (Chapter 9, *Update 1996*). No changes were made in this category in *Update 1998*.

After the publication of *Update 1998*, and based on its evaluation of newly available scientific evidence and the cumulative findings of research reviewed in previous *VAO* reports, the committee responsible for *Type 2 Diabetes* concluded 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 (mellitus). The evidence reviewed in *Update 2000* supported that finding.

The committee responsible for *Update 2000* reviewed the material in earlier reports and the newly 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 chil-

dren of Vietnam veterans. After release of that report, researchers on one of the studies reviewed in *Update 2000* discovered an error in the published data. After reconvening to reevaluate the previously reviewed and new literature regarding that illness, the *Acute Myelogenous Leukemia* report was produced; it reclassified AML in children from "limited or suggestive evidence of an association" to "inadequate evidence to determine whether an association exists."

Inadequate or Insufficient Evidence to Determine Association

By default, any health outcome considered falls into this category prior to accumulation of enough reliable scientific data to promote it to the categories of sufficient or limited-suggestive evidence of an association or to the category of suggestive evidence of *no* association. In this category, available studies may have inconsistent findings or are of insufficient quality or statistical power to support a conclusion regarding the presence or absence of an association. Such studies might fail to control for confounding or might have inadequate assessment of exposure.

The cancers and other health effects so categorized as of *Update 2002* are listed in Table 1-1, but several health effects have been moved into or out of this category since the original *VAO* committee reviewed the evidence then available. Skin cancer was moved into this category in *Update 1996* when inclusion of new evidence no longer supported its classification as a condition with limited or suggestive evidence of *no* association. Similarly, the *Update 1998* committee moved urinary bladder cancer from the category of suggestive evidence of *no* association to this category; although there was no evidence that exposure to herbicides or TCDD is related to urinary bladder cancer, newly available evidence weakened the evidence of *no* association. The committee for *Update 2000* had partitioned acute myelogenous leukemia in the offspring of Vietnam veterans from other childhood cancers and put it in the classification with suggestive evidence; but a separate review, as reported in *Acute Myelogenous Leukemia* (IOM, 2002), found errors in the published information and returned it to the category of inadequate or insufficient evidence with other childhood cancers. In *Update 2002,* chronic lymphocytic leukemia was moved from this category to join Hodgkin's and non-Hodgkins lymphomas in the category with sufficient evidence of an association.

Limited or Suggestive Evidence of *No* Association

In this category, several adequate studies covering the full range of human exposure are consistent in *not* showing a positive association between exposure to herbicides and the outcome, at any exposure. Those studies 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 small increase in risk at the levels of exposure studied can never be excluded. However, a change in classification from inadequate–insufficient evidence to limited–suggestive evidence of *no* association would require new studies that correct for the problems in methodology in previous studies and that have sample sizes that are large enough to limit the range of possible study results attributable to chance.

The original *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 the exposures of interest and a small group of cancers: gastrointestinal tumors (colon, rectum, stomach, pancreas), skin cancer, brain tumors, and bladder cancer. The *Update 1996* committee removed skin cancer 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* or *Update 2002.*

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. Previous reports point out that although there have been many health studies of Vietnam veterans, most are hampered by relatively poor measures of exposure to herbicides or TCDD and by other problems in methodology. 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 settings rather than from studies of Vietnam veterans. The committees that produced *VAO* and the updates found that body of evidence was sufficient for reaching conclusions about statistical associations between herbicide exposures and health outcomes, but that the lack of adequate data on Vietnam veterans themselves complicated 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 persisting large uncertainties about the magnitude of risk posed by exposure to herbicides as defined by the studies reviewed and about the nature and magnitude of exposure to herbicides in Vietnam make quantitative risk assessments difficult. The committees have concluded that, in general, it is impossible to quantify the degree of risk likely to be experienced by veterans because of their exposure to herbicides in Vietnam.

The 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 of 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.

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*, *Update 1998*, *Update 2000*, and *Update 2002*. 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; those data contribute to the biologic plausibility of potential health effects in human populations. Chapter 4 provides an overview of populations repeatedly studied in an effort to understand the chemicals of interest in this report; it also gives design information on those epidemiologic studies new to this update that investigated those populations or that report multiple health outcomes. 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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2

Evaluating the Evidence

This chapter outlines the approach used by this and previous Committees to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides to evaluate the available scientific evidence. A more complete description is 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 for an association between herbicide exposure during service in the Vietnam War and a variety of diseases or health outcomes. Public Law (PL) 102-4, which led to the committee's work, however, did not specify particular health outcomes of interest. *VAO* listed health outcomes addressed in the scientific literature; that list has been amended in the subsequent updates in response to new publications, to requests from the Department of Veterans Affairs (VA) and various veterans' service organizations, and to concerns of Vietnam veterans and their families. Comments received at public hearings and in written submissions from veterans and other interested persons have been valuable for identifying issues to be pursued in greater depth in the scientific literature.

IDENTIFICATION OF RELEVANT LITERATURE

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 in Vietnam. 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 herbicide formulations sprayed in Vietnam. Databases, therefore, were searched for the names of those compounds, their synonyms and abbreviations, and their Chemical Abstract Service (CAS) numbers. The evidence indicates that a tissue protein, the aryl hydrocarbon receptor (AhR), mediates the toxicity of TCDD. As such, the AhR also was used as a key word, as were "dioxin," "Agent Orange," and "Vietnam veteran."

As discussed in Chapter 3, one of the herbicides used in Vietnam, cacodylic acid, is an organic form of arsenic, dimethylarsinic acid (DMA). In addition to being synthesized as an herbicide, DMA is a metabolite of inorganic arsenic in humans. DMA was long thought to be a biologically inactive metabolite of inorganic arsenic, but recent evidence suggests that one form—DMA^{III}—might be responsible for some of the adverse effects of inorganic arsenic. That evidence, however, is not sufficient to support a conclusion that exposure to cacodylic acid results in the same adverse health effects as does exposure to toxic concentrations of inorganic arsenic. Therefore, the literature on the health effects of inorganic arsenic was not considered in this report. Further details on the effects of inorganic arsenic can be found in *Arsenic in Drinking Water* (NRC, 1999) and *Arsenic in Drinking Water: 2001 Update* (NRC, 2001). For cacodylic acid and picloram, the search terms were the synonyms for the herbicides' chemical names and their CAS numbers.

This report concentrates on the evidence published after the completion of work on *Veterans and Agent Orange: Update 2002* (IOM, 2003a). For each health outcome, new evidence was reviewed in detail. The conclusions, however, are based on the accumulated evidence, and not just on recently published studies. When statistics have been generated on the same study population over time (as noted in Chapter 4), there will be multiple entries corresponding to successive updates in the summary tables of Chapters 6–9, but only the most comprehensive version of the information on a given population is factored into the committee's conclusion on any health outcome. A detailed description of the committee's general approach to the evaluation of scientific evidence is delineated in Chapter 5 of *VAO*. Later committees have adopted the original committee's approach.

The information the committee used was compiled from a comprehensive electronic search by keyword of public and commercial databases—biologic, medical, toxicologic, chemical, historical, and regulatory—that provide citations from the scientific literature. In addition, the reference lists of some review and research articles, books, and reports were examined for potentially relevant articles. Literature identification continued through June 1, 2004. That search strategy helped ensure that all potentially relevant articles were identified, however, it also resulted in a large number of non-relevant studies being identified. More than 3,000 citations were identified in those searches, including some

studies that were identified multiple times. For the majority of the citations, it was evident from the abstract that the article did not address health effects in association with exposure to the chemicals of interest. For example, many of the identified citations investigated the efficacy of the herbicides in killing weeds. All studies that discussed the health effects were considered if there was an indication that the herbicides of interest (or any of their components) were investigated. Because of the large number of non-TCDD-like polychlorinated biphenyls (PCBs), epidemiology studies of PCBs were reviewed only if they had data on the TCDD-like activity. More than 550 potentially relevant citations were identified, and each of those articles was retrieved and reviewed for the report.

COMMITTEE'S APPROACH

The committee had three specific tasks: determine whether there is a statistical association between exposure to the herbicides used in Vietnam and health outcomes, determine the degree of increased risk of effects among Vietnam veterans, and determine whether plausible biologic mechanism(s) provide support for a causal relationship with a given health outcome. This section discusses the committee's approach to each of those tasks.

Statistical Association

The issues in determining whether a statistical association exists are detailed in Chapter 5 of *VAO*. The committee found that the most relevant evidence came from epidemiologic studies—investigations in which large groups of people are studied to identify an association between exposure to a chemical of interest and the occurrence of particular health outcomes. Epidemiologists estimate associations between exposure and outcome in a specific population or group by use of such measures as relative risk, standardized mortality ratio, or odds ratio. Those terms describe the magnitude by which the rate of disease differs between two populations. For example, if the rate in an exposed population doubles relative to a non-exposed population, the relative risk, or rate ratio, is 2. Similarly, if the odds of a health outcome in an exposed population are 1:20 but 1:100 in an unexposed population, then the odds ratio is 5. In this report *relative risk* refers to the results of cohort studies; *odds ratio* (an estimate of relative risk) usually refers to the results of case–control studies. (The results of cohort studies sometimes are reported with odds ratios, again to estimate relative risk.) An estimated relative risk greater than 1 indicates a positive association (that is, it is more likely that the outcome will be seen with exposure), whereas values between zero and 1 indicate a negative or inverse association (that is, the outcome is less likely with exposure). A ratio of 1 suggests the absence of association. A statistically significant association is one that would be unlikely to occur by chance if there were truly no association (that is, null hypothesis is true).

Determining whether an estimated association between an exposure and an outcome represents a "real" relationship requires careful scrutiny because there can be more than one explanation for the estimate. *Bias* is a distortion of the measure of association that results from flawed selection in the assembly of the study population or from error in measurement of the characteristics studied. *Confounding* is the distortion of the measure of association that results from the failure to recognize or account for some other factor related both to exposure and to outcome. *Chance* is the degree to which the estimated association might vary across separate samples of the population studied. The width of the confidence interval is used to quantify the likely variability of the exposure-disease association. Even when a relative risk or standardized mortality ratio exceeds 1, a conclusion regarding increased risk must be qualified when the confidence interval is broad. In drawing its conclusions, the committee examined the quantitative estimates of association and evaluated the potential influences of bias, confounding, and chance. When integrating the findings from various studies, the committee considered the degree of statistical significance associated with every estimated risk (a reflection of the magnitude of the observed effect and the power of the study design), rather than simply tallying the "significant" and "non-significant" outcomes as dichotomous items of evidence.

In pursuing the question of statistical association, the committee recognized that an absolute conclusion about the absence of association is unattainable. As in science generally, studies of health effects associated with herbicide exposure cannot demonstrate that a purported effect is impossible or could never occur. Any instrument of observation, even the most excellent epidemiologic study, is limited in its resolving power. In a strict technical sense, therefore, the committee could not prove the absolute absence of an association between a health outcome and exposure to any one of the compounds of interest.

Factors such as consistency of evidence, biological plausibility, temporality, dose-response, and strength of association may be considered when deciding whether an observed statistical association is actually causal. The committee's charge, however, did not extend to making determinations of causality, so no conclusions regarding cause-and-effect relationships have been made.

Interaction or synergism among the chemicals of interest or with yet other agents is another theoretical concern. Because the committee is not charged with making attribution to one of the chemicals of interest specifically, joint effects among them should be adequately identified by the committee's approach. The number of combinations of these chemicals with other agents that might be problematic is virtually infinite. Real life experience, as investigated by epidemiology studies, effectively integrates any effect of a target substance over all other possibly detrimental or mitigating exposures that a population might have; it may never be possible to tease apart the contributions of the various factors definitively.

Increased Risk in Vietnam Veterans

When all of the available epidemiologic evidence has been evaluated, it is presumed that Vietnam veterans are at increased risk for a specific health outcome when there is evidence of a positive association between one or more of the chemicals of interest and that outcome. The best measure of potency for the quantification of risk to veterans would be the rate of the outcome in exposed Vietnam veterans compared with the rate in non-exposed veterans, adjusted for the degree to which any other differing factors between exposed and non-exposed veterans might influence those rates. A dose-response relationship established in another human population suitably adjusted for such factors would be similarly suitable.

It is difficult, however, to quantify risk when exposures have not been measured accurately in a population. Fairly accurate TCDD exposure data for Vietnam veterans are available only for a small subgroup enrolled in the Air Force Health Study (Ranch Hand population). Therefore, the absence of reliable measures of exposure to the chemicals of interest among Vietnam veterans limits the committee's ability to quantify risk of specific diseases in this population.

Plausible Biologic Mechanisms

Chapter 3 details the experimental basis for assessment of *biologic plausibility* or the extent to which an observed statistical association in epidemiology studies is consistent with biologic or medical knowledge. In other words, would causation of the particular health effect observed make sense based on what is known about how the chemical acts at the tissue, cellular, or molecular level? The relationship between a particular exposure and a specific human health outcome is addressed in the context of research on the effects of those compounds on biologic systems and of evidence from animal studies. In this report, the committee reviews studies that were published after *Update 2002* (IOM, 2003a), and considers those and earlier studies in its conclusions about biologic plausibility.

A positive statistical association between exposure and outcome does not necessarily mean that the exposure to the compound is the cause of the health effect. Data from toxicology studies may support or refute a hypothesis that a specific compound can cause a particular disease. Many toxicology studies are conducted with laboratory animals so that variables, including the amount and duration of exposure, can be controlled precisely. Studies that use isolated cells in culture also can be used to elucidate the way a compound alters cellular processes. The objectives of those toxicology studies are to determine what toxic effects are observed at different exposure concentrations and to identify the mechanisms by which the effects are produced. Ultimately, the results of the toxicology studies should be consistent with what is currently known about the human disease process to support a conclusion that the development of the

disease was caused by exposure. That approach is not without shortcomings; for example, the dose of a chemical required to produce an effect in experimental animals is often many times higher than human exposures. (For TCDD, however, effects have been observed in animals having body burdens within a factor of 10 or less of those at the high end of the general population in the industrialized world.) Furthermore, animal and cell culture models do not always accurately mimic human responses. When the epidemiological evidence is strong, the absence of evidence for biologic plausibility from toxicology studies does not rule out the possibility that an association may exist. In fact, such cases often drive new toxicology research.

EVALUATION OF THE EVIDENCE

The associations between exposures to the chemicals of interest and specific health outcomes are determined through an analysis of available epidemiologic studies, informed by an understanding of the toxicology of the chemicals and their exposure pathways. In reaching conclusions, *VAO* committees consider the nature of the exposures, the nature of the health outcomes, the populations exposed, and the quality of the evidence examined. Some specific issues this and prior committees have considered are addressed below.

Human Studies

The committee reviewed studies of Vietnam veterans and of other populations that might have been exposed to the chemicals of interest. Those studies included cohorts of workers in chemical production and agriculture, populations that reside near sites of environmental contamination, and residents of Vietnam. The committee believes that studies of such non-veteran subjects can help in the assessment of whether the chemicals of interest are associated with particular health outcomes. Some of the studies, especially those of workers in chemical-production plants, provide stronger evidence about health outcomes than do studies of veterans, because industrial exposures are frequently measured sooner after occurrence and are often more thoroughly characterized than has been the case for Vietnam veterans. Furthermore, in the chemical-production plant studies, the magnitudes and duration of exposure to the chemicals were generally greater, and the studies were frequently large enough to examine the health risks among groups of people with different levels of exposure. It is, however, the practice of *VAO* committees to evaluate all studies according to the same criteria whether or not their subjects are Vietnam veterans and then to weight findings of similar strength and validity equivalently when drawing conclusions.

The committee has concluded that it would be inappropriate to use quantitative techniques, such as meta-analysis, to combine individual study results into a single summary measure of statistical association. The committee reached this

conclusion because of the many differences among studies in their definitions of exposure, health outcomes considered, criteria for defining study populations, correction for confounding factors, and degree of detail in reporting results. The appropriate use of meta-analysis requires more methodologic consistency across studies, especially in the definition of exposure, than is present for the literature reviewed by the committee (Egger et al., 2002; Petitti, 2000). It is more informative to include a detailed discussion of the results from individual studies in appropriate categories (occupational, environmental, and Vietnam veterans), along with a thorough examination of each study's strengths and weaknesses.

In general, the committee did not consider case reports, case series, or other published studies that lacked control or comparison groups. An exception was made, however, for early-onset transient peripheral neuropathy. Individual case reports were reviewed because the rapid appearance and transient nature of that condition imposes methodologic constraints that might have precluded the application of standard epidemiologic techniques.

Because the effect of Agent Orange in individuals or groups of veterans is evaluated in terms of disease or medical outcome, attention to disease classification was important to the committee in accurately assembling all pertinent data related to a particular endpoint from various investigations prior to integrating the information. The researchers conducting the studies reviewed by the committee faced the same challenge in reliably interpreting the available documentation when assigning a diagnostic label to a given subject and in then grouping those labels for analysis.

Pathologists, clinicians, and epidemiologists use several classification systems, including the International Classification of Diseases (ICD), International Classification of Diseases—Clinical Modification (ICD-CM), and International Classification of Diseases for Oncology (ICD-O). International Classification of Diseases, 10th Edition (ICD-10) is currently used to classify mortality information. The majority of subjects investigated in the studies cited in this update were diagnosed under earlier systems and most of the articles report results using the International Classification of Diseases, 9th Edition (ICD-9), if they use ICD codes at all, so the committee has also employed ICD-9. ICD codes are a hierarchical system for indicating type of disease and site (for example, ICD-9 162 specifies cancers of the lung, trachea, or bronchus; while more exactly 162.2 represents cancer of the main bronchus of the lung; 162.3, cancer of the upper lobe of the lung; and 162.4, cancer of the middle lobe of the lung). In this report, ICD codes appear almost exclusively in the introductory sections of health outcome discussions (particularly for cancers) to specify precisely what endpoint is being addressed. (See Appendix C for cancer groupings with corresponding ICD-9 and ICD-10 codes.)

For a patient to be correctly diagnosed, careful staging of the extent of disease is necessary and a biopsy of the tissue must be analyzed by microscopy, often with special immunohistochemical stains, to confirm a clinical impression.

Unfortunately, many of the epidemiology studies reviewed by this committee did not use the ICD approach to classification of disease and relied instead on clinical impression alone. Death certificate diagnoses are notoriously inaccurate when they are completed by medical officers who are not familiar with the decedent's medical history. Self-reported diagnoses, which are obtained from survey questionnaires, often are partially or completely inaccurate. For instance, a patient may state that he was treated for stomach cancer when the correct diagnosis could be gastric adenocarcinoma, gastric lymphoma, pancreatic cancer, large bowel cancer, or peritoneal cancer.

Many epidemiologic studies report the disease outcome by organ system. For instance, the term "digestive system" may be used for conditions that are benign or malignant; affecting the esophagus, stomach, liver, pancreas, small bowel, large bowel, or rectum. Therefore, if a report indicated that a cohort has an increased incidence of digestive system cancer, it would be unclear whether the association was attributable to excess cases of esophageal, gastric, hepatic, pancreatic, or intestinal cancers—or to some combination. Such generalization is further complicated by the fact that the cause of cancer may differ at various anatomical sites; for instance, there are strong associations between gastric cancer and *Helicobacter pylori* infection, between smoking and squamous cell carcinoma of the esophagus, and between chronic hepatitis B infection and liver cancer. Furthermore, a single site may also experience a carcinogenic response to multiple agents.

Interpretation of the epidemiology literature is further complicated because many studies lack information on the latent period (time from exposure to recognition or diagnosis of disease). Issues surrounding the latent period are discussed in detail in *Veterans and Agent Orange: Length of Presumptive Period for Association Between Exposure and Respiratory Cancer* (IOM, 2004). The latent period must be considered when evaluating whether there is an increased risk of disease. If a study is looking for an increase in cancer, for example, ample time must have passed since exposure to allow cancers to develop.

The committee has also noted concerns expressed by veterans at public meetings regarding cases of multiple health outcomes, such as multiple cancers, in individuals. Little research has been done to address whether the rate of concurrence is greater than would be expected by chance. Simultaneous analysis of multiple health outcomes could potentially provide more insight into the effect of the chemicals of interest in causing multiple health effects, competing risks between various health outcomes, and the interactive effects of some health endpoints on others.

Rare diseases are also difficult to study because it is hard to accumulate enough cases to permit analysis. This often results in whatever cases are observed being included in a broader, less specific category. Thus, epidemiologic data may not be available for assessing whether a certain rare disease is associated with Agent Orange exposure. In some instances, as for chronic lymphocytic leukemia,

VAO committees have reached conclusions on the basis of the data available and the etiology of the disease. *VAO* committees could not possibly address every rare disease, however, and they simply do not draw any conclusions on diseases that they have not discussed.

Exposure Assessment

Much of the evidence that the committee considered is drawn from studies of populations who were not in Vietnam during the period when Agent Orange and other herbicides were used as defoliants. The most informative studies are well-documented investigations of occupational exposures to TCDD or specific herbicides, such as 2,4-D or 2,4,5-T. In many studies, TCDD exposure is combined with exposures to an array of "dioxin-like" compounds and the herbicides are often analyzed as members of a functional class, which is less informative for the committee's purpose than individual results for each specific compound. In the real-world experience investigated in epidemiology studies, exposure to multiple possibly toxic chemicals is the rule rather than the exception; for example, farmers or other agricultural populations are likely to be exposed to insecticides and fungicides, as well as to herbicides. In such studies, the committee looked for evidence of health effects that are associated with the specific compounds contained in the chemicals used as defoliants in Vietnam, with consideration to and adjustment for other possibly confounding exposures.

The quality of exposure information in the scientific literature reviewed by this committee spanned a broad range. Some studies relied on interviews or questionnaires to determine the extent and frequency of exposure. Such self-reported information generally carries less weight than would more objective measures of exposure. To the extent that questionnaire-based information can be corroborated or validated by other sources, its strength as evidence of exposure is enhanced. Written records of chemical purchase or production can provide one type of objective information. Even more useful are scientific measurements of exposure. In many occupational studies, for example, workers wear air-sampling instruments that measure the concentration of a contaminant in each worker's breathing zone. Measurement of chemicals or their products in biologic specimens, such as blood or urine, also can provide reliable indications of exposure for specific periods. Studies that categorize exposure from well-documented environmental sources of contaminants can be important in the identification of exposed populations, but the results of these studies may be inaccurate when individuals with different levels of exposure are assigned to the same general category of exposure. Studies that explore environmental exposure and disease frequency of populations (e.g., states, counties) are known as ecologic studies. Although ecologic studies vary in their ability to specifically link an exposure to a health outcome, most are considered preliminary or "hypothesis generating"

studies because they lack information on exposure and disease on an individual basis and are unable to address potential confounding factors.

Exposure or dose reconstruction is a particularly challenging aspect of exposure assessment for a population such as Vietnam veterans in which few measurements were made during the period of exposure. Much subsequent work has relied on records of herbicide production and use and on military records of troop locations. A recent effort overseen by the Institute of Medicine has developed a new algorithm for application to Vietnam veterans (the "Stellman model") using records of herbicide applications in Vietnam and revised data about troop movements (IOM, 2003b). The new information holds promise for use in the estimation of what is called *exposure opportunity* for veterans; that is, estimates of the amount of herbicides (with characteristic TCDD contamination) applied at particular places over particular time periods. Recent studies of veterans known to have been exposed to herbicides in Vietnam have included collection of blood samples and analysis of TCDD in those samples. The readings from those contemporary samples have been used to identify subgroups of Vietnam veterans with relatively high exposures and also to validate estimates from the Stellman model when extrapolated to the present. Although such analysis clearly is valuable, it also must be viewed with caution. In most cases, the measurement of compounds in blood has taken place many years after exposure. There are numerous difficulties in extrapolating back from contemporaneous TCDD tissue concentrations to estimate TCDD (and indirectly herbicide) doses at the time of first exposure or to maximum exposure; the estimates so derived are subject to substantial uncertainty.

Animal and Mechanistic Studies

Animal models used as surrogates for the study of a human disease must reproduce, with some degree of fidelity, the manifestations of the disease in humans. However, a given effect of herbicide exposure in an animal species cannot always be used to establish its occurrence in humans. In addition to possible species differences, there are many factors that affect the ability to extrapolate from studies in animals to health effects in humans. Animals used in experimental studies are most often exposed to purified chemicals and not to mixtures. Even if herbicide formulations or mixtures are used, the conditions of exposure might not realistically reproduce those exposures that occur in the field. Furthermore, Vietnam veterans were likely exposed to other agents—tobacco smoke, therapeutics, drugs, diesel fumes, or alcohol, for example—that may positively or negatively affect the ability of chemicals contained in herbicides to produce a particular adverse health outcome. There have been few, if any, studies either in humans or experimental animals that have examined those interactions.

As discussed in Chapter 3, TCDD, a contaminant of 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 known species-, sex-, and endpoint-specific differences in susceptibility to TCDD toxicity. Some data indicate that humans might be more resistant than are other species to TCDD's toxic effects (Ema et al., 1994; Moriguchi et al., 2003); other data suggest that, for some endpoints, human sensitivity could be the same as or greater than that of some experimental animals (DeVito et al., 1995). Differences in susceptibility may also be affected by variations in the rate at which TCDD is eliminated from the body. (See Chapter 3 for details on the toxicokinetics of TCDD.)

It also is important to consider TCDD's mode of action when considering species and strain differences. There is a consensus that most, if not all, of the toxic effects of TCDD involve interaction with the AhR, a protein that binds TCDD and other aromatic hydrocarbons with high affinity. Formation of an active complex, involving the intracellular receptor, the ligand (the TCDD molecule), and other proteins is followed by interaction of the activated complex with specific sites on DNA. That interaction can alter the expression of genes involved in the regulation of cellular processes. The development of mice that lack the AhR has helped to establish a definitive association between the AhR and TCDD-mediated toxicity. The affinity of TCDD for the AhR is species- and strain-specific, and responses to binding of the receptor vary among cell types and developmental stages. Also, genetic differences in the properties of the AhR are known to exist in human populations, as they do in laboratory animals. Thus, individuals may be at greater or lesser risk of the toxic effects of TCDD.

Although studying AhR biology in transformed human cell lines minimizes the inherent error associated with species extrapolations, caution must be exercised because it is still not clear to what extent toxicity is affected by the transformation itself or by the conditions under which cell lines are cultured in vitro.

Publication Bias

Some studies are more likely to be published than others. This is the concept of publication bias, which has been documented in biomedical research (Song et al., 2000; Stern and Simes, 1997). Most commonly, bias can be introduced when studies that are statistically significant or are otherwise deemed favorable by their authors are selectively submitted for publication. Conversely, "negative" studies, in which the hypothesis being tested is not supported by the study findings, often go unpublished. Therefore, conclusions about associations between exposure and outcome that are based solely on published results could be subject to bias. Despite that, the committee does not believe its conclusions have been unduly affected by publication bias for two reasons: the extensive publicity surrounding the possibility of health effects associated with the herbicides used in Vietnam

has created considerable pressure to publish all findings on the subject, and the many published studies assembled and reviewed contain among their results the full range of possible statistical associations, from convincingly negative to indeterminate to strongly positive.

Role of Judgment

This committee's process of reaching conclusions about statistical associations involved more than a formulaic application of quantitative procedures to the assembled evidence. First, the committee had to assess the relevance and validity of individual reports. Then, it had to evaluate the possible influences of measurement error, selection bias, confounding, and chance on the reported results. Next, the committee integrated all of the evidence within and across diverse fields of research. Finally, the conclusions drawn were based on consensus within the committee. 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 realized approach, as described here, was determined to a large extent by the nature of the exposures, of the health outcomes, and of the resultant evidence available for examination; therefore, it has evolved in the course of the work of this and previous *VAO* committees. The quantitative and qualitative procedures underlying this review have been made as explicit as possible, but 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* (IOM, 1994; hereafter referred to as *VAO*), *Veterans and Agent Orange: Update 1996* (IOM, 1996; hereafter, *Update 1996*), *Veterans and Agent Orange: Update 1998* (IOM, 1999; hereafter, *Update 1998*), *Veterans and Agent Orange: Update 2000* (IOM, 2001; hereafter, *Update 2000*), and *Veterans and Agent Orange: Update 2002* (IOM, 2003; hereafter, *Update* 2002), this chapter summarizes recent experimental data that provide the scientific basis for assessment of the biologic plausibility of the effects of herbicide exposure as reported in epidemiologic studies. Establishment of biologic plausibility through laboratory studies strengthens the evidence of the effects of herbicide exposure that are believed to occur in humans. Toxic effects are influenced by dosage (magnitude and frequency of administration); by exposure to other substances, including compounds other than herbicides; by pre-existing health status; by genetic factors; and by the route and rate of the substance's absorption, distribution, metabolism, and excretion. Attempts to extrapolate from experimental studies to human exposure must therefore carefully consider those variables.

Many chemical compounds were used by the US armed forces in Vietnam. The nature of the substances themselves is discussed in more detail in Chapter 6 of *VAO* (IOM, 1994). Four herbicides documented in military records were of particular concern and are examined 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 (dimenthylarsenic acid, DMA). This chapter also focuses on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, or dioxin), a contaminant of 2,4,5-T, because its potential toxicity is of concern and because

considerably more information is available on TCDD than is available for the herbicides. Except as noted, the laboratory studies of those compounds were done with pure formulations of the compounds. The epidemiologic studies discussed in later chapters often track exposures to mixtures.

This chapter begins with a summary of major conclusions presented in past reports. The rest of the chapter consists mostly of overviews and discussions of the relevant experimental studies that have been published since *Update 2002* (IOM, 2003) on 2,4-D; 2,4,5-T; picloram; cacodylic acid; and TCDD. Within the update for each substance is a review of the toxicokinetic investigations and a summary of the toxic endpoints and their underlying mechanisms of action. The process of estimating human health risk 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*, *Update 2000*, and *Update 2002* review the results of animal and in vitro studies published through 2002 that investigated the toxicokinetics, mechanisms of action, and disease outcomes of exposure to the herbicides, and the contaminant TCDD, used in Vietnam. The herbicides have not been studied extensively, but in general none of them is considered particularly toxic. High concentrations usually are required to modulate cellular and biochemical processes. In contrast, experimental data reviewed in previous Agent Orange reports led those committees to conclude that TCDD elicits a diverse spectrum of sex-, strain-, age-, and species-specific effects: 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 enzyme induction and other biochemical effects.

TOXICITY PROFILE UPDATE OF 2,4-D

Toxicokinetics

Toxicokinetics (also called pharmacokinetics) identifies the routes and rates of uptake, tissue distribution, transformation, and elimination of a toxic substance. Those processes, in part, determine the amount of a particular substance that reaches target organs or cells to influence toxicity. Understanding the toxicokinetics of a compound is an important component for valid reconstruction of exposure.

Several studies have examined the pharmacokinetics and metabolism of 2,4-D in animals and humans since the publication of *Update 2002*. Those data support the previous conclusions that metabolism and elimination of 2,4-D are relatively rapid and that tissue uptake is small.

Van Ravenzwaay et al. (2003) compared the metabolism and elimination of 2,4-D in rats and in dogs to explain dogs' greater sensitivity. Elimination of 2,4-D from rat plasma was significantly faster than in dogs after oral doses of 5 or 50 milligrams per kilogram (mg/kg) body weight. In the rat, excretion essentially was complete after 24 hours (h) for the low dose and after 48 h for the high dose. For the dog, only about half the dose was eliminated in 5 days. Thus, for an equivalent dosage, the body burden of 2,4-D is significantly higher in dogs, and that finding is consistent with the increased sensitivity of dogs to 2,4-D.

Notably, an interspecies pharmacokinetic analysis by Timchalk (2004) suggested that the dog is not a relevant animal for comparative evaluation of human health risk attributable to 2,4-D exposure. The plasma half-life for 2,4-D in dogs (92–106 h) is substantially longer than in rats (~1 h) or in humans (~12 h) because dogs have less efficient renal clearance mechanisms. The result is a higher body burden in dogs for a substantially longer period than is exhibited by other species. A recent study examining concentrations of 2,4-D and its metabolites in the urine of herbicide applicators was consistent with 2,4-D urinary half-life estimates of 13–40 h for humans (Hines et al., 2003).

Three studies reported that use of sunscreen and chronic consumption of alcohol could significantly increase dermal penetration of 2,4-D. Pont et al. (2004) determined that the total percentages of 2,4-D penetrating excised hairless mouse skin within a diffusion chamber in 24 h ranged from 55% for the no-sunscreen control to 87% in skin treated with sunscreen. All but one of the ingredients tested led to a significant increase in 2,4-D penetration. Penetration enhancement also occurred for human skin (Pont et al., 2004). Brand et al. (2002) observed that of nine sunscreen formulations tested, six led to significant increases in the dermal penetration of 2,4-D in hairless mice. In one case, the penetration was more than twice that of the control. The same laboratory investigated the dermal penetration of 2,4-D through the skin of rats fed either an ethanol-containing or a control diet for 6–8 weeks (Brand et al., 2004). Ethanol consumption more than doubled the rate of 2,4-D penetration. Those studies imply that people who regularly use sunscreens or consume ethanol could be at increased risk for internal exposure to and toxicity from 2,4-D.

Durkin et al. (2004) describe the development of a physiologically based pharmacokinetic (PBPK) model to estimate risk to workers who use backpack pesticide sprayers. There was good correspondence between modeled and observed elimination rates of 2,4-D in rats and humans. Although it might underestimate variability because of a lack of consideration of interindividual differences in the kinetics of 2,4-D, with further refinement, the model could result in

more accurate and complete assessments of risk to those who use backpack sprayers and to others who are exposed to 2,4-D.

Chemically reactive metabolites of 2,4-D are believed to mediate the hepatotoxicity of 2,4-D observed in some animal species. Li et al. (2003) determined that 2,4-dichlorophenoxyacetyl-S-acyl-CoA, a metabolite of 2,4-D, binds covalently to human serum albumin and to proteins in rat hepatocytes after in vitro incubation. The authors suggested that the metabolite contributes to induced hepatotoxicity.

Toxic Endpoints and Underlying Mechanisms of Toxic Action

Studies of disease outcomes published since *Update 2002* are consistent with the earlier conclusion that 2,4-D is relatively non-toxic and has weak carcinogenic potential. The developing fetus appears to be the most sensitive for several toxic endpoints after maternal exposure. Recent animal studies of disease outcomes of 2,4-D exposure and possible mechanisms are discussed below.

Carcinogenicity and Mechanisms Related to Genotoxic Effects

Previous updates indicated little experimental evidence that 2,4-D produces any carcinogenic activity. No relevant studies on its carcinogenic effects have been published since *Update 2002*.

Studies reviewed in previous updates indicated that 2,4-D has no genotoxic potential, or that potential is weak, at best. Several more recent reports, however, suggest a weak but positive association between 2,4-D exposure and genotoxic potential in some biologic-model systems. Whereas no effects were observed with pure 2,4-D, in some cases commercial mixtures produced dose-related responses. In others, genotoxicity was observed only when there was evidence of cytotoxicity. Although overall the studies suggest only a weak genotoxicity for 2,4-D, they suggest that the constituents of commercial formulations (like Agent Orange) enhance the toxicity—and, specifically, the genotoxicity—of 2,4-D.

Grabińska-Sota et al. (2002) tested a commercial formulation of 2,4-D in several strains of bacteria. Some genotoxicity was observed, but only at very high concentrations.

The genotoxicity of 2,4-D in fish was evaluated by Ateeq et al. (2002), who assessed the induction of micronuclei and erythrocyte alterations in catfish exposed to 2,4-D at concentrations of as much as 75 parts per million (ppm) in water. The formation of micronuclei and cytotoxicity (vacuolization and echinocyte formation) was concentration dependent. Essentially the same results with similar exposures were observed in a freshwater air-breathing fish, *Channa punctatus* (Abul Farah et al., 2003). Arias (2003) evaluated the induction of sister chromatid exchange (SCE) and altered cell cycle kinetics in 4-day-old chick embryos

exposed either to a commercial herbicide preparation containing 37% 2,4-D or to pure 2,4-D. Pure 2,4-D failed to induce a statistically significant change in SCE frequency, and the commercial product produced a change only at the highest dose. It was suggested that genotoxic effects observed with some 2,4-D preparations could be attributable to impurities or adjuvants in the technical-grade products. In these studies, both types of exposure inhibited the mitotic index, but only at the two highest doses (2 and 4 mg/embryo).

Since the last update, two studies have examined the genotoxic effects of 2,4-D exposure in human lymphocytes. Zeljezic and Garaj-Vrhovac (2004) treated cultured lymphocytes with two concentrations (0.4 and 4.0 micrograms per milliliter [µg/mL]) of a commercial formulation of 2,4-D with and without a metabolic activator (rat liver microsomes; S9). The lower concentration of 2,4-D is the acceptable daily intake recommended by the World Health Organization (1–4 picogram/body weight). Both concentrations induced increases in chromatid and chromosome breaks, in the number of micronuclei, and in the number of nuclear buds. Metabolic activation increased the number of chromatid breaks and micronuclei. The investigators also suggested that the genotoxicity could be attributable to compounds other than 2,4-D within the formulation.

A similar study (Holland et al., 2002) examined the effects of exposure to pure and commercial formulations of 2,4-D. Induction of micronuclei was observed in both whole blood and isolated lymphocytes only at a cytotoxic concentration (0.3 milli molar [mM]) of pure or commercial 2,4-D. The replicative index, a measure of cell division kinetics, was decreased at this cytotoxic level, but at a lower concentration (0.005 mM) showed a slight increase which was more pronounced for the commercial formulation. The commercial formulation contained 9.4% pure 2,4-D, which suggests that other ingredients might be responsible for or enhance the effect of 2,4-D on the replicative index. The authors concluded that the genotoxicity of 2,4-D as measured by the micronucleus assay is negligible at environmentally relevant concentrations, but that it might be enhanced in the presence of other chemicals.

Neurotoxicity

Previous updates indicated no evidence that 2,4-D causes effects on the neurologic system in adult animals at doses in the µg/kg/day range. Case reports of acute poisonings of humans exposed to large amounts of 2,4-D formulations (>20 mL) indicated neurologic manifestations of drowsiness, coma, hyperreflectivity, hypertonia, and cerebral edema (Brahmi et al., 2003). One case of ingestion of an unknown quantity of 2,4-D resulted in death. No other relevant studies involving neurotoxicity in adult humans have been published since *Update 2002*.

Reproductive and Developmental Toxicity

Stebbins-Boaz et al. (2004) examined the in vitro sensitivity of amphibian oocytes to 10 mM 2,4-D. Treatment caused depolymerization of perinuclear microtubules and altered cell morphology that was blocked with cotreatment with cytochalasin B, a microfilament inhibitor. 2,4-D treatment also blocked progesterone-induced maturation of the oocytes. Those data indicated that 2,4-D disrupts amphibian oocyte maturation through effects on cytoskeletal organization. Another study (Greenlee et al., 2004) examined the effects of 2,4-D on mouse preimplantation embryo development—a period that corresponds in humans to the first 5–7 days after conception. The 2,4-D exposure of embryos at 10 ng/mL in vitro increased the percentage of cellular apoptosis but had no effect on the development of the embryo to the blastocyst stage or on the mean cell number per embryo. Another group reported that exposure of pregnant mice to herbicide mixture containing low concentrations of 2,4-D (0.01 and 0.1 mg/kg/day), mecoprop (0.004 and 0.04 mg/kg/day), and dicamba (0.0009 and 0.009 mg/kg/day) resulted in significant reductions in implantation sites and live births (Cavieres et al., 2002), but no significant fetotoxicity was observed. Together, those data suggest that the preimplantation embryo might be especially sensitive to 2,4-D. Additional in vivo studies using 2,4-D exclusively are necessary.

Sulik et al. (2002) examined kidney morphology of newborn rats exposed before and after birth to 2,4-D. Dams were exposed to a daily dose of 250 mg/kg (one-third the $LD_{50,}$ the dose that is lethal in 50% of test subjects) in drinking water for 2 months before fertilization and during pregnancy and lactation. Varying degrees of damage to kidney tubules were observed that were more intense after exposure in pregnancy than in the postnatal period. After withdrawal of 2,4-D, the more severe changes observed in the fetus regressed.

Several studies cited in previous updates suggested effects of 2,4-D on the developing brain, and more recent studies present concordant results. Garcia et al. (2001) exposed pregnant rats to 70 mg 2,4-D/kg/day from gestation day 16 through postnatal day 23. Some of the pups were maintained on this exposure until postnatal day 90. Exposure during pregnancy and lactation produced an increased serotonin neuronal area and increased serotonin immunoreactivity in the mesencephalic nuclei. For pups exposed until postnatal day 90, only the serotonin neuronal area from the dorsal raphe nucleus was increased. Changes also were observed in the presence of reactive astrocytes in the mesencephalic nuclei and hippocampus areas; those changes differed with treatment. Those data provide evidence that 2,4-D exposure alters the serotoninergic system during brain development. Another study with the same design also sought to determine whether 2,4-D affected lateralization in the monoamine systems of the basal ganglia and whether there was any correlation with behavioral changes (Bortolozzi et al., 2003). Asymmetrical variations in brain concentrations of dopamine and serotonin were observed that were dependedent on brain region

and sex. Some changes appeared to be irreversible, and serotonin changes in left and right striata appeared to correlate with the behavioral alterations (spontaneous circling activity) previously reported.

Ferri et al. (2003) examined the effect of 2,4-D exposure on iron, zinc, and copper concentrations in the brain, serum, and liver of well-nourished and undernourished developing rats. Those metals are known to be essential for normal-brain development. Metal concentrations in tissues were found to be altered only at the highest dose (100 mg/kg) to the dam in well-nourished pups exposed through dams' milk. A lower dose (70 mg/kg) produced no alterations. However, undernourished pups displayed greater sensitivity to the lower dose of 2,4-D, as indicated by altered metal concentrations and decreased tissue weight. Those data suggest that undernourishment might exacerbate the effects of 2,4-D on developing tissues.

Immunotoxicity

Previous updates indicated that 2,4-D has at most a weak effect on the immune system. Recent publications are consistent with this. One study reported a possible beneficial effect of exposure to low concentrations of 2,4-D (Balagué et al., 2002).

Some studies suggest a relationship between the frequency of errors in antigen receptor gene assembly and an increased risk of lymphoid malignancy. That correlation has been reported for agricultural workers exposed to pesticides (Lipkowitz et al., 1992). Knapp et al. (2003) examined the effects of 2,4-D exposure to mice on the frequency of errors in V(D)J recombination (that is, recombination of the V-gamma and J-beta segments of the T-cell receptor) in thymocytes. At doses of 3–100 mg/kg/day for 4 days, no significant increase in aberrant V(D)J rearrangements was observed. Alterations to bone marrow B-cell populations after exposure of mice to single doses of 50–200 mg/kg were studied by de la Rosa et al. (2003). Decreases in the pre-B and IgM+ B-cell populations were observed 7 days after treatment for the highest dose of 2,4-D.

Escherichia coli is responsible for many urinary tract infections in humans. Weak acids, such as salicylate, that interfere with interaction betweeen bacteria and epithelial cells are sometimes used to treat those infections. Balagué et al. (2002) reported that daily low doses (2.6 and 25 mg/kg) of 2,4-D (also a weak acid) for 20 days significantly decreased or eliminated bacterial cells in mouse bladder and kidneys. A higher dose (70 mg/kg) was not effective after 9 days of treatment. The authors suggested that low exposure to 2,4-D might have a protective effect against urinary tract infections. Several previous investigations (IOM, 2001, 2003) reported kidney toxicity for high 2,4-D concentrations in several animal species. More recent reports detail effects in fish (Gómez et al., 2002).

Mechanisms Related to Effects on Energy Metabolism or Mitochondrial Function

Several reports cited in previous updates suggested that the toxicity of relatively high concentrations of 2,4-D might be related, at least in part, to its effect on calcium homeostasis and energy metabolism. Those actions might be mediated by a direct action on mitochondria. Some publications suggest it might be attributable to the surfactant in the formulations and not to the 2,4-D itself. However, two recent studies are consistent with the hypothesis that 2,4-D itself causes mitochondrial damage. De Moliner et al. (2002) and Tuschl and Schwab (2003) reported that the exposure of rat cerebellar granule cells and human hepatoma cells, respectively, to 1–16 mM concentrations of 2,4-D elicited cell cycle alterations and apoptotic cell death. Their studies also suggested that the events were caused by a direct effect on mitochondrial membrane potential.

Mechanism Related to Effects on Thyroid Hormones

Effects of 2,4-D on serum concentrations of thyroid hormones, particularly to decreases in thyroxine, were noted in previous updates. Ishihara et al. (2003) examined the effects of industrial and agricultural chemicals on the binding of 3,5,3-L-triiodothronine (T3) to serum thyroid hormone binding proteins (THBPs) and thyroid hormone receptors (TRs). 2,4-D had little or no effect on the binding to THBPs from several species, including humans, or to TRs from chicken or bullfrog.

Mechanisms Related to Effects of Cell Stress Responses

Several investigations examined the ability of 2,4-D to promote or inhibit oxidative damage to cell membranes. Together they suggest that, at high concentrations, 2,4-D is incorporated into cellular membranes to modify membrane structure and integrity.

Duchnowicz and Koter (2003) reported that exposure of isolated erythrocytes to 1 mM 2,4-D resulted in a small but significant increase in hemolysis and in membrane lipid peroxidation. Increased hemolysis was not observed at 0.1 mM (Kleszczyńska et al., 2003). This might be related to a noted concentration-dependent decrease in erythrocyte glutathione (Bukowska, 2003). However, concentrations of 0.5 and 1 mM 2,4-D were found to protect erythrocyte membranes against partial peroxidation induced by ultraviolet irradiation (Bonarska et al., 2003). Özcan Oruç et al. (2004) examined oxidative stress responses in the gills, brains, and kidneys of fish exposed to 2,4-D. Although no significant changes in tissue malondialdehyde, a measure of lipid peroxidation, were observed, there were significant changes in the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GST), and catalase. GST activity in the liver was

increased at all times from 24–96 h after 2,4-D exposure; SOD activity was increased only at 72 and 96 h (Özcan Oruç and Üner, 2002). It was suggested that tissues adapt to protect cells against oxidative stress elicited by toxins such as 2,4-D. The authors also suggested that increases in tissue SOD and GST activity might serve as good biologic markers of oxidative stress. Orfila et al. (2002) reported that administration of the maximum recommended daily doses of vitamins E and C was ineffective for preventing or altering liver damage produced in rats orally dosed with 200 mg/kg 2,4-D amine daily for 15 days.

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 2002*. Several previous reports indicated that 2,4,5-T has only weak mutagenic potential but that it might alter the profile of enzymes involved in the metabolism of procarcinogens. Previous reports concur that 2,4,5-T is only weakly toxic or carcinogenic.

TOXICITY PROFILE UPDATE OF CACODYLIC ACID

Toxicokinetics

Cacodylic acid was present at 4.7% in a herbicide used for defoliation in Vietnam. Cacodylic acid is DMA, which also is a metabolic product of exposure to inorganic arsenic. Methylation of inorganic arsenic generally has been considered a detoxification process: it produces less acutely toxic methylated species (monomethyl arsenic (MMA) and DMA), and it increases excretion of arsenic. More recently, however, some of the methylated metabolic intermediates have been thought to be more toxic than is the parent compound. The methylation pathway of inorganic arsenic results in the formation of pentavalent DMA (DMA^{V}) and trivalent DMA (DMA^{III}) (IOM, 2003). DMA^{V} appears to be less toxic than DMA^{III} (IOM, 2003); about 80% of DMA is excreted unchanged and more rapidly than is inorganic arsenic (reviewed in Duzkale et al., 2003). DMA^{III} in fingernails and DMA^{V} in fingernails and hair can serve as biologic markers of arsenic exposure (Mandal et al., 2003).

Endpoints and Underlying Mechanisms of Toxic Action

Since *Update 2002*, the only new literature concerning the toxic activity of cacodylic acid addressed genotoxicity, a major mechanism of carcinogenesis. In addition to being produced as an herbicide, cacodylic acid, or DMA, is a metabolic product of organic arsenic exposure in humans. The committee considered the relevance of data on inorganic arsenic to DMA. Although inorganic arsenic is

a human carcinogen, there is no evidence that direct exposure to its metabolite, DMA, produces cancer in humans. DMA also is not demethylated to inorganic arsenic. It has not been established nor can it be inferred that the effects observed from exposure to inorganic arsenic also occur from exposure to DMA. Therefore, the literature on inorganic arsenic is not considered in this report. The reader is referred to *Arsenic in Drinking Water* (NRC, 1999a) and *Arsenic in Drinking Water: 2001 Update* (NRC, 2001). Cancer has been induced in the urinary bladder, kidneys, liver, thyroid glands, and lungs of laboratory animals exposed to high concentrations of DMA (IOM, 2003). DMA might act through induction of oxidative damage or damage to DNA, and exposure results in necrosis of the urinary bladder epithelium followed by regenerative hyperplasia (IOM, 2003).

Since *Update 2002*, further studies have investigated the potential carcinogenicity of DMA itself. In a 2-year bioassay, F344 rats were administered drinking water that contained 0, 12.5, 50, or 200 ppm DMA for 104 weeks (Wei et al., 2002). Between weeks 97 and 104, urinary bladder tumors were observed in 8 of 31 rats treated with 50 ppm DMA and in 12 of 31 rats administered 200 ppm DMA. No urinary bladder neoplasms were observed in the groups given 0 or 12.5 ppm DMA. The urinary bladder tumors had a low rate of H-ras mutations, but no alterations of the p53, K-ras, or B-catenin genes were reported. In another study, F344 rats were exposed to 100 ppm DMA for 2 weeks. DMA produced cytotoxicity and regenerative hyperplasia of the urothelium of the urinary bladder (Cohen et al., 2002).

Salim et al. (2003) administered DMA in drinking water (0, 50, or 200 ppm for 18 months) to P53 heterozygous (+/-) knockout mice and wild-type (+/+) C57BL/6J mice. Treatment resulted in a significant increase in total numbers of tumors (at the 50- and 200-ppm doses) in the wild-type mice and significant earlier induction of tumors in more organs and tissues of both the p53 +/- knockout (50 and 200 ppm) and the wild-type mice (50 and 200 ppm). The lack of organ specificity or mutations in the residual allele or in wild-type alleles in both genotypes suggests that DMA is a non-genotoxic carcinogen.

In an initiation–promotion experiment, carcinogenesis in F344 rats was initiated with a single injection (200 mg/kg) of diethylnitrosamine and promoted with 100 ppm DMA (Nishikawa et al., 2002). DMA treatment resulted in a significant increase in numbers and areas of GST-P positive foci (preneoplastic lesions) in the liver.

Duzkale et al. (2003) found that DMA exerted differential antiproliferation and cytotoxic activity against leukemia and multiple myeloma cells, but not against normal peripheral blood progenitor cells, and induced apoptosis in the malignant cells. However, the concentrations of DMA necessary to achieve those effects were 500–1,000 times those required when arsenic trioxide was used. Other researchers have observed increased apoptosis in cell cultures exposed to DMA and noted that DMA requires intracellular reduced glutathione to induce apoptosis (Sakurai, 2003; Sakurai T et al., 2002).

DNA strand breaks, a form of oxidative damage, were generated by DMA in cultured human cells and in isolated bacterial DNA (Schwerdtle et al., 2003). DMA also was cytotoxic to cultured Chinese hamster V79 cells and caused chromosome structural aberrations (Ochi et al., 2003). Nesnow et al. (2002) showed that the DNA-damaging activity of DMA is an indirect genotoxic effect mediated by reactive oxygen species formed concomitantly with the oxidation of DMA^{III} to DMA^{V}. Induction of the tumor suppressor protein p53, another indicator of DNA damage, was produced by DMA in cultured human cells in a dose- and time-dependent manner (Filippova and Duerksen-Hughes, 2003). DMA also has induced chromosome aberrations in Chinese hamster ovary cells and in the SCE assay (Kochhar et al., 2003). However, DMA cultured with primary rat astroglia cells did not produce changes in cell viability or cause DNA damage at micromolar concentrations; treatment of the astroglia cells with inorganic arsenicals resulted in decreased cell viability and increased DNA damage (Jin et al., 2004).

DMA was a clastogen in human lymphocytes and a mutagen at the Tk+/- locus in mouse lymphoma cells (Kligerman et al., 2003). Those authors did not consider DMA to be a gene mutagen.

Coexposure of human liver ferritin and DMA resulted in more DNA damage (Plasmid pBR322) than did exposure to DMA alone (Ahmad et al., 2002). The authors proposed that iron-dependent DNA damage could be a mechanism of action of human arsenic carcinogenesis.

Cacodylic acid, at doses on the order of 200 ppm, has been shown to act as a tumor promoter in the kidneys and urinary bladders of laboratory animals (IOM, 2003).

TOXICITY PROFILE UPDATE OF PICLORAM

The compounds 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2,4-dichlorophenoxyacetic acid (2,4-D) are components of Agent White, an herbicide formulation used in Vietnam. Studies reviewed in previous updates and in *VAO* reported fairly rapid elimination of picloram and suggest that some carcinogenic and neurologic effects can be attributed to exposure, although only at extremely high doses. Some cellular abnormalities in liver and inconsistent developmental effects also have been reported, and there is some evidence that picloram causes male-mediated birth defects including persistent histologic effects in testes, in animals (IOM, 2003). However, blood concentrations of either agent associated with a dose that is high enough to elicit such effects were not likely to occur in occupational exposure to Agent White.

No relevant studies of picloram have been published since the preparation of *Update 2002*.

TOXICITY PROFILE UPDATE OF TCDD

Toxicokinetics

The terms "toxicokinetics" and "pharmacokinetics" refer to processes and rates involved in the movement of a toxic chemical or drug into, within, and from an animal's body. Thus, toxicokinetics encompasses the routes and rates of uptake, tissue distribution, transformation, and elimination of a toxic substance from the body. Those processes can determine the amounts of a particular substance that will reach specific target organs or cells, thereby influencing toxicity in those organs or cells. It is important to determine the concentrations of TCDD and other polychlorodibenzo-*p*-dioxin (PCDD) congeners in different organs in the process of reaching conclusions about toxic effects in target organs.

The distribution of TCDD and other chlorodibenzo-*p*-dioxin congeners has been examined extensively in experimental animal models over the past 25 years. Other planar halogenated aromatic hydrocarbon (PHAH) compounds are thought to act by similar mechanisms, especially the polychlorinated dibenzofurans and non-*ortho*-polychlorinated biphenyls, and they also have been examined extensively. In animal models it is possible to control exposure and thus to test the validity of PBPK or other models. In humans, the utility of such models is determined by examining TCDD tissue and blood concentrations as related to accidental or background exposure.

As discussed in numerous papers reviewed in previous reports, those compounds are hydrophobic and tend to be absorbed readily across cell membranes. TCDD is distributed to all compartments of the body, although the amounts differ from organ to organ. Properties of the compounds, properties of the organs and cells, and the route of exposure affect the partitioning, absorption, and accumulation of the chemicals. Lipid content affects the accumulation of TCDD and other PHAHs in different organs and in the body as a whole. Biologic processes, especially metabolism, subsequently can affect their distribution and elimination. The concentration in a given organ or tissue thus depends on dose, absorption, lipid content, and metabolism in the organ of concern. Adipose tissue–serum partition coefficients derived from concentration ratios can indicate the degree of accumulation in fatty tissues, and the amount of fat in a particular organ can determine partitioning into that organ. Interindividual differences in absorption, distribution, and elimination can lead to a range in the organ–serum partition coefficients within a population. Whole-blood or serum concentrations of TCDD can also fluctuate with differences in physiologic state and metabolic processes that can affect the mobilization of lipids and possibly the mobilization of compounds from lipid stores. Moreover, the processes in one organ can influence distribution to others. Thus, binding proteins in some organs, such as cytochrome P450 1A2 (CYP1A2) in liver, can influence accumulation in extrahepatic organs.

Modeling the toxicokinetics or pharmacokinetics of TCDD has several objec-

tives: to estimate organ distribution from concentrations measured in surrogate tissue, such as blood; to determine organism concentrations from diet or other external sources of exposure; and to back-extrapolate from current tissue concentrations to those at the time of original exposure. Pharmacokinetic or toxicokinetic parameters can be similar or differ substantially among species, depending on similarities or differences in anatomy, physiology, and metabolic biochemistry—particularly for processes involved in foreign chemical metabolism—which vary greatly. PBPK models apply knowledge of mechanisms, including organ size and blood flow, binding proteins in blood, xenobiotic metabolism, and excretion and clearance rates to the process of predicting concentrations and kinetic behavior of a compound, given a particular exposure. Allometric models rely largely on relationships between body size and lipid content and the kinetic parameters of elimination. PBPK models can offer more information about tissue distribution, but it still is possible to predict elimination of TCDD with knowledge of fat content and body mass index (BMI). In this way, toxicokinetic modeling and exposure assessment are linked.

Animal Studies

There have been several additions to the literature since the last report (IOM, 2003) that detail the processes that affect distribution of TCDD. Studies in rodent models have continued to support the value of PBPK models for predicting the disposition of TCDD. Kim et al. (2002) used a mechanistic PBPK model to estimate body burden after exposure of female rats to TCDD, as compared with a kinetic model that applies effective dose for a specific known molecular effect. That study showed that the use of a simpler kinetic approach to body burden gave results similar to those based on a PBPK model for estimating body burden, with a defined exposure regimen.

Efforts have continued to identify dietary or other approaches that can enhance the elimination of dioxins, decreasing their uptake and half-life. The goals of such studies are to decrease absorption of dioxins from contaminated food in the digestive tract and to stimulate the excretion and elimination of dioxins from highly contaminated individuals. Studies over the past several years have attempted to enhance elimination of TCDD, for example with activated charcoal, crude dietary fiber, Olestra, and seaweed (reviewed in previous updates). Elimination of TCDD residues is generally in the feces, which can include ingested matter that is not absorbed, and in residues excreted from the body in bile. Aozasa et al. (2003) fed mice soluble or insoluble dietary fiber—the latter with or without several porphyrin (chlorophyllin) derivatives differing in the metal complexed with the porphyrin—after the mice had been exposed to the dioxin congener 1,2,3,4,7,8-HxCDD. Mice were given an oral dose of 10 µg of the HxCDD congener per kilogram of body weight. Three days later, the mice were fed diets that contained various amounts of fiber. Results showed that a diet

with 10% insoluble fiber promoted dioxin excretion by about 60% over a soluble fiber diet, and that with Cu-porphyrin added, the stimulation was 144%. A similar objective was approached in a study with rats, using nori, a traditional constituent of Japanese diets that is prepared from red algae, to affect TCDD uptake and elimination (Morita and Tobiishi, 2002). The experiment included administration of nori simultaneously with a mixture of polychlorinated dibenzofurans (PCDF) and PCDD congeners, including TCDD, and administration of nori 7 days after administration of the PCDD–PCDF. The two approaches should indicate effects on absorption and elimination from the body, respectively. The results with a 10% nori diet showed a 5-fold increase in fecal excretion of TCDD when nori and TCDD were given together, indicating reduced uptake of TCDD. When the 10% nori diet began 7 days after TCDD, there was a 2.4-fold increase in TCDD excretion and a slight but significant decrease in TCDD body burden, indicating an effect on elimination. Similar results were obtained for total toxic equivalents (TEQ), with a greater amount of dioxins and dibenzofurans eliminated with the nori diet in both experimental approaches.

Such dietary approaches are not yet sufficiently effective to reliably accomplish a therapeutic enhancement of elimination for TCDD. Sakurai K et al. (2002) carried out a study in guinea pigs to establish the tissue distribution of TCDD given at different doses and for different periods. The objective was to establish a baseline in the guinea pig for eventual use of this species as a model to test the ability of dietary additions to enhance elimination of TCDD. The TCDD concentrations achieved in the guinea pig showed tissue distribution (adipose/liver ratios) similar to that in humans, suggesting that the guinea pig could be a useful model for low-dose studies. The TCDD concentrations were higher in adipose tissue than they were in liver, suggesting that CYP1A2 might not be as effective at sequestering TCDD in guinea pigs as it is in rats or mice, or that the dose was too low to induce CYP1A2. The longer half-life of TCDD in guinea pigs (94 days) than in rats (20 days), as reported by Olson (1986), may involve a slower rate of excretion of metabolites or parent compound at low doses and a higher body fat content in the guinea pig rather than a difference in their rates of metabolism.

The major source of exposure to dioxins is the diet. Cavret et al. (2003) used an in vitro model (Caco-2 cells derived from a human colon carcinoma) and an in vivo model system (pig fitted with vascular catheters, allowing sampling of portal and brachiocephalic blood) to define the factors that influence uptake of TCDD and other compounds (phenanthrene and benzo[*a*]pyrene) in the intestine. The studies showed proportional absorption of the compounds in the cells and in the in vivo model, suggesting that the companion systems might be useful in defining conditions and factors involved in accumulation of TCDD.

Emond et al. (2004) refined a PBPK model for pregnant rats, to predict TCDD concentrations in both maternal and fetal tissues, throughout gestation. The model was simplified by incorporating only those tissue compartments known to significantly influence the distribution of TCDD. This simplified model

gave similar fits to the data as the full model, a result of the fact that liver and fat compartments variably represent 80–95% of the total body burden of TCDD. The validated rodent model could be useful in assessing fetal human exposures.

Human Studies

Since *Update 2002*, few studies have addressed toxicokinetic modeling of TCDD in humans, although those under way continue to support the validity of PBPK modeling to estimate internal dose from amount ingested. Bois (2003) used a PBPK model to assess the effect that ingestion of TCDD at concentrations above background would have on internal concentration. The model predicted that ingestion of 100 picograms (pg) of TCDD twice a week for 3 weeks would increase the internal (blood) concentrations by less than 1% relative to the background TCDD exposure in Europe.

In a study in Japan, Maruyama et al. (2002a) determined tissue–blood partition coefficients for use in a PBPK model for humans, measuring the concentrations of 17 PCDD and PCDF congeners in various tissues (liver, kidney, gut, viscera, skin, muscle, fat, bile) taken at autopsy from 8 men and women who had not been accidentally exposed to dioxins. The partition coefficients were used in a model previously described by Lawrence and Gobas (1997), and the model was used to estimate the concentrations in two other sets of people for whom PCDD data were available. There was good agreement between the concentrations estimated by the model and the actual concentrations measured in the tissues. The estimated concentration in adipose tissue of the samples from Japan was 4.1 pg/g lipid, which falls between the concentrations reported earlier for a group of Massachusetts Vietnam veterans (6.9 pg/g) (Schecter et al., 1990) and those reported in a population in Munich, Germany (2.6 pg/g) (Thoma et al., 1990).

Maruyama et al. (2002b) explored individual variation in human TCDD concentration using the PBPK model. As discussed in previous *Updates*, individual variation arises from differences in biologic variables, such as body weight, sex, and BMI, as well as from differences in metabolism rates and exposure. TCDD concentrations in Japanese adults vary greatly, ranging from 0.0025 to 0.025 pg/g lipid in blood, and from 0.036 to 1.11 pg/g lipid in liver. From their analysis, Maruyama et al. (2002b) concluded that differences in concentration of TCDD in the diet affect TCDD accumulation more than do any of the possible differences in half-life of the chemical. The levels of various PCDDs in multiple organs of the Japanese individuals analyzed in the study by Maruyama et al. (2002a) also showed that the concentrations of some congeners were greater in liver than in adipose tissue, which could be an indication of sequestration by CYP1A2.

Since *Update 2002*, new studies have reported on TCDD and other PHAHs in a limited number of human tissues, focusing on blood. Several reported on TCDD, PHAH, and TEQ in human milk as well.

Petreas et al. (2004) examined the differences between concentrations of persistent lipophilic chemicals, including PCDDs, in abdominal adipose tissue and in breast tissue that came from surgical specimens. The objective was to determine whether concentrations in one kind of tissue could be used to predict concentrations in the other. The study provided further information on individual variation in the content of the compounds. Adipose tissue is generally thought to be suitable for estimating the steady state of highly lipophilic contaminants. The average concentration of TCDD alone in abdominal–breast pairs was 3.9 pg/g lipid, with a range of 0.3–9.5 pg/g. In general, the concentrations in one tissue were highly correlated with those in the other. In this study, the correlation was weakest for TCDD, and there was a suggestion of preferential deposition of TCDD in abdominal adipose rather than in breast tissue, although in other studies TCDD is often evenly distributed in different fat compartments (IOM, 2003).

Kim et al. (2001) examined current blood concentrations in Korean veterans of the Vietnam conflict for exposure assessment related to health outcomes. Blood concentrations were measured in pooled samples from each of 4 groups defined on the basis of service in Vietnam and from a control group of veterans who did not serve in Vietnam. TCDD analyses were performed at the US Centers for Disease Control and Prevention. Concentrations averaged 0.3 pg/g serum lipid in the non-Vietnam group and 0.63–0.84 pg/g in the 4 exposed groups. The authors suggest a trend related to conditions of service in Vietnam, but they acknowledge that it is nearly impossible to use those numbers to determine what exposure had occurred 25 years earlier. The TCDD concentrations in the presumably exposed groups were all substantially lower than were concentrations in control groups from the Ranch Hand or other studies.

Concentrations of contaminants in workers at a hazardous-waste incinerator, a source of dioxins, were studied by Agramunt et al. (2003) to evaluate the worker exposure to metals and organic chemicals that emanate from the incinerator, which began operation in 1999. The study compared incinerator operators (4 composite samples), laboratory workers (1 composite), and administration workers (1 composite). Total PCDDs and PCDFs did not differ for the groups. Moreover, TCDD concentrations declined from an average of 4.3 pg/g blood lipid in 1999 to an average of 0.9 pg/g in 2003. The total PCDD and PCDF concentrations followed a similar pattern.

Dwernychuk and colleagues (2002) examined concentrations of TCDD in environmental matrices and in Vietnamese residents from 4 sites in Vietnam that had been exposed at different rates during the Vietnam conflict. The TCDD concentrations in soil, fish fat, and duck fat were highest in a village in the Aluoi Valley on the site of a former US base that, among the sites considered in this study, had been used the longest by US special forces. Samples of blood and milk were obtained from village residents in 1999. Blood samples from each site were pooled by sex and age (younger or older than 25 years). Values in the 4 pooled-blood samples from the most contaminated location were 31 and 41 pg/g lipid in

males and 14 and 16 pg/g in females. Concentrations were lower in samples from the other sites and were below the limits of detection in those from the least-contaminated site. Milk samples from 4 women in each location had average values that fell in the same ranking, although the individual variation was as high as 4-fold within a group. Total TEQ followed the pattern, though TCDD values differed more between sites than did the TEQ. The results suggest that contamination that occurred decades earlier still contributes to elevated exposure of the populations in those areas, presumably via contamination of fish and fowl that are used for food.

In another study in Southeast Asia, Schecter et al. (2003) examined chlorinated dioxin, dibenzofuran, and biphenyl concentrations in blood and milk from residents of Laos. TCDD in blood was detectable in 3 of 6 individuals and in 1 of 4 pooled samples. Detected values ranged from 1.2 to 4 pg/g blood lipid. Those values are similar to the concentrations detected in blood from residents of unsprayed areas of Northern Vietnam, measured at 1.2–2.3 pg/g blood lipid. The report also notes that the samples from Laos are comparable to those in 2 pooled-blood samples from Dallas, Texas (1 and 3.8 pg/g lipid), and to the mean blood concentrations of TCDD from 13 persons in Munich (2.4 pg/g lipid). TCDD in milk from 3 of the Laotian women ranged from 0.06 to 0.35 pg/g, as compared with the average of 1.6 pg/g in milk from 69 individual samples taken in Germany.

Eskenazi et al. (2004) recently analyzed archived samples from individuals from Seveso, Italy, collected soon after the accident. The analyses confirmed that the highest concentrations of TCDD were in people residing in the most highly contaminated areas, but although the average values for blood levels of TCDD in zone A residents are higher than in zone B, the levels in some zone B individuals were higher than those in some individuals from zone A. Age at first exposure was the other strong predictor of TCDD serum concentration. It is noteworthy that the actual concentrations of TCDD measured in the immediate postexposure samples by Eskenazi et al. (2004) were similar to the estimates that Landi et al. (1998) had obtained by extrapolating back from levels measured in blood samples collected in 1996, 20 years after the accident. Eskenazi et al. (2004) also reported greater than anticipated total TEQ in blood samples archived in 1976 from women from zone non-ABR (unexposed individuals) and found that substantial TEQ (about 80% of the total TEQ) was due to compounds other than TCDD. It is likely that compounds other than TCDD contributed to the overall TEQ in women from exposed areas also. These findings are consistent with the results from more contemporary Seveso samples reported by Weiss et al. (2003) and discussed below. This suggests that inclusion of compounds additional to TCDD contributing to TEQ could be important in health assessments and might in fact confound the determination of the effects of TCDD alone.

Weiss et al. (2003) reported on measurement of dioxin, dibenzofuran, and polychlorinated biphenyl (PCB) congeners in milk samples from residents near

Seveso (zones R and B), as compared with milk from women in Milan, Italy, and from a rural area that was unaffected by the Seveso accident. Samples were collected 25 years after the heaviest exposure at Seveso. Samples collected from 12 women at each site in the first week postpartum and 3 months after delivery were pooled separately. TCDD concentrations were elevated in the samples from Seveso—4.45 pg/g lipid in the first-week sample from Seveso as compared with 1.63 and 1.58 pg/g in the other locations. However, TEQ from PCDD, PCDF, and PCB congeners were similar in all areas.

Elevated TCDD concentrations in breast milk have been observed by numerous investigators over the years. The occurrence of TCDD in breast milk is important because it indicates a flux of chemical through the organ, which could have implications for effects in breast tissue. Breast milk also is the dominant source of TCDD contamination in infants. Analyses show that breast-fed infants accumulate substantially more TCDD than do bottle-fed infants. Several variables influence the concentration of TCDD and other contaminants in breast milk, including the time from delivery that milk is obtained, the number of children who have been nursed, and the variation in milk composition (for example see NRC, 1999b). Lorber and Phillips (2002) note the importance of including such information in pharmacokinetic models for estimating infant exposures. They suggest that models enhanced by adding information on metabolism of specific congeners as a function of age and of the initial exposure could estimate the persistence of elevated concentrations as children grow.

Analysis of dioxins in tissues of infants who died suddenly in Westphalia, Germany (Bajanowski et al., 2002) gave results consistent with several of the observations mentioned above. The cases were divided into two groups: one of 15 children who died in 1991–1992; a second of 12 children who died in 1996–1997. The results showed similar TCDD concentrations in adipose and liver tissue, although several PCDD and PCDF congeners were more concentrated in liver, which would reflect binding by CYP1A2, as in the Japanese study by Maruyama et al. (2002a). There was a consistent difference between breast-fed and non-breast-fed infants. TCDD concentrations in adipose tissue were 2.5 and 0.6 pg/g lipid, respectively, in breast-fed and bottle-fed infants. The data also showed that duration of breast-feeding was directly proportional to dioxin concentration. Total TEQ was 22 pg/g fat in the 1991–1992 group and about 6 pg/g in the tissue from the child who died in 1996–1997. This is attributed to a general decrease of about 50% from 1989 to 1998 in background levels of PCDDs and PCDFs in human milk.

The trend of decreasing levels is supported by studies of Aylward and Hays (2002), who examined human TCDD body burdens in studies over nearly 30 years in the United States, Canada, Germany, and France. The analysis identified temporal trends in human blood levels, with a nearly 10-fold decrease in levels from the early 1972 to 2000, from nearly 20 pg/g blood lipid to just over 2 pg/g blood lipid. Using half-life estimates that are average for humans (about 7.5 years)

and first-order elimination kinetics, they conclude that the decreases must result from decreased intake levels in the diet. The study's best estimate of daily TCDD absorption was 0.04 pg TCDD/kg. The authors pointed out that, despite limitations, the conclusion of a reduction in intake over this time is strong.

The dose of a compound such as TCDD to an infant through milk could constitute an important proportion of lifetime exposure. Aylward et al. (2003) reviewed several studies to examine the extent to which concentrations in milk corresponded to blood concentrations in the same people. The objective was to determine whether blood concentrations might be used to estimate general exposure for infants. Their analyses of the somewhat limited data suggest that the concentrations of TCDD and other compounds human milk generally reflect those in blood.

Metabolism and Half-Life Studies

It is generally agreed that the toxicity of TCDD is related in part to the fact that it is so persistent in the body. But recent estimates of TCDD half-life in humans have been reported to range much more widely than once thought. The half-life of TCDD in humans varies with BMI, age, sex, and, most substantially, with initial exposure. Miniero et al. (2001) reviewed data on the half-life of TCDD and how it was correlated with body weight. Michalek and Tripathi (1999) observed that the elimination rate of TCDD in Ranch Hand personnel was related to body fat. Although the major determinants of TCDD half-life are thought to be lipophilicity, metabolism, and sequestration in the liver, the half-life seems to be correlated empirically with body weight in mammals. However, elimination also appears to occur biphasically, with initial exposure determining the initial rate of elimination. Some studies addressed in *Update 2002* are mentioned here, given their significance to determination of half-lives of TCDD.

As reviewed in *Update 2002*, the elimination of TCDD from highly exposed individuals was examined by Geusau and coworkers (2002), who studied 2 patients who had extremely high concentrations of TCDD (144,000 pg/g blood fat in one and 26,000 pg/g blood fat in the other). Overall TCDD half-lives of 1.5 and 2.9 years (Table 3-1) were reported for the more and less severely contaminated individuals, respectively. Those values are considerably shorter than are the commonly reported values, which range from 6.9 to 9.8 years (Table 3-1). The implication that the rate of elimination is greater for more highly contaminated persons was supported by the analysis of TCDD toxicokinetics in adults from Seveso (Michalek et al., 2002). Serum samples obtained within days after exposure, which provided a measure of the initial dose accumulated by the individuals, gave a mean half-life of only 0.34 years in the Seveso males over the first 3 months after exposure. The mean half-life during the period from 3 years to just over 16 years after exposure was 6.9 years. By comparison, the mean half-life in Ranch Hand personnel, at 9–33 years after exposure, was 7.5 years—only slightly

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

Reference	Half-life[a]	Confidence Interval	Comment
Human Studies			
Aylward et al., 2004	<3 years		Calculated for exposures >10, 000 pg/g serum lipid
	>10 years		Calculated for exposures <50 pg/g serum lipid
Flesch-Janys et al., 1996	7.2 years		Adult males, Boehringer cohort
Geusau et al., 2002	1.5 years[b]		Adult female, severe exposure 0–3 years PE
	2.9 years[b]		Adult female, severe exposure 0–3 years PE
Michalek et al., 2002	0.34 years[b]		Adult males, Seveso cohort, 0–3 months PE
	6.9 years		Adult males, Seveso cohort, 3–16 years PE
	9.8 years		Adult females, Seveso cohort, 3–16 years PE
	7.5 years		Adult males, Ranch Hands 9–33 years PE
Needham et al., 1994	7.8 years	7.2–9.7 years	Adults, Seveso cohort
Pirkle et al., 1989	7.1 years	5.8–9.6 years	Adult males, Ranch Hands 9–23 years PE
Animal Studies			
Neubert et al., 1990	73.7 days	60.9–93.8 days	Monkeys, Marmoset, single injection
DeVito and Birnbaum, 1995	15 days		Mice, female B6C3F1
Gasiewicz et al., 1983	11.0 days[c]		Mice, C5BL/6J
	24.4 days[c]		Mice, DBA/2J
	12.6 days[c]		Mice, B6D2F1/J
Koshakji et al., 1984	20 days		Mice, male ICR/Ha Swiss
Hurst et al., 1998	8 days		Rats, Long-Evans, excretion from liver
Pohjanvirta et al., 1990	21.9 days		Rats, male Han/Wistar resistant strain
Viluksela et al., 1996	20.2 days		Rats, Long-Evans TurkuAB strain
	28.9 days[d]		Rats, Long-Evans Charles River strain
Weber et al., 1993	16.3 ± 3.0 days		Rats, male Sprague-Dawley

[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] Total cumulative excretion of ^{3}H-TCDD-derived radioactivity.
[d] Attributed to differences in dilution due to different growth rates.
ABBREVIATION: PE, postexposure.

different from the rate in Seveso males. The pattern of elimination in the Seveso cohort is consistent with a two-compartment model that should be considered in toxicokinetic models of TCDD accumulation and in attempts to extrapolate original exposures in other contaminated populations.

Aylward et al. (2004) reexamined the toxicokinetic modeling to estimate exposure history of adults from several groups (Seveso, the National Institute for Occupational Safety and Health [NIOSH] cohort, and the highly exposed Austrian women). They optimized the fit of the model to the data by varying the elimination rates, based on evidence that there are concentration-dependent elimination rates. The modeling indicates substantial variability in elimination rates between individuals, as well as confirming a faster elimination in males than females and in younger than older individuals. The half-lives for TCDD in males, resulting from the data and models, range from less than 3 years at serum levels greater than 10,000 pg/g lipid to over 10 years at serum lipid levels less than 50 pg/g lipid. Employing varying elimination rates may provide more reliable ranges for estimating exposure concentrations by back-extrapolation for individuals.

Once accumulated in a person, internal exposure to a compound such as TCDD, which has a half-life of years, would continue over time, as it is slowly eliminated from the body. Time-dependent cumulative dose estimates or "area under the curve" dose estimates are based on the concentrations measured in blood and the time over which those concentrations were present in the individual. In a study of the NIOSH cohort, Salvan et al. (2001) used a PBPK model to calculate cumulative dose as related to all cancer risk. In the case of the Ranch Hands, it was suggested that the elimination rate (the area under the curve) should be tested for a relationship to the incidence of diabetes in the Ranch Hand cohort (Michalek et al., 2003). Differences in elimination rates have been observed among Ranch Hands, and those variations would relate to differences in cumulative dose; that is, to an accurate calculation of the area under the curve for total internal exposure. The analysis did not show a relationship between elimination rate and diabetes. In their analysis of elimination rates, Michalek et al. (2003) also noted that the data on elimination rates based on blood samples collected years after the exposure could not be extended to earlier periods.

The issue of cumulative internal dose over time also concerns potential effects of background exposure. In many parts of the world, background exposure to TCDD and related chemicals that contribute to total TEQ continues. If the area under the curve were to be used as the exposure metric related to outcomes, then background exposure would be important, either alone or as added to an accidental exposure. There is no consensus about whether the area under the curve over time, the actual tissue concentration at any given time, or some other expression is the most valid dose metric for any particular health outcome. For cancer, however, it is quite clear that total area under the curve, as was attempted by Salvan et al. (2001), is not optimal.

It is generally thought that the elimination rate of TCDD is related to the rate of metabolism of the compound. Thus, the shorter half-life in rodents than in humans could be related to a lower percent body fat, but could also involve more rapid metabolism of TCDD in rodents. However, there are few measurements of the TCDD metabolism rate by human enzymes. Inouye et al. (2002) examined 12 human cytochrome P450 forms (expressed in yeast) for their ability to catalyze the metabolism of PCDDs. The expressed CYP1A1 showed the highest rates of activity with a series of mono-, di-, and trichlorinated dibenzo[p]dioxins. However, the rates of TCDD metabolism were below the limits of detection in this study. There are virtually no measurements of TCDD metabolism in vivo. Recent studies have addressed how the structure of rat cytochrome P450 1A1 is related to the mechanism and rate of dioxin metabolism by that enzyme. Shinkyo et al. (2003) used structural models of rat CYP1A1 to suggest parts of the enzyme molecule that might govern the rate at which the enzyme metabolizes TCDD. Subsequently, they used genetic engineering to modify the rat enzyme and reported that altering the structure in accord with the model results did increase the rate of TCDD metabolism by the enzyme.

In humans, there are individual differences in the rate of TCDD elimination. It is not known whether there are human CYP1A variants that oxidize TCDD at different rates or whether that could influence the disposition or elimination of TCDD. It should be noted, however, that the rates of TCDD metabolism in humans still are slow enough that the influence on accumulation patterns could be minimal. In any case, the major determinants of the half-life of TCDD in all mammals examined, including humans, are the percent body fat at background exposures and hepatic sequestration at high doses.

Summary

Studies that model the disposition and effects of TCDD in rodents continue to be refined and to support the development and use of PBPK models to estimate congener-specific concentrations in human tissues. It will be important to continue to refine PBPK models for evaluating tissue distribution in humans.

The information on TCDD toxicokinetics is expanding, and new studies tend to support the conclusions of earlier work. PBPK models could predict distribution and elimination rates, but allometric models also appear to explain elimination rates, including differences between individuals. The data show that BMI and body fat content are important determinants of TCDD half-life, particularly at low exposure. It is worth emphasizing again that persons who accumulate high concentrations of TCDD show an initial phase of elimination that is rapid, with half-lives that are much shorter than average. The mechanism underlying the rapid phase of elimination is not known. Regardless, biphasic elimination continues to confound back-extrapolation to initial exposure for persons who might have experienced high exposures years before blood or other tissue samples were

obtained for analysis. Efforts to enhance the rate of absorption of TCDD from the diet and to enhance elimination by inclusion of fiber or other dietary supplements continue to show promise. Back-extrapolation is still an important issue to be addressed. Accurately assessing risk of TCDD or other PHAH exposure above background could require information about the amounts accumulated at the times of greatest exposure. To make such estimates usually requires researchers to work backwards from current blood concentrations in exposed subjects. Although differences in dioxin concentrations in blood serum obtained years or decades after external exposure could relate to differences in that exposure, individual differences in elimination rates could be substantial.

Toxic Endpoints and Underlying Mechanisms of Toxic Action

Studies published since *Update 2002* are consistent with the hypothesis that TCDD produces its biologic and toxic effects by binding to a gene regulatory protein, the AhR, which can modulate gene expression through several mechanisms. Research indicates that the binding of TCDD to the AhR, the dimerization of the AhR with a nuclear protein (AhR nuclear transport protein, or Arnt), and the interaction of that complex with specific DNA sequences (often called Ah-responsive elements, AhREs; dioxin-responsive elements, DREs; or xenobiotic-responsive elements, XREs) present in the 5′-promoter regions of particular 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 compounds that bind to the AhR, the genetics of mutant genes that express the AhR, AhR-deficient mice, and the molecular events that contribute to and regulate AhR expression and its activity. Many studies published since *Update 2002* are consistent with this mechanism of AhR action. However, newer studies also indicate that the TCDD-bound AhR modulates genes and cellular signaling pathways by mechanisms that do not involve direct binding to DNA but that occur by its interaction with other cellular proteins. The exact relationships among the mechanisms, the modulated expression of known genes, and the diversity of toxic effects elicited by TCDD in humans and numerous animal species 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 that lead to a particular toxic event are complex. Much of the data are consistent with the notion that the cellular processes that involve growth, maturation, and differentiation are most sensitive to TCDD-induced modulation as mediated by the AhR. The findings in animals indicate that the reproductive, developmental, and oncogenic endpoints are sensitive to TCDD. The data support the biologic plausibility of similar endpoints in exposed humans. Many of

the responses, however, are tissue and species specific. As such, the appearance of some toxic endpoint in one or even several animal species exposed to TCDD does not necessarily indicate the same endpoint will occur in exposed humans, or vice versa. The exact mechanisms responsible for the differences are not known.

The conclusions indicated above are similar to those in *Update 2002*. Since that update, many cellular and molecular interactions of the AhR have been reported. However, in many cases, it is not clear how they might be related to a particular toxic endpoint. Therefore, although the text below cites related work published since *Update 2002* that was identified by the committee, closer attention is given only to studies that add substantial new information, particularly as it might be relevant to the exposure of Vietnam veterans. As discussed in *Update 2002*, 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

AhR Gene and Protein Published data cited in previous updates are consistent with the conservation of AhR structure and function among species. Nevertheless, specific differences in amino acids or amino acid sequences within domains of the AhR protein are associated with altered AhR function and with responsiveness to TCDD and other ligands. Some differences might account for species–species differences in sensitivity to TCDD. Hahn et al. (2004) identified 25 single nucleotide polymorphisms in the *AhR* gene of a population of Atlantic killifish that has evolved genetic resistance to TCDD and related chemicals. However, none of the encoded proteins from the identified alleles differed functionally when expressed in cultured cells. Backlund and Ingelman-Sundberg (2004) reported that a tyrosine at the 320 position of the rat AhR is critical in its responsiveness to several AhR ligands, but not to TCDD. Tyrosine 9 in the mouse AhR was found to be essential for recognition of DREs (Minsavage et al., 2003).

Bunger et al. (2003) reported that mice carrying a mutation in the nuclear localization sequence of the AhR were resistant to TCDD-induced hepatomegaly, thymic involution, and cleft palate formation. The data suggest that most, if not all, TCDD-induced toxicity requires nuclear localization of the TCDD-bound AhR. Two publications by Simanainen et al. (2003, 2004a) reported that genetic differences in the C-terminal transactivation domain of the rat AhR modify some, but not all, of the TCDD-induced toxic responses in these animals. The authors postulate that there are at least two different AhR-mediated signaling pathways that lead to different toxic endpoints. Furthermore, the related resistance of the rat strains carrying the genetic variances in the AhR develops differently as they age (Simanainen et al., 2004b). The concepts are consistent with the tissue-specific sensitivity to TCDD observed in vertebrate species. Two forms of the AhR, AhR1 and AhR2, are expressed in the zebrafish. Consistent with the finding that

the AhR2 but not the AhR1 binds TCDD, Prasch et al. (2003a) reported that the AhR2 mediates the developmental toxicity of TCDD in zebrafish.

The amount of the AhR protein could influence a tissue's relative responsiveness to TCDD. Several publications have reported on factors that control the expression and stability of AhR protein in cells. Racky et al. (2004) identified 7 polymorphisms of the human *AhR* promoter region, although they did not appear to alter *AhR* gene expression in lymphocytes. However, the analysis also demonstrated the involvement of Sp1 protein in *AhR* gene regulation. Exposure of cells and animals to TCDD has been shown to stimulate pathways that mediate the degradation of the AhR protein, leading to a subsequent decrease in AhR-mediated gene alterations. Additional data have improved our understanding of those pathways (Ma and Baldwin, 2002; Pollenz, 2002; Santiago-Josefat and Fernandez-Salguero, 2003; Song and Pollenz, 2003; Wentworth et al., 2004). Notably, TCDD plasma concentrations in people exposed 20 years earlier in Seveso were associated with a reduction in AhR expression in lymphocytes. Plasma TCDD concentrations showed a negative association with induced cytochrome P450 (CYP)1A1-mediated enzyme activity after exposure of lymphocytes from those persons to TCDD in vitro (Baccarelli et al., 2004; Landi et al., 2003). Those studies might suggest that long-term exposure of humans to TCDD perturbs *AhR* gene regulation. Song and Pollenz (2002) reported that treatment of cells with geldanamycin, which binds to 90 kilodalton heat shock protein (hsp90) and disrupts the conformation of the hsp90–AhR complex, resulted in a substantial degradation of the AhR and to decreased cellular responsiveness to TCDD. Spink et al. (2003) reported that a continued presence of estrogen was required to maintain high concentrations and activity of AhR protein in MCF-7 breast cancer cells. Although hypophysectomy significantly lowered AhR expression in rat liver, no change in induction of CYP1A1 by AhR ligands was observed (Timsit et al., 2002). Hestermann et al. (2002) reported that serum withdrawal from cultured teleost hepatoma cells led to decreased AhR expression and to lowered inducibility of CYP1A1. *AhR* gene silencing using small inhibitory RNA decreased the responsiveness of cells to TCDD (Abdelrahim et al., 2003).

One of the many difficulties of extrapolating from experimental data in animals is the fact that, although AhRs in humans and animals are homologous with a high percentage of identity, there may be functional differences. At least one form of human AhR binds and responds differently to TCDD and related xenobiotics than animal counterparts. Moriguchi et al. (2003) generated a mouse possessing the human AhR (hAhR) in place of the mouse AhR. Mice homozygous for the hAhR gene exhibited weaker induction of target genes such as *cyp1a1* and *cyp1a2* than did wild-type mice. After maternal exposure to TCDD, mouse fetuses homozygous for *hAhR* developed hydronephrosis but not cleft palate; wild-type mice developed both. Although some of those differences might reflect the previous findings indicating that the human AhR has lower affinity for TCDD than does the AhR in other species (IOM, 2003), it is apparent from the investigations

of Moriguchi et al. (2003) that the relative sensitivity could depend not only on the type of AhR present, but also on the dose and the endpoint examined. This could also vary for different human AhR allele products.

Interaction of the AhR with Other Proteins As indicated in *Update 2002*, 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 Arnt (Chapman-Smith et al., 2004; Korkalainen et al., 2003), a protein called X-associated protein 2 (XAP2; also called ARA9 and AhR-interacting protein, or AIP) (Berg and Pongratz, 2002; Lees and Whitelaw, 2002; Lees et al., 2003; Petrulis et al., 2003; Ramadoss et al., 2004), hsp90 (Cox and Miller, 2003, 2004), p23 (Cox and Miller, 2004; Shetty et al., 2003), the export protein CRM-1 (Berg and Pongratz, 2002), and the nuclear import protein importin (Petrulis et al., 2003). Those proteins function in stabilizing the AhR in cells and in regulating the intracellular localization of the AhR. Ikuta et al. (2004a) observed that changes in cell density regulate the intracellular localization and transcriptional activity of the AhR. This could be related to altered interaction of the AhR with the above proteins, as mediated by the phosphorylation of the AhR at particular amino acid residues (Ikuta et al., 2004a,b).

Ramadoss et al. (2004) noted that the hAhR is biochemically different from the mouse AhR in its ability to interact with factors that regulate cytoplasmic–nuclear localization. Interactions of the AhR with TRAP/DRIP/ARC/Mediator protein complex (Wang S et al., 2004), transcription elongation factor (Tian et al., 2003), histone deacetylase (Wei et al., 2004), and ubiquitin-like protein Nedd8 (Antenos et al., 2002) also modulate, through a variety of mechanisms, the ability of the AhR to regulate genes.

Updates 2000 and *2002* 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 are consistent with previous information. Tsuchiya et al. (2003a) used a variety of human cell lines to show that several polycyclic aromatic hydrocarbons, including TCDD, induced the expression of the AhRR gene, but in a compound- and cell-specific manner. Yamamoto et al. (2004) observed that in human tissues the expression of AhRR was extremely high in testis. It was very high in lung, ovary, spleen, and pancreas from adults but low in the same tissues from fetuses. They also observed variable expression of AhRR in mononuclear cells from various sources. The authors suggest that this might account, at least in part, for differences in sensitivity of human tissues to TCDD and related compounds. Korkalainen et al. (2004), however, determined that the relative expression and inducibility of AhRR in several rat strains did not account for strain differences in sensitivity to TCDD.

Several investigations demonstrated AhR-mediated alteration of the estrogen receptor (ER) signaling pathway, and vice versa, that could occur through a variety of mechanisms, including inhibition of ER protein expression, enhanced

metabolism of estrogens, induction of inhibitory factors, binding of the AhR to inhibitory DREs, competition for common nuclear coregulatory proteins, and proteosome-dependent degradation of the AhR and ER (Reen et al., 2002; Safe and Wormke, 2003). Ohtake et al. (2003) reported that the AhR–Arnt complex can associate directly with ER-α and ER-β, which could lead to the recruitment of the ERs to estrogen-responsive promoters and to estrogenic effects. Those data are particularly relevant because TCDD exposure in animals is known to affect estrogen-dependent responses in several tissues, especially those involved in reproduction.

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 show that the AhR can mediate the toxicity of those substances; newer information supports that conclusion. Since *Update 2002,* several new assays and reagents have been developed to detect AhR ligands and to determine how they modulate AhR function (Behnisch et al., 2002; Fukuda et al., 2004; Han et al., 2004; Ramadoss and Perdew, 2004; Sun et al., 2004; Swanson et al., 2002). Several compounds alter AhR function by binding directly to the AhR. The indole derivatives indirubin and indigo (Adachi et al., 2004; Guengerich et al., 2004; Knockaert et al., 2004; Sugihara et al., 2004), several pesticides (Long et al., 2003), and some dietary flavonoids (Gouedard et al., 2004; Zhang et al., 2003) appear to act as AhR agonists. Other compounds, such as other naturally occurring flavonoids (Zhang et al., 2003); synthetic flavonoids (Palermo et al., 2003; Roblin et al., 2004; Zhou and Gasiewicz, 2003); the drug salicylamide (MacDonald et al., 2004); and omeprazole, 2-mercapto-5-methoxybenzimidazole, and promaquine (Backlund and Ingelman-Sundberg, 2004) also bind to the AhR, but they act as AhR antagonists. Notably, several compounds used for their ability to inhibit specific kinases also act either as AhR agonists (Andrieux et al., 2004) or as antagonists (Joiakim et al., 2003; Shibazaki et al., 2004). Their ability to produce agonist or antagonist activity appears to depend on their relative affinity for the AhR and on properties related to their intrinsic efficacy, that is, on their ability to produce a response once bound to the AhR. The latter could be related to ligand structure (Beger et al., 2002) and to the relative ability of a chemical to elicit a particular conformational change in AhR structure (Henry and Gasiewicz, 2003). Phenylthiourea is a weak activator of the AhR, but it can inhibit TCDD-induced responses under some conditions (Wang W-D et al., 2004). The relative agonist or antagonist activity of a compound also is species and gene specific (Gouedard et al., 2004; Zhou and Gasiewicz, 2003; Zhou et al., 2003).

Recent reports indicate the presence of AhR ligands in airborne particulate organic matter (Arrieta et al., 2003), in fish exposed to effluents from a bleached-pulp and paper mill (Hewitt et al., 2003), and in plant food extracts (Amakura et al., 2004).

Other chemicals, such as *o,p′*-DDT (Jeong and Kim, 2002), estradiol, and

dexamethasone (Lai et al., 2004), appear to alter TCDD- and AhR-dependent gene transcription by mechanisms that are not related to their ability to bind the AhR. Pearce et al. (2004) reported that 6-methyl-1,3,8-trichlorodibenzofuran, a weak AhR agonist and partial antagonist, also is a partial ER agonist.

It is postulated that TCDD is toxic by mimicking an endogenous ligand for the AhR and activating the receptor at inappropriate times or for inappropriately long periods. The actual physiologic ligand for the AhR, if any, is not known. Roblin et al. (2004) determined that a variant rat hepatoma cell line contains some agent that acts as an AhR agonist. Song et al. (2002) identified a compound, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester, from porcine lung that binds to the AhR from several vertebrates, including human, and activates it to a transcriptionally active form. It is not clear whether this is truly a physiologically relevant ligand for the AhR. Previous investigations (IOM, 2001) noted that metabolites of tryptophan can act as AhR agonists. A study by Bittenger et al. (2003) reported that aspartate aminotransferase could convert tryptophan into indole-3-pyruvate, which then spontaneously reacts in aqueous solution to form several compounds that act as AhR agonists.

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 and altered intracellular signaling pathways. Several genes that are modulated by TCDD and by dioxin-like compounds in a variety of biologic systems, including human cells, are listed in Table 3-2, which includes citations to papers published since *Update 2002*. Genes whose mRNA or protein concentrations have been altered are included, but enzymes or proteins whose biologic activities are altered by some other mechanism are not. Several genes are regulated by direct interaction of the AhR–Arnt complex with DREs in the promoter regions. Other genes are suspected, but not yet proven to be induced by this mechanism. The expression of several genes is inhibited by the ability of the AhR to bind to DREs near the DNA-binding sites for other transcription factors, such as the ER. The expression of other genes appears to be altered by posttranscriptional mechanisms. Table 3-2 also lists genes that are modulated in a variety of cells and tissues in several species, including humans, although the mechanisms of alteration are not understood. It is likely that the induction or repression of many of those genes is 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 lead to cell and tissue alterations. That they are tissue-, species-, and developmental-stage-specific events also emphasizes the complex nature of the biochemical events that lead to particular toxic response (Wilson, 2004; Wood et al., 2002).

Since *Update 2002*, several investigations have examined the molecular mechanisms underlying the specificity of the responses elicited by TCDD and

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	
Thomsen et al., 2004	hairy and enhancer of Split homolog-1 (HES-1)
Son and Rozman, 2002	plasminogen activator inhibitor-1
Baba et al., 2001	aryl hydrocarbon receptor repressor (AhRR)
Porter et al., 2001	heat shock protein 27 (inhibition)
Jeon and Esser, 2000	interleukin-2
Gao et al., 1998	ecto-ATPase
Gillesby et al., 1997	pS2 (inhibition)
Kraemer et al., 1996	cyclooxygenase-2
Krishnan et al., 1995	cathepsin D (inhibition); Sp1 (inhibition)
Gaido and Maness, 1994	plasminogen activator inhibitor-2
Lamb et al., 1994	UDP glucuronosyltransferase1
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
Tukey and Nebert, 1984	CYP1A2
Poland and Knutson, 1982	CYP1A1
Genes Suspected to Be Directly Regulated by the AhR	
Gouedard et al., 2004	paraoxonase
Santiago-Josephat et al., 2004	TGF-β binding protein 1 (inhibition)
Niermann et al., 2003	T cadherin (inhibition)
Huang et al., 2002	proopiomelanocortin (ACTH precursor)
Ma, 2002	poly(ADP-ribose) polymerase
Matikainen et al., 2002	Bax
Park and Rho, 2002	Cu–Zn superoxide dismutase
Rivera et al., 2002	CYP2S1
Zhao et al., 2002	RANTES
Ogi et al., 2001	polκ
Ohbayashi et al., 2001	DIF-3
Sugawara et al., 2001	steroidogenic acute regulatory protein
Kim et al., 2000	c-myc
Lai et al., 1996	transforming growth factor-β (TGF-β); interleukin-6; interferon-γ
Masten and Shiverick, 1995	BSAP
Genes and Proteins Modulated by Posttranscriptional Mechanisms	
Henley et al., 2004a	interleukin-1β
Dong et al., 1997	MHC Q1
Gaido et al., 1992	TGF-α; urokinase plasminogen activator
Puga et al., 1992	c-fos; c-jun

continued

TABLE 3-2 *Continued*

Reference	Genes
Other Genes Reported, Since Update 2002, to Be Altered by AhR Ligand Exposure	
Adachi et al., 2004	ADP ribosylation factor 4; basic transcription factor 2 34-kDa subunit; cadherin 2; CDC-like kinase; complement component 5; cyclin-dependent kinase inhibitor 1A; cyclin-dependent kinse 1; CYP19A1; DNA mismatch repair protein; early growth response protein; 110-kDa heat-shock protein; heat shock factor-binding protein 1; heat shock protein 60-kDa protein; insulin-like growth factor-binding protein 10; insulin-like growth factor binding protein 1; insulin-like growth factor II; integrin β; interleukin 1 receptor type 1; 45-kDa interleukin enhancer-binding factor 2; NEDD5 protein homolog; Niemann-Pick C disease protein; retinoblastoma-binding protein 3; Rab geranylgeranyl transferase β subunit; RNA polymerase II elongation factor SIII p15 subunit; Sec61-γ; sex-determining region Y box-containing gene 9; short/branched chain-specific acyl-CoA dehydrogenase; solute carrier family 2 member 2; T-complex protein 1 τ and δ subunits; thyroid receptor-interacting protein 15; topoisomerase I and II α; transcription factor HTF4; translation initiation factor 4E 25-kDa subunit
Fisher et al., 2004	Bcl-2 family genes bik, bid, Hrk, bok/mtd, mcl-1, bcl-x, and bcl-w; IAP family genes X-linked IAP, NAIP1, and NAIP5; Myd88; p21; p53; RIP; TNFR family genes OX40, Fas, CD30, Ltβ-R, and TNFR1; TNF family genes LIGHT, OX40L, and Bar-like; TRAF2
Johnson et al., 2004	actin α; Ahr; alcohol dehydrogenase 1, complex; angiopoietin-like 4; angiotensinogen; brain derived neurotrophic factor; cadherin 16; calbindin-28k; carbonic anhydrase 3; carboxylesterase 3; Cd44 antigen; coagulation factor II; cytokine receptor-like factor 1; epiregulin; fibroblast growth factor 7; fibroblast growth factor receptor 4; follistatin; forkhead box a2 and f2; Fos-like antigen 1; glutamyl aminopeptidase; Gro1 oncogene; high mobility group at-hook 2; α-2-hs-glycoprotein; hydroxysteroid 11-β dehydrogenase 2; insulin-like growth factor 2; insulin-like growth factor binding proteins 3, 5, and 6; integrin α 3, α 6, and β 4; IL-6; interferon activated gene 202a; lymphocyte antigen 6 complex, loci e, A and H; lysyl oxidase; matrix metalloproteinase 3 and 9; mitogen regulated protein proliferin 3; NADH dehydrogenase 1; osteopontin; p21; peripherin; phospholipase a2 group VII; proliferin 2; Ras-related protein; rennin 1 structural; retinol binding protein 4, plasma; RNA binding motif, single stranded interacting protein 1; secreted phosphoprotein 1; small proline-rich proteins 2b, 2c and 2f; spleen tyrosine kinase; squalene epoxidase; stratifin; thrombomodulin; TNF receptor family member 1b; tumor-associated calcium signal transducer 2

TABLE 3-2 *Continued*

Reference	Genes
Karyala et al., 2004	ADP-ribosylation-like factor 6 interacting protein 5; calcium binding protein A11; CCAAT/enhancer-binding protein; esterase 10; immediate early response 3; nicotinic acetylcholine receptor subunit α 6; nuclear factor erythroid derived 2, like 2; prenylated SNARE protein; RIKEN-CDNA FLJ13933 FIS, clone Y79AA1000782; RIKEN-phosphogluconate dehydrogenase inhibitor; S100 calcium-binding protein A4; vanin 1; Vomeronasal organ family 2, receptor, 11; distal-less homeobox 5
Moennikes et al., 2004 (constitutively active AhR)	DEAD/H box polypeptide 3; DnaJ (hsp40) homolog, subfamily B, member 1; fatty acid binding protein 2 (intestinal); heat shock 70 kDa protein 5; heat shock protein 1α, hsp90; heat shock protein 105; hepatic nuclear factor 4 (HNF4); HIV-tat interactive protein 2; homocysteine-inducible, ER stress-inducible, ubiquitin-like domain member 1, Herp; C-type lectin-like receptor 2; lectin (galactose binding, soluble 1); malic enzyme; mannoside acetylglucosaminyltransferase 2; phosphoribosyl pyrophosphate amidotransferase; pleckstrin homology domain containing (family B number 1); Ras homolog gene family member E; ribosomal protein L12; S-100 calcium binding protein A10 (calpactin); signal transducer and activator of transcription 2; solute carrier protein 21 (organic anion transporter, member 10); TNFα-induced adipose-related protein; ubiquitin-specific protease 2; vaccinia related kinase 2; zinc finger protein 191
Murphy et al., 2004	matrix metalloproteinase-1
Vogel et al., 2004	CCAAT/enhancer-binding protein
Hoegberg et al., 2003	lecithin:retinol acyltransferase
Son et al., 2003	CK8 polypeptide; glutathione peroxidase; Ig λ-1 chain C region; Ig λ-2 chain C region
Bhathena et al., 2002	CYP2C11
Bruno et al., 2002	albumin; ATP synthetase β subunit; calreticulin precursor; cytochrome B5; CYP2D4; 25DX; endoplasmic reticulum protein ERP29 precursor; ferritin light chain; 78 kDa glucose-regulated protein precursor; glutamate dehydrogenase; glyceraldehydes-3-phosphate dehydrogenase; heat shock protein 72; 3-α-hydroxysteroid dehydrogenase; IκB kinase 2; 150 kDa iodothyronine 5′ monodeiodinase; isocitrate dehydrogenase; oxygen-regulated protein; peroxiredoxin IV; prohibitin; protein disulfide isomerase ER60 precursor

continued

TABLE 3-2 *Continued*

Reference	Genes
Martinez et al., 2002	activin receptor type II B; acyl-coenzyme A oxidase; aminoaclase 1; B-cell lymphoma protein 3; basic transcription element binding protein 1; bone morphogenic protein; β-catenin; Cdc42; CDK-2 associated protein; cellular retinoic acid binding protein 1; collagen IV α 3 chain; collagen VI α 3; cyclin-dependent kinase 4 inhibitor C; cyclin-dependent kinase inhibitor 2B isoform; CYP27A1; discoidin receptor tyrosine kinase; E2F dimerization partner 2; early growth response 1; EGF-containing fibulin-like extracellular matrix protein; ephrin A1, isoform a; epidermal growth factor receptor substrate 15; epithelial-cadherin; fibroblast growth factor; fibronectin receptor β subunit; Fos-related protein; GABA A receptor; GATA binding protein 1; glucocorticoid receptor; GTPase activating protein; homospermidine synthase; hsp 70 kDa protein insulin-like growth factor 1 receptor; GABA A receptor ε subunit; 25 kDa GTP binding protein; l hsp 70 kDa 2; hyaluronidase 1; insulin induced protein 1; interferon-induced protein 56 and p78; interferon γ receptor 1; interferon regulatory factor 4; IL-6 receptor β; IL-8; Kruppel-like factor 5; lamanin B2 chain and α 3b chain; leukemia inhibitor factor; low density liproprotein receptor-related protein; macrophage inflammatory protein 1-β; MAP kinase-activated protein kinase 2; MAP kinase phosphatase-1; matrix metalloproteinase 1 and 9; mesoderm specific transcript isoform; mitotic arrest defective protein; multifunctional DNA repair enzyme; neurotrophic tyrosine kinase; NFκB p100/p49 subunits; nuclear receptor coactivator 2; ornithine cyclodeaminase; 8-oxo-dGTPase; p53; p53-binding protein Mdm4; peripheral benzodiazepine receptor; polyamine oxidase; protein kinase C α; protein kinase C-like 2; protein tyrosine phosphatase type 1; pyruvate dehydrogenase kinase; replication licensing factor; retinoic acid receptor β; RNA polymerase II; S100 calcium binding protein; serine/threonine kinase 4; serine/threonine specific protein phosphatase; serum/glucocorticoid regulated kinase; STAT1; thioltransferase; thioredoxin reductase; thrombin receptor; thrombomodulin; thymosin β 10; tissue inhibitor of metalloproteinase-3; translation initiation factor 3 and 4H; transmembrane 4 superfamily member; tumor-associated calcium signal transducer 4; tyrosine-protein kinase receptor; ubiquitin-like interferon, α-inducible protein; vasoactive intestinal polypeptide receptor; VEGF; vitronectin; WAP four-disulfide core domain 2, isoform 1 precursor; zinc finger protein 42

TABLE 3-2 *Continued*

Reference	Genes
Zeytun et al., 2002	angiogenin; Bad; bcl-w (Bcl2-like 2); casper; caspses 1, 3, 7, 8, 11, and 14; CRADD; cyclin-dependent kinase inhibitor p21 Waf1; DAXX (Fas-binding protein); DR5 (TRAIL death-inducing receptor); Fas ligand; IAP 1 and 2 (inhibitor of apoptosis proteins 1 and 2); fibroblast growth factor; G-CSF; GADD45 (DNA-damage inducible transcript 1); HGF (hepatocyte growth factor); ILs 3, 4, 5, 6, 7, 9, 10, 12α, 15 and 18; mdm2; NFκb1; NF-κB inducing kinase; p53 responsive protein; PDGFα; retinoblastoma supsceptibility protein; RIP (cell death protein); thrombospondin 3; TNFβ; TRAF2 (TNF receptor associated factor 2); (TRAF3 (death adaptor molecule); TRAF6 (CD40 associated factor); Trail (TNF-related apoptosis inducing ligand); TRIP (TRAF-interacting protein); tumor necrosis factor I and II receptors; VEGF-B, C, D and I

mediated by the AhR. The relative expression of the AhR and AhRR could influence those mechanism, as could the relative presence or activity of other molecules, such as Sp1 (Tsuchiya et al., 2003b), the retinoid receptor (Soprano and Soprano, 2003), antioxidants (Münzel et al., 2003), histone acetylases and DNA methylases (Nakajima et al., 2003), and nuclear factor erythroid 2-related factor 2 (Ma et al., 2004). Nickel and other hypoxia-mimicking agents (Davidson et al., 2003) could influence AhR activity at specific gene sites and in specific cells or tissues.

There also have been several reports that TCDD exposure to cells alters the activity of enzymes such as cAMP/protein kinase A (Vogel et al., 2004), mitogen-activated protein kinases (Kwon et al., 2003; Tan et al., 2002), and protein kinase C (Williams et al., 2004) by mechanisms that might not depend directly on the ability of the AhR–Arnt complex to bind to specific DNA sites. It has been postulated that the chronic activation of those and other kinase pathways is significant in the toxic actions of TCDD (Matsumura, 2003). The ability of TCDD to elicit formation of the nuclear AhR complex also could activate proteasomal degradation of the ER (Wormke et al., 2003). That mechanism, in addition to the ability of the AhR–Arnt complex to bind to inhibitory response elements in estrogen-regulated genes, could be responsible for the observation that AhR ligands suppress estrogen-induced responses in several estrogen-responsive tissues. Caruso et al. (2004) also reported that the AhR could affect the trafficking or processing of cellular proteases found in endosomes and lysosomes. TCDD might cause many of its effects by altering the expression of genes involved in matrix remodeling. Murphy et al. (2004) reported that TCDD in-

duces the expression of matrix metalloproteinase-1 and that the AhR-signaling pathways interact with the retinoic-acid-signaling pathways for the regulation of the expression. Several other investigators reported TCDD-induced expression of matrix metalloproteinases in experimental systems (Table 3-2).

As noted in *Update 2002*, TCDD and other AhR ligands alter cell proliferation and differentiation in a variety of model systems. This is believed to be an important factor in the mechanism of TCDD-induced carcinogenesis and toxic effects in many tissues, especially in reproductive tissues and in developing fetuses. Several studies have been published that identify the ability of TCDD, via the AhR, to disrupt the cell cycle (Abdelrahim et al., 2003; Hestermann et al., 2002; Levine-Fridman et al., 2004; Puga et al., 2002; Shimba et al., 2002), possibly by mechanisms that are related to the ability of the AhR to form complexes directly with cellular proteins, including retinoblastoma protein, that are critical in cell cycle regulation. The AhR also could regulate genes for cell cycle regulatory proteins (Table 3-2).

Data on several individual genes are discussed below in the context of particular tissue systems or toxic endpoints that could 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 tissue. Although the effects of TCDD are high 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 Endpoints

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. Effects are most often dose dependent; that is, some toxic endpoints appear to be more sensitive to low exposures, and others occur only at high concentrations. Toxic effects also 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 endpoints are the most sensitive to TCDD, and this is consistent with the notion that growth, maturation, and differentiation are the most sensitive cellular processes. The data support the biologic plausibility of similar toxic endpoints in humans. Although the exact biologic mechanisms of those endpoints and the observed differences are not yet understood, recent data show 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 or inappropriate cell activation, hormones and growth factors and the responses to them, or the biochemical pathways that lead to oxidative stress. Those mechanisms are implicated in many of the toxic endpoints discussed below.

Lethality and Wasting Syndrome As indicated above and in *Update 2002*, there is some variation among species in susceptibility to the lethal effects of TCDD that is attributable in part to differences of primary amino acid sequences and expression of the AhR protein. 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 wasting syndrome have not been identified. Several groups posit that TCDD, via the AhR, alters a body weight set point. The hypothalamus contains neuroendocrine cells that regulate several physiologic processes, including energy balance.

Recent research has focused on the ability of TCDD to perturb hypothalamus function. Cheng et al. (2003a) reported that the treatment of rats with TCDD caused a down-regulation of nitric oxide synthase and NADPH–diaphorase activity in the hypothalamus. They suggested that decreased production of nitric oxide results in the reported increased activity of the enkephalinergic system that modulates food intake (Cheng et al., 2003b). The expression of several hypothalamic neuropeptides—neuropeptide Y, proopiomelanocortin, cocaine- and amphetamine-regulated transcript, and melanin-concentrating hormone—were increased in TCDD-treated rats that displayed a decrease in body weight gain (Fetissov et al., 2004). The investigators also reported that the AhRR was present in the nuclei of neurons found in the hypothalamus, and they suggested that the AhR has a physiologic role in regulating food intake. Yang et al. (2004) reported that TCDD treatment elicited an induction of Sim1 in a neuronal cell line as well as in mouse kidney and hypothalamus. Sim1 is essential for the differentiation of the paraventricular nucleus of the hypothalamus, and the authors suggested that Sim1 mediates the effect of TCDD on feeding behavior in laboratory animals. Uno et al. (2004) reported that mice that lack expression of a functional *cyp1a1* gene (*cyp1a1* -/- mice) were protected against TCDD-elicited lethality and wasting syndrome. Although it is known that this cyp1a1 enzyme generates oxidants and metabolites of endogenous and exogenous compounds, how those materials operate in the induced wasting syndrome is not clear.

Effects on Skin and Adipose Tissue Skin lesions, including chloracne, often are reported for animals and humans after exposure to TCDD and related compounds. Chloracne is characterized by altered proliferation and differentiation of epidermal cells. TCDD affects the temporal expression of protein markers of keratinocyte terminal differentiation during murine skin morphogenesis (Loertscher et al., 2002). Henley et al. (2004a) reported that TCDD exposure induced an increased expression of IL-1β in human keratinocytes by a posttranscriptional mechanism (Table 3-2). The investigators also reported that ERK and JNK MAP kinase pathways are necessary for this to occur (Henley et al., 2004b).

As indicated in previous updates, TCDD inhibits the differentiation of some preadipocyte cell lines to adipocytes (fat cells); that process is AhR dependent.

Several groups have examined the mechanism because it could help explain how TCDD acts in various tissues. Fibroblasts stimulated by a hormone mixture undergo a cascade of molecular events to initiate adipocyte differentiation. Those events include regulation of c-myc, fos, and jun and then up-regulation of CCAAT-enhancer-binding proteins and of the peroxisome proliferators-activated receptor (PPAR) γ. Hanlon et al. (2003) and Cimafranca et al. (2004) determined that AhR- and ERK-dependent pathways function synergistically to mediate TCDD-induced suppression of PPARγ and subsequent inhibition of adipocyte differentiation. Vogel and Matsumura (2003) reported that the inhibitory effect of TCDD was absent in fibroblasts from c-Src-deficient mice, suggesting that c-Src kinase mediates the antiadipogenic action of TCDD. Notably, the AhR protein is depleted during adipose tissue differentiation, resulting in the loss of responsiveness to TCDD and related xenobiotics. Shimba et al. (2003) determined that the *Ahr* gene is regulated during adipogenesis at the transcriptional level by an unidentified trans-acting factor that was higher in preadipocytes than it is in adipocytes. Down-regulation of this factor during adipose differentiation could result in suppression of *Ahr* gene transcription.

Several investigations cited in previous updates noted that TCDD exposure alters plasma and tissue lipid content in animals. Stanton et al. (2002) reported increased plasma concentrations of total triacylglycerides and specific fatty acids in immature male chickens exposed to TCDD but no increase in free fatty acid concentrations. Treatment with TCDD antagonized estrogen-induced increases in fatty acids. The data suggested that TCDD treatment increased plasma lipids through a mechanism other than increased adipose tissue mobilization. Chen et al. (2002) examined the lipid content of pregnant female rats and fetuses exposed to mixtures of dioxin-like compounds. The lipid content of placenta, liver, and serum from treated dams was lower than it was in the control group at gestational days 16 and 21 and at postnatal day 4. The lipid content of the offspring was not affected until after birth, when they were exposed by lactation, at which time the lipid content in treated mice was higher than it was in controls.

Effects on Bone and Teeth Previous studies have suggested that defects in children's tooth development may be associated with environmental exposure to dioxins and dioxin-like chemicals (Alaluusua et al., 2002; Funatsu et al., 1971; Lind et al., 1999, 2000a,b; Rogan et al., 1988). Tooth development in rats and mink appears to be a target of TCDD toxicity that is at least partially a result of coexpression of the AhR and Arnt during early tooth development (IOM, 2003). TCDD can arrest molar tooth development in rats after in utero and lactational exposure. Recent studies in rats have identified the "critical window" of in utero exposure that effects development of the molars (Miettinen et al., 2002) and incisors (Kiukkonen et al., 2002), as well as impairment of tooth development in tissue culture (Partanen et al., 2004). Pregnant female dioxin-sensitive line C rats were exposed to a single oral dose of TCDD at 1 µg/kg on gestation days 11, 13,

or 19 and on postnatal days 0, 2, and 4 (Miettinen et al., 2002). Pups were killed at 40 days of age. The offspring exposed in utero and via lactation lacked third molars; the greatest effect was exhibited in pups exposed on gestation day 11. Postnatal exposure to TCDD did not affect third-molar development, and none of the treatments affected development of the first and second molars. TCDD accelerated the eruption of the lower incisors and retarded the eruption of the third molars. That effect was most pronounced if exposure had occured during early morphogenesis and from tooth initiation to the early-bud stage, after which sensitivity decreased substantially.

In a study in which mouse embryonic molar tooth explants were exposed to TCDD at 1 µM in tissue culture, morphogenesis of the first molar teeth was not inhibited, but the development of the second molars was arrested when they were explanted before the early-bud stage (Partonen et al., 2004). Later exposure led to smaller tooth size and altered cuspal morphology. The results of in vitro studies also revealed that TCDD enhanced apoptosis of dental epithelial cells predetermined to undergo apoptosis during normal development. The authors concluded that TCDD can arrest tooth development in vitro if exposure starts at the initiation stage and that TCDD interferes with tooth development by stimulating apoptosis in cells of the dental epithelium.

Hans/Wistar (TCDD-resistant) and Long-Evans (TCDD-sensitive) female rats were administered subcutaneous total doses of TCDD at 0.17, 1.7, 17, or 170 (H/W rats only) µg/kg (Kiukkonen et al., 2002). The treatments began when the rats were 10 weeks old and continued for 20 weeks. The exposures covered two life cycles of the incisor. At the high doses (17 and 170 µg/kg), there were color defects and pulpal perforation of the lower incisors and arrest of dentin formation of the incisor teeth. The authors concluded that there was a dose-dependent effect in the mesenchymal and, to a lesser extent, the epithelial elements of the forming tooth.

No relevant studies on bone have been published since *Update 2002*. In general, possible effects of TCDD on bone have not been thoroughly investigated.

Cardiovascular Toxicity TCDD can affect the developing cardiovascular system, but there is little evidence that the cardiovascular system is a major target of TCDD toxicity in adult animals (IOM, 2003). It has been proposed, however, that exposure to dioxin increases the incidence of ischemic heart disease by exacerbating its severity (Dalton et al., 2001).

Male marmosets (*Callithrix jacchus*) treated with a single subcutaneous dose of TCDD at 1, 10, or 100 ng/kg TCDD showed no overt signs of toxicity by 2 or 4 weeks after treatment, and there was no difference in heart weight, compared with control monkeys. Histochemistry (Riecke et al., 2002) revealed an increase in collagen (picrosirius red stain) that presented in different patterns in the intracellular matrix of the myocardium; the changes were associated with activation of TGFβ1, which the authors suggested was a mediator.

Cardiomyopathy and chronic active arteritis increased in a dose-related manner in Harlan Sprague-Dawley rats gavaged 5 days per week for 2 years with TCDD at 0, 3, 10, 22, 46, or 100 ng/kg/day (Jokinen et al., 2003; NTP, 2004). The severity of the cardiomyopathy was minimal on average, and the chronic active arteritis occurred mainly in the mesentery and pancreas at the 46 and 100 ng/kg doses.

Treatment of female rats with the dioxin-like 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) for 12 weeks affected several cardiovascular risk factors, including heart weight, serum cholesterol, and blood pressure (Lind et al., 2004). Niermann et al. (2003) reported that TCDD exposure of rat vascular smooth muscle cells repressed, through an AhR-dependent mechanism, the expression of T-cadherin, an adhesion molecule that is highly expressed in vascular tissues and cells (Table 3-2).

Pulmonary Toxicity This and previous updates report evidence suggestive of an association between herbicide exposure in Vietnam and respiratory cancer (see Carcinogenesis below). Although several published reports have suggested an association between TCDD exposure and chronic obstructive pulmonary disease, this committee found insufficient evidence to support a relationship between herbicide exposure and respiratory disorders that are not considered cancer. This is, in part, based on the findings that the pulmonary system of animals is somewhat resilient to the toxic effects of TCDD. Low doses (1–10 µg/kg) of TCDD that result in toxicity to other organ systems do not appear to damage the lungs of animals. No data have been reported since *Update 2002* to elucidate the toxic effects of TCDD on the respiratory system. However, increased lung weight and bronchiolar metaplasia of the alveolar epithelium has been observed in Harlan female Sprague-Dawley rats gavaged 5 days per week for 2 years with TCDD at 0, 3, 10, 22, 46, or 100 ng/kg/day (NTP, 2004).

Martinez et al. (2002) examined the effects of TCDD exposure on global gene expression profiles in human lung cell lines. Althered gene responses included xenobiotic metabolizing genes, genes known to be involved in cell cycle, and genes involved in cell-signaling pathways and that mediate cellular communication (Table 3-2). Some differences were observed between malignant and non-malignant cells. The authors concluded that TCDD exposure can modify signaling pathways associated with pulmonary disease.

Hepatotoxicity The liver is a primary target of TCDD and related compounds in many animals, but the severity of effects varies considerably among species. The liver and its cells often are used to study the effects of TCDD on biochemical pathways that could be responsible for toxic endpoints. In a 2-year NTP study (2004), rats administered TCDD at a dose of at least 10 ng/kg (5 days per week for 104 weeks) exhibited several non-neoplastic lesions in the liver. The lesions included hepatocyte hypertrophy, multinucleated hepatocytes, inflammation, pig-

mentation, diffuse fatty change, necrosis, bile duct hyperplasia, bile duct cyst, nodular hyperplasia, portal fibrosis, and cholangiofibrosis.

In the process of crossbreeding TCDD-sensitive and TCDD-resistant rat strains, Niittynen et al. (2003) observed a new type of TCDD-induced toxicity in the livers of those animals. The toxicity was characterized by the accumulation of bile pigments, made up primarily of biliverdin and related compounds, progressive sinusoidal distension, and hepatic peliosis with membrane-bound cysts. Patterson et al. (2003) reported that TCDD could potentiate hepatic apoptosis during non-lethal hepatic endotoxemia by an unknown mechanism.

AhR-null allele mice develop liver fibrosis characterized by increased hepatic retinoid concentrations, tissue transglutaminase type II activity, TGFβ overexpression, and accumulation of collagen and by reduced expression of PPARγ. Andreola et al. (2004) reported that the effects were reversed when the mice were fed a diet deficient in vitamin A. Previous updates have reported that TCDD exposure in laboratory animals significantly disrupts the homeostasis of vitamin A. Several recent reports are consistent with the ability of the AhR-signaling pathway to interact with the retinoid receptor pathways by different mechanisms. Schmidt et al. (2003) reported that TCDD exposure in rats results in the significant elevation of concentrations of tissue all-*trans*-retinoic acid and in decreased concentrations of several other retinoid acid metabolites. A review by Soprano and Soprono (2003) pointed out that several retinoids, in addition to affecting the RXR–RAR signaling pathway, also can activate the AhR pathway.

Several research groups have suggested that induction of cellular oxidative stress is a mechanism by which TCDD could elicit damage via the AhR to lead to many of the toxic endpoints observed, including liver injury. Since *Update 2002*, several groups have examined the mechanism. Senft et al. (2002) noted that the production of hydrogen peroxide (H_2O_2) by mitochondria from AhR-null-allele mice was one-fifth that found in wild-type mice. TCDD treatment also caused an increase in succinate-stimulated mitochondrial H_2O_2 production in wild-type, but not in AhR-null-allele, animals. Their data suggested that constitutive and TCDD-induced mitochondrial reactive oxygen production are associated with a function of the AhR. Shertzer et al. (2004a) reported that, although TCDD treatment decreased microsomal production of H_2O_2, the addition of other halogenated aromatic hydrocarbons to TCDD-induced microsomes in vitro actually stimulated the release of H_2O_2 production. The authors postulated that the pathway contributes to oxidative stress response and to toxicity of those compounds. CYP1A2, an AhR-regulated protein (Table 3-2), was found to protect against reactive oxygen production in mouse liver microsomes (Shertzer et al., 2004b). Hilscherova et al. (2003) reported that when chick eggs were exposed before incubation there was significant effects on the indicators of oxidative stress in liver, but not in the brain, of the hatchling chicks. The treatment increased susceptibility to lipid peroxidation and oxidative DNA damage that was only partially mitigated by administration of vitamins E and A. Mice treated with chitosan

oligosaccharide II were protected against TCDD-induced lipid peroxidation, inhibition of glutathione peroxidase, and glutathione S-transferase activities and to losses in body weight and in liver weight (Shon et al., 2002). Similar chitooligosaccharides were found to scavenge superoxide and hydroxyl radicals in vitro; to inhibit the growth of cancer cells; and to increase the activity of macrophages, T cells, and natural killer cells in tumor-bearing mice.

Abraham et al. (2002) tested the ability of the caffeine test for the measurement of CYP1A2 induction in human subjects with variable exposure to TCDD. CYP1A2 was induced at least 10 times in 2 subjects who had been highly exposed (initial blood fat concentrations of TCDD at 144,000 and 26,000 ppt, corresponding to doses of TCDD at 25 and 6 µg/kg). However, the test was not able to show induced CYP1A2 activity in a person who had moderate TCDD exposure (initial blood fat TCDD concentration of 856 ppt). The researchers concluded that direct quantification of TCDD was more specific and sensitive for determining exposure. The body burdens of TCDD at 25 and 6 µg/kg observed in 2 of the subjects are among the highest ever recorded in humans and are at least 100-fold higher than body burdens in most, if not all, Vietnam veterans. However, those concentrations are comparable to the doses used in most studies with experimental animals.

Pancreatic and Gastrointestinal Tract Effects An NTP study by Nyska et al. (2004) evaluated the effects in the pancreas of chronic exposure of female rats to TCDD (2 years; 3–100 ng/kg/day). Several dose-related non-neoplastic changes were observed, including cytoplasmic vacuolation, chronic inflammation, atrophy, and arteritis. Low incidences of pancreatic acinar adenoma and carcinoma also were observed. The data suggest that the pancreatic acini are target tissues for TCDD.

Neurotoxicity Few studies have examined the possibility of nervous system damage in adult animals exposed to TCDD. For those studies that have been performed, the developing brain appears to be more sensitive (see Developmental Toxicity) than does the brain in the juvenile or adult animal. Some studies cited in *Update 2002* suggest that the nervous system may also be affected in adult animals, albeit at higher doses. More recent studies are consistent with this (see Lethality and the Wasting Syndrome).

Several investigators have suggested that TCDD-induced oxidative stress elicits damage in the nervous system. Some of those studies were cited in *Update 2002*. Hassoun et al. (2003) observed that subchronic exposure of rats to TCDD (10–46 ng/kg/day for 13 weeks) caused dose-related increases in the production of superoxide anion and lipid peroxidation in the cerebral cortex and hippocampus. The changes were associated with decreases in superoxide dismutase. Lee et al. (2002) also reported that alteration of cellular redox balance could mediate a TCDD-induced inhibition of proliferation of human neuronal cells. In that case,

however, TCDD treatment of isolated SK–N–SH neuroblastoma cells suppressed the basal generation of reactive oxygen species, caused an inhibition of lipid peroxidation, and increased the glutathione concentration. Increases in superoxide dismutase and catalase were reported, but decreases in glutathione peroxidase and glutathione reductase also were observed. Disruption of the blood–brain barrier by TCDD through oxidative stress could compromise neuronal homeostasis and potentiate neurotoxicity. Filbrandt et al. (2004) reported that the AhR was present and functionally active in cerebral vascular endothelial cells and astrocytes.

Previous investigations reported neuropathy and changes in synaptic transmission in the brain of TCDD-exposed rats (IOM, 1996, 1999, 2001). Cho et al. (2002) investigated the possibility that TCDD affects the expression of synaptic proteins in E18 rat cortical cells. After 4 days of exposure, cell viability was significantly reduced and there were decreased numbers of secondary or higher order dendritic processes. NMDA receptor subunits were found to be up-regulated, but there was a down-regulation of several synaptic organizing proteins (PSD-95, Densin-180, septin6 homologue) and a synapse-enriched enzyme (αCaM kinase II). The authors suggested that the altered expression of synaptic proteins is responsible for the altered synaptic transmission and neuropathy observed after TCDD exposure.

Studies cited in previous updates indicate that TCDD affects the homeostasis of vitamin A. Huang et al. (2003) used cellular retinal-binding protein (CRBP-1) knockout mice to determine whether the effect of TCDD on gene expression in the brain and pituitary might be modulated by the retinoid system. In general, the relative induction of CYP1A1 and the AhR was lower in the brains of CRBP-1 knockout animals. However, the basal expression of the AhRR was higher in the pituitary from knockout mice.

Immunotoxicity The immune system of laboratory rodents is highly sensitive to the toxic effects of TCDD, and the immune suppression observed after TCDD exposure is mediated through the AhR. Many cell types make up the immune system, and most have been shown to express the AhR. Developing an understanding of the specific cells that are altered by TCDD and how they contribute to alterations in immune function induced by TCDD is of great interest to the research community. Understanding how TCDD affects the immune system in rodents enhances the ability to extrapolate the experimental results to assessment of human risks. Since *Update 2002*, several papers have addressed the mechanisms of TCDD's effects on the immune system.

Involution of the thymus is a hallmark of TCDD exposure. The mechanism of action of TCDD on the thymus is open to question—some data support indirect effects on the thymocytes via the thymic epithelial or dendritic cells, others support direct effects on thymocytes. Two recent studies, one using differential expression of the AhR (Laiosa et al., 2003) and one using differential ARNT

expression (Tomita et al., 2003), showed that the AhR–ARNT-signaling component that leads to thymic involution is required in the thymocytes but not in the dendritic cells or in other thymic stromal cells. Laiosa et al. (2003) also showed that TCDD blocked the ability of early thymocytes to divide, perhaps because they are incapable of homeostatic repopulation of the thymus. TCDD also induced the expression of adseverin (a protein that could influence cell differentiation) in thymocytes but not thymic epithelial cells, whereas CYP1A1 was induced in both types of cells (Svensson et al., 2002). A direct effect of TCDD on the function of thymic epithelial cells was supported by Riecke et al. (2003), who reported that exposure to low concentrations of TCDD promotes the terminal differentiation of thymic epithelial cells in vitro and alters their expression of several adhesion molecules. ARNT2, another protein that dimerizes with the AhR, is not involved in thymic atrophy (Laiosa et al., 2002). Those new results favor the hypothesis that thymic involution observed after TCDD exposure results from direct AhR-mediated toxicity to the developing thymocytes that could be exacerbated by additional effects on thymic epithelial cells.

Mature lymphocytes in the secondary lymphoid organs also are affected by TCDD. Recent studies using wild-type or AhR-knockout cells show that the AhR must be expressed in $CD4^+$ and $CD8^+$ T cells for TCDD to suppress the generation of a cytotoxic T lymphocyte response (Kerkvliet et al., 2002). Induction of CYP1A1 in purified T cells exposed to TCDD in vitro validates the presence of a functional AhR (Doi et al., 2003). Camacho et al. (2002) suggested that activation of the Fas gene by TCDD induces apoptosis of activated T cells, leading to immune suppression. Kwon et al. (2003) used human Jurkat T cells to show that the mitogen-activated protein kinase signaling pathway was activated by TCDD, including activation of caspase 3, a mediator of apoptosis. Gene array analysis has identified other genes altered in the thymus or spleen of TCDD-treated mice (Park et al., 2001; Zeytun et al., 2002), but their functional significance to TCDD's immunotoxicity has not been determined. Low concentrations of TCDD also were shown to directly alter the activation of dendritic cells derived from bone marrow cell culture (Ruby et al., 2002). Dentritic cells are required for presenting antigens to T cells to initiate an immune response.

Several new projects attempted to explain how TCDD enhances mortality of mice infected with influenza virus. Previous studies had shown that the virus was cleared from the lungs of TCDD-treated mice even though the T cell and antibody-mediated immune responses to the virus were suppressed. Vorderstrasse et al. (2003) reported that virus-specific IgA concentrations were significantly higher in TCDD-treated mice and that all other classes of antibodies were reduced. There also was an increase in the number of neutrophils in the lungs of virus-infected, TCDD-treated mice; natural killer cell numbers and inflammatory cytokine concentrations (TNFα, IL-1, IFNα/β) were not changed (Neff-LaFord et al., 2003). Damage to the lung caused by the increase in neutrophils was postulated to explain the toxicity of TCDD in influenza-infected mice. Increased

numbers of neutrophils in mouse spleen and blood with a growing tumor allograft also were reported by Choi et al. (2003). The neutrophils from TCDD-treated mice were defective at killing tumor cells but were enhanced in respiratory burst capability, which could lead to tissue damage. However, those results contrast with a recent in vitro study in which TCDD suppressed the oxidative burst of human neutrophils (Abrahams et al., 2003). Kim HJ et al. (2003) reported that repeated dosing of rats with TCDD resulted in higher numbers of $CD11b^+$ cells (macrophages or neutrophils) in the spleen and in an increase in serum IL-6.

An alternative hypothesis for mortality in TCDD-treated mice infected with influenza is based on mitochondrial toxicity, similar to that seen in Reye's syndrome in humans (Luebke et al., 2002). However, no effect of TCDD on serum NH_3 or on glucose concentrations, two biologic markers of Reye's syndrome, was observed. Rather, enhanced pulmonary inflammation, consisting of increased macrophage and neutrophil numbers, was reported; there were no effects on the concentrations of TNFα, MIP-1α, MIP-2, overall protein, or LDH in the lung. Those results confirm and extend the findings of Neff-LaFord et al. (2003).

B cells also appear to be direct targets of TCDD; they express high concentrations of CYP1A1 after exposure to TCDD (Doi et al., 2003). Several genes could be regulated by TCDD in B cells, including $p27^{kip1}$, a regulator of cell survival and differentiation (Crawford et al., 2003); AP-1, a transcription factor that influences B cell function (Suh et al., 2002); and Pax5, a repressor of B cell differentiation (Yoo et al., 2004). Recent studies have validated earlier work that showed that PCB congeners that are chlorinated in the di-*ortho* positions inhibit TCDD-induced suppression of B cell functions; the non-*ortho*-substituted PCB (PCB77) produced additive effects on B cells with TCDD (Suh et al., 2003).

TCDD has long been known to suppress primary antibody responses, primarily IgM production. Two new studies address the effect of TCDD on antibody class switching to IgG production, an important aspect of the immune response that is crucial for vaccination effectiveness. Ito et al. (2002) showed that TCDD suppressed the production of key cytokines (IL-2, IL-4, IL-5, IL-6) produced by T cells that are important for B cell switching. IL-5 was particularly sensitive to suppression by TCDD because of its effect on T cells rather than antigen-presenting cells. The proliferation of germinal center B cells, which is essential for the production of high-affinity antibodies, also was suppressed in TCDD-treated mice; and TCDD did not enhance B cell apoptosis (Inouye et al., 2003).

A novel effect of AhR signaling to deplete peritoneal B1 cells was described in mice that express a constitutively active AhR (Andersson et al., 2003). B1 cells are thought to operate in the innate response to infection by viruses and bacteria. The ability of TCDD to activate the AhR to cause a similar depletion of peritoneal B1 cells has not been investigated.

TCDD increases the number of hematopoietic progenitor cells in the bone marrow. Sakai et al. (2003) verified and validated that finding, and they reported that stem cells from TCDD-treated mice (40 µg/kg) were incapable of reconstitut-

ing the bone marrow of irradiated recipient mice. Those effects were not linked to changes in cell proliferation or cell death, and they were not seen in AhR-deficient mice.

Perinatal exposure of rats to low doses of TCDD was found to suppress the contact hypersensitivity response to dinitrofluorobenzene as measured in 6-month-old female offspring (Walker et al., 2004).

Rheumatoid arthritis is a chronic inflammatory condition that affects joints. It is characterized by the proliferation of cells called synoviocytes and by the production of proinflammatory cytokines and chemokines. Tamaki et al. (2004) reported that several AhR agonists, including TCDD, could increase the mRNA for IL-1 β in a human-fibroblast-like synoviocyte line, and that it occurred via the AhR. AhR agonists are contained in cigarette smoke, so the authors posit that might provide the basis for a link between cigarette smoking and rheumatoid arthritis.

Carcinogenesis TCDD has been demonstrated to be a carcinogenic agent and potent tumor promoter in several model systems. A 2-year bioassay in female rats revealed carcinogenicity that was evidenced by increased incidence of cholangiocarcinoma and hepatocellular adenoma of the liver, by cystic keratinizing epithelioma of the lung, and by gingival squamous cell carcinoma of the oral mucosa (NTP, 2004).

The ability of TCDD to induce cell proliferation and to alter differentiation is believed to be an important factor in its mechanism of carcinogenesis. Ray and Swanson (2003, 2004) investigated whether the tumor-promoting activity of TCDD results from its ability to alter proliferation, differentiation, or senescence of normal human epidermal keratinocytes. TCDD accelerated differentiation, as demonstrated by increased expression of the differentiation markers involucrin and filaggrin. TCDD also increased proliferation, as indicated by an increased production of NADH–NADPH and changes in cell cycle. Dioxin exposure attenuated senescence and repressed expression of the tumor suppressor proteins p53 and $p16^{INK-4a}$. The ability of TCDD to immortalize human keratinocytes was indicated by those authors to be a novel mechanism by which the compound could lead to malignancy. Parfett (2003) examined the ability of TCDD and other tumor promoters to induce the expression of proliferin (PLF), a glycoprotein suggested to influence the regulation of cellular proliferation and differentiation, in C3H/10T/1/2 cells. TCDD induced the expression of basal- and serum-induced PLF mRNA. The authors suggested that the induction of PLF in that cell line could be used as a short-term marker for chemical agents with promotional activity.

Several of the enzymes induced by TCDD, including CYP1A1, CYP1A2, and CYP1B1, are responsible for the metabolic activation of many promutagens, so activation of the AhR is considered important for the carcinogenic activity of many compounds. Lin P et al. (2003) reported an association between increased CYP1B1 and AhR expression in human non-small-cell lung cancer tissues.

CYP1B1 expression was detected in 3 of 19 normal lungs, but in 42 of 89 non-small-cell lung cancers. A similar study found that CYP1B1 mRNA concentrations were elevated in peripheral leukocytes of lung cancer patients (Wu et al., 2004). Both studies controlled for age, smoking status, and sex. Previous reports indicate the development of lung cancer in female rats but not male rats exposed to TCDD. Lin et al. (2004) reported that cotreatment with 17 β-estradiol (E2) and TCDD significantly enhanced the toxicity observed in human bronchial epithelial cells, and that TCDD cotreatment increased the production of E2 metabolites. The authors suggested that the metabolites enhance the effects on cells.

Chronic bioassays have shown TCDD exposure to increase the incidence of hepatic tumors in female rats but not in male rats. Moennikes et al. (2004) used a transgenic mouse line for expression of a constitutively active AhR to investigate the role of that protein in hepatocarcinogenesis. The presence of the constitutively active AhR dramatically increased the incidence of N-nitrosodiethylamine-initiated tumors. Whereas only 1 tumor was observed in the 15 wild-type mice, 19 tumors were present in the 19 transgenic mice. Notably, and as indicated in *Update 2002*, increased stomach tumors were observed in the transgenic mice not treated with N-nitrosodiethylamine (Andersson et al., 2002). Chen et al. (2003) confirmed that AhR activation and induction of CYP1A1 was required for benzo[*a*]pyrene-7,8-dihydrodiol-induced apoptosis in human HepG2 cells. That activity also was linked to increased mitogen-activated protein kinase activity that was AhR dependent. However, Schrenk et al. (2004) reported that TCDD inhibited apoptosis in rat hepatocytes initiated by ultraviolet irradiation. The suppression of apoptosis by TCDD coincided with increases in concentration and hyperphosphorylation of the tumor suppressor protein p53.

Non-genotoxic carcinogens (such as TCDD) are thought to induce tumor formation by altering the balance between cell proliferation, differentiation, and death. Mally and Chipman (2002) examined the effect of TCDD treatment (2.5–250 ng/kg for 2 days/week for 4 weeks) on gap junction formation in relation to proliferation and apoptosis. Dose-dependent reductions in gap junction formation were observed in rat liver but not in rat thyroid or kidney. Alterations in gap junctions did not correlate with induction of cell proliferation. However, Dietrich et al. (2002) reported that TCDD treatment of confluent WB-F344 rat liver epithelial cells induced a release from contact inhibition and a 2-fold increase in cell number in the presence of serum. This did not occur when TCDD was added to exponentially growing or to subconfluent, serum-deprived cells. The contact-inhibited cells also demonstrated loss of G1 arrest and increases in cyclin D2 and cyclin Q protein. The cdk2–cdc2-specific inhibitor olomoucine abolished the TCDD response. The same research group reported that TCDD exposure to those cells caused a down-regulation of γ-catenin protein and mRNA (Dietrich et al., 2003). Notably, catenin, a cell membrane protein, also is considered a tumor suppressor. Chramostova et al. (2004) reported that strong or moderate AhR

ligands, including TCDD, increased WB-F344 cell numbers that corresponded to an increased percentage of cells in S phase.

Several studies have implicated the β-catenin-signaling pathway in prostate cancer. The cytoplasmic accumulation of free β-catenin leads to increased binding to and activation of particular transcription factors that control genes involved in proliferation and differentiation. Accumulation of intracellular β-catenin occurs in a variety of cancers. Chesire et al. (2004) reported that the overexpression of a mutant hyperactive form of β-catenin leads to increased AhR in human prostate cancer cells. A possible role of this increase in the development of prostate cancer has yet to be determined.

Investigations by Lamartiniere (2002) indicated that if TCDD is given to pregnant rats, the offspring are more susceptible to dimethylbenz[a]anthracene-induced mammary cancer as adults. Offspring exposed prenatally to TCDD had mammary glands with more terminal end bud structures and fewer lobules. The terminal end buds are considered more undifferentiated and more susceptible to for tumor initiation; the lobules are more mature and are the least susceptible structures. Thus, the timing of exposure to TCDD could be extremely important for carcinogenesis. Van Duursen et al. (2003) reported that exposure to TCDD and several dioxin-like compounds decreased the ratio of 4-hydroxyestrogens to 2-hydroxyestrogens but increased the concentrations of potentially carcinogenic estrogen metabolites in human mammary epithelial cell lines. A high estrogen 4-/2-hydroxylation ratio has been identified as a likely indicator of the presence of mammary tissue neoplasms. The authors suggested that the value of this ratio as a prognostic marker for cancer risk should be further examined. Spink et al. (2003) reported that the presence of estrogen was required to maintain high AhR expression in those cells and inducibility of the enzymes responsible for the altered metabolism of estrogen.

Kim AH et al. (2003) used a differential-dose regimen to examine the possible use of area under the curve as a dose metric for tumor promotional responses in female rats exposed to TCDD. The volume fraction of GST-positive foci in livers was higher in the TCDD group given a high peak dose during the first week of treatment than it was in the group that received the same average daily dose for the duration of the experiment. The authors interpret those findings to indicate that the peak magnitude of TCDD in the liver rather than the area under the curve is more important in the tumor-promoting ability of TCDD.

Effects on the Testis Many effects of TCDD in male rodents have been reported previously, including decreases in the size of the accessory sex organs and daily sperm production. Both AhR and Arnt are expressed in rat and human testis, and the data suggest that AhR in that tissue is regulated by follicle-stimulating hormone (FSH) (Schultz et al., 2003). The latter result suggests a possible action by the AhR in controlling spermatogenesis. The expression of the AhRR also is very high in the human testis (Yamamoto et al., 2004), suggesting at least the

possibility that the human testis might be relatively resistant to the effects of TCDD.

Other investigations suggest that TCDD causes tissue damage by induction of oxidative stress. Latchoumycandane and Mathur (2002) observed that subchronic treatment of rats with TCDD (1–100 ng/kg/day for 45 days) resulted in a significant decrease in the weights of the testis, epididymis, seminal vesicles, and ventral prostate. There also was a substantial decline in daily sperm production. Testicular activities of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase were decreased, and there were increased tissue concentrations of H_2O_2 and lipid peroxidation. Acute-exposure studies produced a similar effect of TCDD-induced oxidative stress on epididymal sperm (Latchoumycandane et al., 2003). A report by Kwon et al. (2004) noted a protective effect for dietary ursodeoxycholic acid (UDCA), a biliary component in bears, on the acute effects of TCDD on testes in mice. TCDD induced decreased testicular weight, and decreases in serum-luteinizing hormone and FSH were abrogated in animals also receiving UDCA. The mechanism of the protective effect is unknown. Fukuzawa et al. (2004) reported that TCDD treatment at relatively high doses (20–100 µg/kg) to wild-type, but not AhR-null-allele mice, caused a significant reduction in testicular leutinizing hormone receptor and cytochrome P450 side chain cleavage protein and mRNA, as well as decreased testosterone synthesis.

Effects on the Prostate Prostate cells and prostate cancer cell lines are responsive to TCDD in terms of induction of a variety of genes, including those involved in drug metabolism. Some polycyclic aromatic hydrocarbons exhibited antiandrogenic effects in human prostate carcinoma (LNCaP) cells as determined by the analysis of the 5α-dihydrotestosterone-stimulated induction of prostate-specific antigen (PSA) (Kizu et al., 2003). TCDD was not examined in those studies. Nevertheless, the effects were found to depend on the AhR and were likely mediated through the noted elevation of c-fos and c-jun expression. Those proteins are known to inhibit binding, through formation of the AP-1 complex, of the androgen receptor (AR) to the androgen-responsive element of target genes, such as PSA. Studies by Morrow et al. (2004) demonstrated that TCDD inhibits growth of LNCaP cells and hormone-induced up-regulation of AR protein. However, the effects appeared to depend on the promoter region of the particular gene, suggesting that although there might be cross-regulation between the AR- and AhR-signaling pathways, the interactions are complex.

Effects on the Ovary and Female Reproductive Tissue The ovaries of experimental animals provide targets for the action of TCDD. Abnormal follicle development and decreased numbers of ova have been observed. There also is a widening of the mesenchyme that separates the Mullerian ducts while the zone of unfused ducts is increased, and TCDD exposure delays vaginal opening and

causes persistent vaginal threads (IOM, 2003). The AhR and Arnt are expressed in rabbit ovary within the steroid-secreting interstitial cells, in follicular and granulose cells, and in lutein cells (Hasan and Fischer, 2003) as well as in oocytes and surrounding cumulus cells (Pocar et al., 2004). Those cells are responsive to TCDD in terms of AhR-mediated altered transcription. Dasmahapatra et al. (2002) reported that TCDD induced CYP1A1 and CYP1B1 mRNA in rat ovary, but that it was highly dependent on the phase of the estrous cycle. The authors suggested that this could be important for the metabolism of estrogens. Pocar et al. (2004) reported that there is constitutive expression of CYP1A1 in immature oocytes, but not in cumulus cells, and that a significant increase in CYP1A1 occurs in both cell types after maturation. This expression of CYP1A1 was found to be AhR dependent, suggesting that the AhR could have some physiologic role in oocyte maturation. Estradiol was found to enhance, but estriol to inhibit, TCDD-induced expression of CYP1A1 in a mouse ovarian cancer cell line (Son et al., 2002).

TCDD alters processes involved in ovarian steroid synthesis. Moran et al. (2003a,b) noted that treatment with TCDD of human luteinized granulose cells resulted in an approximate 50% decrease in the expression of cytochrome P450 17α-hydroxylase/17,20-lyase, concomitant with a 65% decrease in 17,20-lyase activity. The decreases were proportional to the observed inhibition of estradiol secretion by the cells. The data are consistent with those from a study by Petroff and Mizinga (2003). Diminished serum progesterone and estradiol concentrations after the exposure of rats to TCDD were attributed to altered steroid synthesis and release rather than to an alteration in pharmacokinetics. Mizuyachi et al. (2002) also reported that TCDD treatment blocked ovulation in immature rats primed with chorionic gonadotropin to stimulate ovulation. An analysis of the ovaries indicated increased concentrations of CYP1A1 and Arnt, plasminogen activator inhibitor types 1 and 2, urokinase plasminogen activator, and tissue plasminogen activator 24 h after treatment. TCDD inhibited the expression of cyclooxygenase-2 (COX-2). That effect was thought to be critical because a reduction in COX-2 expression is associated with ovulation failure. Minegishi et al. (2003) reported that treatment of rat granulose cells with TCDD resulted in a significant decrease in the FSH-induced expression of luteinizing hormone receptor. Luteinizing hormone acts on granulose cells to stimulate steroidogenesis, luteinization, and ovulation. Williams et al. (2004) indicated that TCDD exposure induced PCKδ protein expression and phosphotransferase activity in mouse ovarian cancer cells. Benzo[*a*]pyrene, but not TCDD, was found to cell adhesion proteins in human uterine cells (McGarry et al., 2002).

Effects on the Uterus TCDD exposure has been reported to decrease uterine weight in rodents, to alter endometrial structure in rodents, and to increase the incidence of endometriosis in rhesus monkeys (IOM, 2003). Takemoto et al. (2004) demonstrated that TCDD treatment blocks an estrogen-induced increase in mouse uterine weight. However, they also observed that this effect did not

occur in either *Ahr* -/- or *cyp1b1* -/- animals, suggesting that AhR-dependent-increased metabolism of estrogen could be involved in this antiestrogenic response to TCDD.

TCDD exposure increased the prevalence and severity of endometriosis in non-human primates. Some concern exists about the possibility that TCDD also increases the prevalence of endometriosis in humans. Rier and Foster (2002), who reviewed the evidence, concluded that the data are consistent with a hypothesis that TCDD and related compounds promote endometriosis by stimulating chronic inflammation that leads to an alteration of the estrogen- and progesterone-dependent processes that normally limit the development of endometriosis. TCDD has been shown to modulate the expression of many genes involved in regulating inflammatory processes (Table 3-2). Nevertheless, the exact mechanisms are not known. Zhao et al. (2002) reported that functional AhR is present in human endometrial stromal cells and that TCDD induces the expression of RANTES, a chemokine shown to regulate inflammation. The authors suggested that could be a mechanism for relating TCDD exposure and endometriosis. However, in a review of the literature on endometriosis in humans and non-human primates, Guo (2004) concluded that there is insufficient evidence to support the hypothesis that dioxin exposure leads to the development of endometriosis.

Effects on the Mammary Gland TCDD exposure results in a reduction of primary branches, decreased epithelial elongation, and fewer alveolar buds and lateral branches in the mammary glands. Consistent with this, Vorderstrasse et al. (2004) reported that AhR over-activation during pregnancy could disrupt mammary gland differentiation and lactation. Exposure of pregnant mice to TCDD (5 µg/kg) on days 0, 7, and 14 of pregnancy resulted in severe defects in hormone-stimulated breast development, including stunted growth, decreased branching, and poor formation of lobular alveolar structures. The impaired differentiation resulted in the decreased expression of whey protein in the gland, and all pups born to the dams died within 24 hours. The exact mechanism of this effect has not been determined. However, because the effects preceded hormone-induced events, it was proposed that altered hormone concentrations were unlikely to have been a mechanism of impaired mammary development.

Human breast cancer cells have been useful in investigations of the mechanisms of AhR signaling and of the effects of TCDD on hormone-induced responses, especially responses to estrogen. Previous updates reported that TCDD blocks many estrogen-induced responses in human breast cancer cells. Davis et al. (2003) reported that the AhR antagonist 3′-methoxy-4′-nitroflavone blocked the ability of TCDD to inhibit apoptosis induced in mammary epithelial cells by epidermal growth factor withdrawal. A concentration-dependent analysis suggested that TCDD inhibited apoptosis by several mechanisms, including an effect on the expression of transforming growth factor-α.

Endocrine and Other Effects As indicated in previous updates, TCDD and related compounds affect the thyroid and thyroid hormones in several animal species. Possible mechanisms include the displacement of hormones from serum transport proteins, the alteration of deiodinase activity, and the increasing thyroid hormone catabolism via glucuronidation. Viluksela et al. (2004) recently reported that alterations in tissue 5′-deiodinases I and II in rats after TCDD treatment were secondary to elicited decreases in serum thyroxine (T4) concentrations, and that altered deiodinase activities did not significantly influence the circulating levels of T4 and triiodothryonine (T3). Yamada-Okabe et al. (2004) reported that many genes found to be altered by T3 in HeLa cells overexpressing the thyroid hormone receptor were further modulated in cells exposed to both T3 and TCDD. The results indicated that TCDD augments the cellular responses to T3 by hyperactivating thyroid-hormone-mediated gene expression. The National Toxicology Program (2004) reported that female rats exposed chronically (2 years) to TCDD demonstrated altered thyroid follicles characterized by decreased luminal size and increased height of the follicular epithelial cells. Tani et al. (2004) characterized that effect as the result of a reversible hypertrophic response of the thyroid follicular cell.

Huang et al. (2002) reported that TCDD induced several genes in the pituitary after in vivo and in vitro exposure. Most notably, an increased expression of the adrenocorticotrophic hormone precursor proopiomelanocortin (POMC) gene was observed in TCDD-treated mice and in a pituitary cell line. This could be an AhR-responsive gene; DRE sequences were found in the promoter region. The data suggest that the pituitary gland is a direct target for TCDD and that, in particular, the up-regulation of POMC expression is significant in many endocrine alterations induced by TCDD. Petroff et al. (2003) observed that TCDD treatment in immature female rats stimulates a premature gonadotropin release by interacting with an estradiol- and phenobarbital-sensitive neural signal but not by acting directly on the gonadotrophin-releasing-hormone releasing neurons in the hypothalamus.

In the National Toxicology Program study (NTP, 2004), female Harlan Sprague-Dawley rats were administered TCDD at 3, 10, 22, 46, or 100 ng/kg body weight by gavage 5 days a week for 104 weeks. Exposure to TCDD increased the incidence of non-neoplastic lesions in the liver, lungs, oral mucosa, pancreas, thymus, adrenal cortex, heart, clitoral gland, kidney, forestomach, and thyroid glands. In the pancreas, the incidence of chronic active inflammation, acinar atrophy, and arterial chronic active inflammation was increased in the 100 ng/kg group at 2 years. At 2 years, there also was cortical atrophy of the adrenal cortex in the 100 ng/kg group; cortical hyperplasia was observed in groups administered 10 ng/kg or more. Thymic atrophy was observed at 2 years in groups administered 22 ng/kg or more, and follicular cell hypertrophy was noted in some exposure groups. Serum-free T4 concentrations were generally lower and serum T3 concentrations were higher, as were the thyroid-stimulating hormone concen-

trations in a dose–response fashion. Gingival squamous hyperplasia was observed in the oral mucosa, as was squamous hyperplasia of the forestomach. A minimal to mild nephropathy was generally observed, as was an infrequent moderate-to-marked nephropathy.

Developmental Toxicity Extensive data from studies in animal experiments 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. Recent publications are consistent with this. All the following are studies in which effects of TCDD on the developing embryo or fetus were investigated after maternal exposure to TCDD. There have been no studies since *Update 2002* in which the effect of TCDD on the fetus was investigated after paternal exposure.

Work by Ishimura et al. (2002) suggested that the increased fetal death observed in rats after exposure to TCDD is attributable to placental hypoxia. They compared the content of placental proteins from TCDD-treated rats and animals subjected to hypoxic conditions. Increased glyceraldehyde 3-phosphate dehydrogenase, a marker protein of hypoxia, in both sets of placentas at gestational day 20 suggested that the TCDD-exposed placentas were in a hypoxic state at the end of the pregnancy.

Since *Update 2002*, there have been several reports detailing effects of TCDD on the developing cardiovascular system. Some investigations were stimulated by the finding that the AhR appears to influence the development of normal vascular architecture. Vasquez et al. (2003) demonstrated that cardiac hypertrophy is a consequence of AhR inactivation in mice. In their investigations, AhR-knockout animals were not found to be hypertensive, but they developed cardiomyopathy and diminished cardiac output. They did not exhibit the molecular markers characteristic of cardiac hypertrophy. The data suggested that increased cardiomyocyte size is a consequence of the absence of the AhR. Thackaberry et al. (2003) also observed that AhR-null embryos develop cardiac enlargement, and that the phenotype depends in part on the maternal genotype. However, markers of cardiac hypertrophy, β-myosin heavy chain and atrial natriuretic factor, were increased in AhR-null embryos. Thackaberry et al. (2003) also reported that the AhR is required for normal insulin regulation in pregnant and older mice and for cardiac development in embryonic mice. Pregnant and nonpregnant females at particular ages had significantly decreased fasting plasma insulin concentrations and a reduced ability to respond to exogenous insulin. Lund et al. (2003) reported that cardiac hypertrophy in AhR-null mice is correlated with elevated angiotensin II, endothelin-I, and mean arterial blood pressure and that all of these were reduced by treatment with an angiotensin-converting enzyme inhibitor. Additional investigations by Guo et al. (2004) indicate that the expression of TGF-β-regulated genes is deregulated in aortic smooth muscle cells from AhR-knockout mice. The AhR has been shown to mediate resolution and maturation of the fetal vascular structure, in particular closure of the fetal liver

vascular structure known as the ductus venosus. Work by Walisser et al. (2004) demonstrated that Arnt also is essential to AhR developmental signaling that mediates those processes in vascular development. Bunger et al. (2003) reported that liver development in mice carrying a mutation in the nuclear localization sequence of the AhR was identical to that observed in AhR-null-allele mice, indicating that a role of the AhR in vascular development requires nuclear localization of that protein.

Chick embryos are susceptible to cardiac effects after TCDD exposure. Data from work by Ivnitski-Steele and Walker (2003) indicate that TCDD inhibits early coronary vascular outgrowth in chick embryos by a mechanism that depends on vascular endothelial growth factor (VEGF). Treatment of embryos with exogenous VEGF rescued them from TCDD-induced inhibition of coronary vasculogenesis. In addition, hearts from TCDD-treated embryos exhibited a significant reduction in VEGF mRNA and in protein. Kanzawa et al. (2004) noted that TCDD induced a differential expression of the CYP1A family genes in chick embryo heart and liver.

The cardiovascular system, in particular the vascular endothelium of the developing embryo, also has been identified as a primary target of TCDD toxicity in fish. Studies cited in *Update 2002* concluded that circulatory failure and oxidative stress in vascular endothelial cells are primary events that mediate the toxicity. Several recent studies using zebrafish and rainbow trout are consistent with this (Bello et al., 2004; Carvalho et al., 2004; Dong et al., 2004). Dong et al. (2004) provided further evidence that an effect of TCDD on vascular endothelium leads to local circulation failure and apoptosis in zebrafish dorsal midbrain. Zodrow and Tanguay (2003) reported that TCDD inhibits caudal fin regeneration that could occur by a down-regulation of genes that are important in vascularization. Further studies indicated that the pathology observed could not account alone for the inhibitory effects of TCDD on fin regeneration (Zodrow et al., 2004). Bello et al. (2004) reported that TCDD significantly inhibits growth of the common cardinal vein in zebrafish and that is dependent on AhR2. Hill et al. (2004) reported that the TCDD-induced edema seen in zebrafish results from an increased permeability to water across the surface of the developing embryo. Prasch et al. (2003b) observed that hypoxia decreases responses to TCDD in zebrafish embryos, thus suggesting interactions between the signaling pathways for HIF-α (which regulates VEGF) and the AhR. Investigations by Teraoka et al. (2003) suggested that induction of CYP1A1 is required for circulation failure and edema induced by TCDD toxicity in zebrafish.

Several of the developmental defects observed in animals exposed to TCDD can be explained, at least in part, by the failure of the vascular system to develop normally. TCDD induces myocardial defects during chick embryogenesis. Ivnitski-Steele et al. (2004) reported that those effects could be attributable to the ability of TCDD to disrupt oxygen gradients necessary for normal coronary vascular development and to alter expression of HIF-1a and VEGF-A. Prasch et

al. (2003b) likewise observed an interaction between the AhR- and hypoxia-signaling pathways in developing zebrafish. Hypoxia decreased TCDD induction of CYP1A mRNA and decreased the potency of TCDD in causing edema.

Studies by Blankenship et al. (2003) tested the hypothesis that oxidative stress, the generation of radical oxygen species, and induction of CYP1A1 could promote TCDD-induced abnormalities and embryo lethality in chickens. However, under the conditions of the study, cotreatments with inhibitors, antioxidants, and radical scavengers failed to significantly alter the outcomes induced by TCDD.

Abbott et al. (2003) examined the potential roles of epidermal growth factor (EGF) and TGF-α in developmental toxicity—specifically cleft palate and hydronephrosis—elicited by TCDD by the use of EGF, TGF-α, and double-knockout mice. The EGF (-/-) mice were less responsive to induced cleft palate, but more sensitive for the induction of hydronephrosis; the TGF-α (-/-) and double-knockout animals demonstrated sensitivity that was no different from that of the wild-type mice. The data demonstrated that the EGF receptor pathway is important in developmental responses to TCDD but that the relative mechanism appears to be specific to the endpoint. Miettinen et al. (2004) recently reported that EGF receptor deficiency in mice did not alter the ability of TCDD to produce cleft palate and hydronephrosis or change the time to eye opening. The data suggest that although EGF could influence those endpoints mediated by TCDD, signaling through the EGF receptor is not absolutely required. Davis et al. (2002) reported the localization in mouse chromosome 11 of a quantitative trait locus that affects the ability of TCDD to induce alterations in the mandible. The exact genes responsible for this have not been identified. Notably, work by Falahatpisheh and Ramos (2003), who used metanephric cultures from AhR (-/-) and AhR (+/+) mice, suggested that the AhR is involved in normal kidney development. That effect appears to involve regulation of the Wilms's tumor suppressor gene, which is important in mesenchymal–epithelial transition and differentiation during nephrogenesis, and it is consistent with the noted ability of TCDD to disrupt the process.

Previous updates cited several reports indicating that development of the male reproductive system is exceptionally sensitive to in utero and lactational TCDD exposure. Those effects have included alterations in sperm production, increased numbers of abnormal sperm, decreased prostatic weight and growth and seminal vesicle growth, and decreased urogenital–glans penis length (IOM, 2003). Nevertheless, the TCDD-exposed males were able to impregnate females to produce viable fetuses (IOM, 2003). Simanainen et al. (2004a) reported that genetic differences in the C-terminal transactivation domain of the rat AhR modified sensitivity to some TCDD-induced male reproductive effects. TCDD-altered growth of the reproductive organs was not affected by the allelic differences; sperm numbers appeared to be affected differentially. Impaired prostate growth has been shown consistently. Ko et al. (2002) determined that the effect of TCDD

on the prostate was lobe specific. The inhibition of ventral prostate development was characterized by the complete absence of branching morphogenesis. The impaired development of the dorsal, lateral, and anterior prostate is associated with inhibition of processes involved in duct formation. The inhibition of prostate bud formation in the ventral and dorsolateral prostate was not the result of an insufficient amount of 5α-dihydrotestosterone (Lin T-M et al., 2003) or to the impairment of the androgen-signaling pathway (Ko et al., 2004a). Abbott et al. (2003) used EGF- and TGF-α–knockout animals, to demonstrate that both growth factors are important in the formation of prostatic buds and the ability of TCDD to inhibit bud formation in a region-specific manner. An additional study (Ko et al., 2004b) demonstrated that the changes that occur in the urogenital sinus epithelium that are responsible for the inhibited prostate budding are secondary to those initiated in the mesenchyme.

TCDD harms the reproductive systems of immature and adult female animals. Salisbury and Marcinkiewicz (2002) reported that in utero and lactational exposure to TCDD disrupted estrous cycles and inhibited ovulation rates in female offspring. Decreased rates of ovulation occurred in the presence of exogenous gonadotrophins, suggesting that TCDD directly affects the ovaries.

Several reports of studies in animals and exposed humans suggest that perinatal exposure to TCDD or to dioxin-like compounds can impair brain development. Rats exposed in utero to TCDD at 1 µg/kg maternal body weight showed deficits in spatial discrimination–reversal learning (RL) tasks but showed an increase in task spatial learning and memory (*Update 2002*). Prenatal exposure of primates to TCDD facilitated some spatial tasks and impaired visual RL tasks (IOM, 2003). Other research with rats exposed in utero to TCDD has resulted in reports of dose-dependent reductions in the number of revolutions on running wheels, in lever response rates, and in accuracy in lever chambers (IOM, 2003). GABA neurons in the brain are targets of TCDD; virtually all GABA neurons expressed the AhR gene (IOM, 2003). It has been proposed that GABAergic neurons in the brain are targets of TCDD that mediate developmental effects via affecting GAD67 gene expression in the preoptic area of the brain that controls reproductive functions (Hays et al., 2002).

Zareba et al. (2002) used male and female Sprague-Dawley rats to determine the effects of TCDD exposure in utero on brain cortical dominance. The rats were exposed to TCDD at 0, 20, 60, or 180 ng/kg gestational day 18 (Zareba et al., 2002). In TCDD-treated males, several brain areas reversed from right hemispheric dominance to left hemispheric dominance; in the females, brain dominance moved from left (normal) toward right hemispheric dominance. Motor activity was not affected in the TCDD-treated offspring. In agreement, Kakeyama et al. (2003) determined that perinatal exposure of rats significantly altered sexual behavior of male offspring. In normal animals, mating behavior induces c-fos mRNA in the preoptic area and brain-derived neurotrophic factor (BDNF) mRNA

in the frontal cortex. The investigators determined that TCDD exposure decreased the up-regulation of BDNF, but that it had no effect on c-fos expression. Another study examined the effects of gestational and lactational exposure to TCDD on spatial and visual discrimination/RL in Sprague-Dawley rats using two-lever operant testing chambers (Widholm et al., 2003). The mothers were administered TCDD orally at 0.1 µg/kg on gestational days 10–16. TCDD-exposed rats made more errors in spatial RL when they were beginning to learn new reinforcement contingencies, but no overall differences in the number of errors committed were noted between the TCDD-exposed and the control rats. TCDD-induced visual RL effects in males resulted in a reduction in errors on original learning; in females, there was a reduction in errors on the second reversal. The authors concluded that alterations in cognitive function after early exposure to TCDD are subtle and, under some conditions, that learning is facilitated rather than impaired. Ishizuka et al. (2003) reported that low-dose TCDD treatment of pregnant rats on gestational day 15 altered the sex-dependent expression of hepatic CYP2C11. Together those data are consistent with the hypothesis that perinatal exposure to TCDD changes the sexual differentiation of the neonatal brain in male rats.

Yamaguchi et al. (2003) reported that low (0.01–1 pg/mL) exposure to TCDD in vitro disrupted glial differentiation in the presence of low toluene exposure by up-regulating the synthesis of glial fibrillary acidic protein at the translational level. Hill et al. (2003) reported that exposure of zebrafish embryos to TCDD caused a 30% reduction in the total number of neurons in the 168-h brain. It was linked to decreased expression of the developmentally regulated genes *neurogenin* and *sonic hedgehog*. Publications from two laboratories indicate the importance of the AhR for neuronal development in *Caenorhabditis elegans*, although the AhR in this species apparently lacks the ability to bind to TCDD (Huang et al., 2004; Qin and Powell-Coffman, 2004).

Embryos from cynomologus macaques treated with a single dose of TCDD (4 µg/kg) on gestational days 15 or 20 were examined to determine morphology of the developing neural tube (Moran et al., 2004). Maternal blood was analyzed for fatty acid concentrations. The TCDD-treated embryos exhibited increased cell death and intracellular spaces in the neural tube. Significant decreases were observed in the n-3 (40–60%) and n-6 (47–57%) essential fatty acids in the treated pregnancies. The authors suggest that because neural tube development depends, in part on n-3 and n-6 fatty acids, it is possible that the effect resulted in the observed defects in early brain development. Stanton et al. (2003) examined the relationship between TCDD-elicited alterations of serum fatty acid concentrations and alterations in brain symmetry in neonatal chickens. Altered fatty acid concentrations correlated with hemispheric differences in dorsal width and angle and in dorsal–tectal length, suggesting that altered fatty acid concentrations are associated with altered brain morphology induced during development by TCDD.

SUMMARY OF TOXICITY PROFILES

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

2,4-D

Most studies of 2,4-D have reported it to be relatively non-toxic; health effects are exhibited in animals only at high doses. Tissue uptake of 2,4-D is poor and metabolism fairly rapid, which could partially explain its low toxicity.

Earlier studies demonstrated that high doses of 2,4-D can cause behavioral effects, muscle weakness, and coordination problems in animals. A recent study indicated effects on the nervous system in humans, but at very high levels of exposure. Since *Update 2002,* there were no additional relevant studies to examine the effects of 2,4-D on the adult nervous system. The reproductive and developmental effects of 2,4-D also have been examined recently. Some studies suggest that oocytes and the preimplantation embryo may be especially sensitive to 2,4-D. Several studies cited in previous updates had suggested effects of 2,4-D on developing brain and recent studies are consistent with this. Elicited changes in brain neurotransmitter content appeared to correlate with behavioral alterations, and some of those changes appeared to be irreversible. Other studies suggested that undernourishment enhances the effects of 2,4-D on development. Recent studies are consistent with a weak effect on the immune system. Carcinogenicity tests of 2,4-D have generally been negative, and it is either non-genotoxic or only weakly mutagenic in the many assays used. However, recent investigations suggest that chemicals in commercial formulations (like Agent Orange) may enhance the genotoxicity of 2,4-D. Previous studies have suggested that 2,4-D might affect thyroid hormones (more specifically serum thyroxine). A recent study found 2,4-D had no effect on the ability of T3 to bind to serum thyroid hormone binding proteins.

Mechanistic studies have been conducted for 2,4-D that show 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, also have been reported for 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 together, the experimental data reviewed here and in previous reports indicate that pure 2,4-D is relatively non-toxic, but that it causes neurodevelopmental effects after neonatal exposure (at 100 mg/kg body weight per day). The herbicide 2,4-D, however, was often contaminated with dioxins other than 2,3,7,8-TCDD.

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 non-toxic. No relevant studies on the disease outcomes in experimental animals after exposure to 2,4,5-T have been published since *Update 2002*. Previous studies indicate that 2,4,5-T is absorbed into the body after oral exposure; 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 also has been investigated; no indications of carcinogenicity were seen. Studies 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 does 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 non-toxic.

Cacodylic Acid

Cacodylic acid, or DMA, is a metabolite of inorganic arsenic. 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 (Chapter 2), 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. However, the trivalent methylated forms of arsenic, DMA^{III} and MMA^{III}, have been shown to cause toxic effects; after acute exposure MMA^{III} is about 4 times more toxic than is inorganic arsenic, and DMA^{III}'s toxicity is similar 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 blood cells and tissues.

Few animal studies are available on the non-cancer health effects of cacodylic acid, but previous reports indicate that high, maternally toxic doses are fetotoxic and teratogenic in rats and mice. There is evidence that DMA can promote skin tumorigenesis in animals that is initiated chemically or with ultraviolet radiation. Evidence of cacodylic acid's pulmonary and bladder carcinogenic activity has been presented for mice and rats, respectively. In other studies, however, cacodylic acid did not promote kidney tumors or lung tumors in nitrosamine-initiated rats. In recent studies, high exposure levels of DMA have also been

shown to induce urinary bladder tumors in rats, and increase the total number of tumors present in both wild-type and p53 +/- mice.

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 (DMA) acts through induction of oxidative damage or damage to DNA. Recent studies demonstrate that DMA is a potent inducer of apoptosis (or programmed cell death) and this is consistent with previous studies on cacodylic acid.

Picloram

Few studies have examined the toxicity of picloram, but those done indicate that it is relatively non-toxic. No relevant studies of picloram have been published since *Update 2002*. Two of three carcinogenicity studies reviewed in *VAO* indicate that picloram 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 had resulted from hexachlorobenzene (HCB) contamination. 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 have been seen that are considered treatment related. Notably, a study of the male-mediated reproductive toxicity of Tordon 75D® (a commercial mixture of 2,4-D and picloram) reported 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. The immunotoxic effects observed with this commercial mixture may be due to their contamination with dioxin-like compounds. 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 non-toxic.

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 that it is transferred to neonates through lactation. The enzyme cytochrome P450 1A2 (CYP1A2) is important 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 clinical studies of two women

exposed to very high amounts of TCDD, however, revealed an elimination half-life of 1.5 and 2.9 years in the more and less exposed women, 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 it is from 3 to 16 years after exposure. This, however, makes back-extrapolation from current to original levels in exposed individuals tenuous at best, especially because individual differences in elimination rates could be substantial. Those data on half-life are consistent with a two-compartment toxicokinetic model for TCDD, but a more complex PBPK model would provide a more satisfactory fit. Those findings emphasize the difficulties of attempts to extrapolate back to original exposures of exposed individuals, especially Vietnam veterans. 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 study in rats demonstrated that dietary seaweed can increase TCDD excretion. Other studies indicted that the consumption of insoluble dietary fiber or nori, a Japanese dietary item prepared from red algae, can increase the elimination of TCDD, but only slightly. The apparent correlation between TCDD half-life and body weight may actually be best explained in terms of body composition: the greater the portion of body composition represented by adipose tissue, the longer the half-life. TCDD concentrations are often measured in blood, and autopsy studies indicate that blood concentrations correlate with tissue concentrations. Studies also have been conducted to validate PBPK 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 were the active ingredients of the herbicides used in Vietnam. Sensitivity to the lethal effects of TCDD varies among species and strains, but after acutely toxic doses most species studied develop a wasting syndrome that is characterized by a loss of body weight and fatty tissue. Several recent reports suggest that this may be due to direct effects of TCDD on the hypothalamus. One target of TCDD is the liver, in which lethal doses of TCDD cause necrosis, although the effect depends on the species. Effects on the structure and function of the liver are also seen at lower doses. Several recent studies demonstrated that TCDD significantly disrupts the homeostasis of vitamin A in the liver. TCDD could affect, directly or indirectly, many organs of the endocrine system in a species-specific manner. Thyroid hormone concentrations have been shown to be affected, although some study results are contradictory and species specific, making interpretation of those data and a determination of their relevance to humans difficult.

The adult nervous system has been shown to be sensitive to TCDD only at high doses. After in utero exposure, however, the developing brain appears to be more sensitive. In utero TCDD exposure decreases performance in some learning

and memory tasks but improves performance in others. Several studies reported that low-dose in utero exposure altered learning and memory, hearing, sexual behavior, and the sex-dependent expression of a hepatic protein in male offspring.

The immune systems of animals are particularly sensitive to TCDD. Recent studies have demonstrated that TCDD can alter the number 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.

Previous studies indicated that TCDD exposure increases the prevalence and severity of endometriosis in non-human primates. However, the data in rodent models show mixed results. Several recently published reviews of the literature are not in agreement as to whether there is evidence to support the hypothesis that dioxin exposure may lead to the development of endometriosis. Reproductive and developmental effects have been seen in animals exposed to TCDD; TCDD exposure affects sperm count, sperm production, and seminal vesicle weights in male offspring and affects the reproductive systems of female offspring. In some recent studies, however, the reproductive-system effects were not accompanied by effects on reproductive outcomes. Effects on the developing cardiovascular system also have been seen in several animal species after TCDD exposure. However, there is little evidence that the cardiovascular system is a target of TCDD in adult animals. TCDD has been shown to affect the thyroid and thyroid homones in several animal species. Possible mechanisms include the displacement of hormones from serum transport proteins, altering deiodinase activity, and increasing thyroid hormone catabolism. The relevance of these data to exposed veterans is not clear.

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 recently completed 2-year bioassay in female rats indicated increased incidences of cholangiocarcinoma and hepatocellular adenoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. A decrease in cancers of the uterus; the pancreas; and the pituitary, mammary, and adrenal glands also has been seen previously. Most of those tumors decreased only at the high dose and the decrease was associated with decreases in body weight gain. The decrease in mammary tumors was seen in one study. A previous study showed an increase in hepatic foci in rats at TCDD doses as low as 0.01 ng/kg body weight per day—the lowest dose of TCDD known to promote tumors. In addition, promotion of liver tumors by TCDD in female rats depends on continuous exposure. In a recent study, TCDD was found to immortalize keratinocytes, and this was suggested to be a possible mechanism by which this chemical may lead to malignancy.

Data published since *Update 2002* are consistent with the hypothesis that TCDD produces most or all of its effects by binding to a protein that regulates

gene expression, the 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 cellular events that include the induction of numerous proteins. Research on animals that have been engineered not to express the AhR and on animals with slightly different forms of the AhR provides evidence that the AhR is a necessary mediator for the toxicity of TCDD. Modulation of genes by the AhR appears to have species-, cell-, and developmental-stage-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 on the cellular effects of TCDD, details of the mechanisms that underlie its effects have not been elucidated. Possible mechanisms discussed in this chapter include effects on protein kinase expression, effects on vitamin stores, effects on cellular differentiation and the cell cycle, and oxidative stress. Although the mechanisms underlying the carcinogenic effects of TCDD remain 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

Exposure to TCDD has been associated with cancer and non-cancer endpoints in animals, and most, if not all, TCDD effects are mediated through the AhR. Although structural differences in the AhR have been identified, it operates similarly in animals and humans, and a connection between TCDD exposure and human health effects is, in general, considered biologically plausible. Animal research indicates that exposure to TCDD can cause cancers and benign tumors, and that it can increase the incidence of some cancers or tumors in the presence of known carcinogens. However, experimental animals differ greatly in susceptibility to TCDD-induced effects, and the sites at which tumors are induced vary from species to species. Non-cancer health effects also vary according to dose, time, and species. Whether the effects of TCDD and other exposures are threshold dependent—that is, whether some exposures are too low to induce any effect—is an open question. The relationship between mechanism and the shape of the dose–response curve—linear or non-linear—is complex, not well understood, and could be different for different endpoints.

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 non-dioxin 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 non-human studies to an evaluation of the potential health effects of herbicide or dioxin exposure in Vietnam veterans. Although 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 studies of laboratory 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 those 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

Epidemiology

In the tradition of previous *Veterans and Agent Orange* (*VAO*) reports, this chapter summarizes certain epidemiology studies considered in the ongoing effort to evaluate and integrate all study results on human subjects pertinent to health effects that might result from exposure to any of the chemicals of interest (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]; 4-amino-3,5,6-trichloropicolinic acid [picloram]; and cacodylic acid [dimenthylarsenic acid]). Primary emphasis is put on describing work new to this review contained in publications appearing since *Veterans and Agent Orange: Update 2002* (hereafter, *Update 2002*) (IOM, 2003).

The framework adopted in this chapter provides a means of organizing the thousands of citations considered over the successive updates. The organization into occupational studies, environmental studies, and studies of Vietnam veterans, which is carried over into the discussions and results tables in the health outcomes chapters, is not intended to imply that any of these populations is intrinsically more valuable for the committee's purpose. Each study design has strengths and weaknesses (see Chapter 2) influencing its potential to contribute evidence of an association with the health outcomes considered in Chapters 6–9 of this report. Critical commentary has been reserved for the individual health outcome chapters and is not included in this chapter. The associated cumulative tables in Appendix A include the basic type of study design; criteria for sample selection; the numbers of subjects and comparison populations; how data were collected; and how exposure was determined.

The major purpose of Chapter 4 is to reduce repetition of design information

in the health outcomes chapters from endpoint to endpoint, and also from update to update. Of particular importance to the *VAO* project are a number of continuing studies of populations that have been exposed to the herbicides sprayed in Vietnam or to their components. It is essential that laboriously amassed information on a single population be recognized as such. Placing each new publication in historical context helps the committee avoid factoring what is actually a single observation into their deliberations repeatedly. Such studies are extremely important in describing the time course of a population's response to an exposure. Furthermore, joint consideration of an entire body of research on a population may permit more insightful evaluation of relationships with potential confounding factors. This chapter augments the existing information on these study populations with descriptions of any new publications investigating any of their members, explaining how the new work meshes with earlier efforts.

Many studies, particularly cancer cohort studies, report on multiple health endpoints. The weight appropriately attributed to a study's findings is determined in the context of its design and execution. Because repetition of this information in the health outcomes chapters for every specific endpoint report would be cumbersome and tedious, discussions of design and evaluation comments for these studies are presented in this chapter and distilled in the design tables in Appendix A. Of course, a multi-endpoint study might also qualify for inclusion in this chapter because it addresses a previously studied population.

Studies new to this update that report on a single endpoint and were conducted on a population that has not been studied by others are not included in this chapter. Their designs characteristics are present in the one place where their results are reported in one of the chapters on health outcomes.

The chapter is organized into three major sections—occupational studies, environmental studies, and studies of Vietnam veterans. Detailed descriptions of many of the study populations can be found in Chapter 2 of the original report of this committee, *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (hereafter referred to as *VAO*; IOM, 1994), and the criteria for inclusion in the review are discussed in Appendix A of that report. In addition to a review of studies that involved exposures to the chemicals of interest (2,4-D; 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, hexachlorophene, and chlorophenols, including trichlorophenol. In some published reports, the study investigators did not indicate the specific herbicides to which study participants were exposed or the magnitude of exposure; those complicating factors were considered when the committee weighed the relevance of a study. Available details of exposure assessment and use of exposure in analyses are discussed in Chapter 5.

The occupational section covers studies of production workers, agriculture and forestry workers (including herbicide and pesticide appliers), and paper and

pulp workers. The environmental section covers studies of populations unintentionally exposed to unusually high 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 discusses studies of Australian and Korean Vietnam veterans.

Many cohorts potentially exposed to any of the chemicals of interest are monitored periodically, typically every 3–5 years. Those groups include 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), residents of Seveso, and Ranch Hand personnel. 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 to new studies. However, in making its conclusions, the committee focused on the most recent update when multiple reports on the same cohorts and endpoints were 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 of the studies are discussed in this report, but when making its conclusions, the committee focused on the studies of the larger, multicenter cohorts.

OCCUPATIONAL STUDIES

Several occupational groups in the United States and elsewhere have been exposed to the compounds of interest. Exposure characterization varies widely in the exposure metric used; the extent of detail; confounding by other exposures; and whether individual, surrogate, or group (ecologic) measures are used. Some studies use job titles as broad surrogates of exposure, others rely on disease registry data. Occupational groups include workers in chemical production plants; agriculture and forestry workers, including farmers and herbicide appliers; and workers in paper and pulp manufacturing.

Production Workers

National Institute for Occupational Safety and Health

Starting in 1978, NIOSH began a study to identify all US workers who might have been exposed to TCDD between 1942 and 1984 (Fingerhut et al., 1991).

From a total of 12 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 substances for which TCDD was a contaminant: 2,4,5-trichlorophenol (TCP); 2,4,5-T, Silvex®; Erbon®; Ronnel®; and hexachlorophene. Another 172 workers identified previously by their employers as being exposed to TCDD also were 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 compounds, some of which could be toxic and 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 chemical manufacturing at a plant in Newark, New Jersey, between 1951 and 1969, and at a plant in Verona, Missouri, between 1968 and 1969 and 1970 and 1972. Control subjects were recruited from surrounding neighborhoods (Alderfer et al., 1992; Calvert et al., 1991, 1992; Sweeney et al., 1989, 1993). The New Jersey plant manufactured TCP and 2,4,5-T; the Missouri plant manufactured TCP, 2,4,5-T, and hexachlorophene.

Later studies examined 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 non-cancer endpoints, including liver function, gastrointestinal disorders, chloracne, serum glucose concentration, hormone and lipid concentrations, and diabetes in a subgroup of the original cohort studied by Calvert et al. (1991). More recent studies of the main cohort examined cardiovascular effects (Calvert et al., 1998); diabetes mellitus, thyroid function, and endocrine function (Calvert et al., 1999); immune characteristics (Halperin et al., 1998); and cancer incidence (Kayajanian, 2002). Cross-sectional medical surveys reported serum TCDD concentrations and surrogates of cytochrome P450 induction (Halperin et al., 1995) in that cohort. A follow-up study (Steenland et al., 1999) examined the association between TCDD exposure and cause of death; it examined specific health outcomes, including cancer (all and site-specific), respiratory disease, cardiovascular disease, and diabetes. Steenland et al. (2001) published a paper that reanalyzed data from two 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). *VAO*, *Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996* [IOM, 1996]), *Update 1998* (IOM, 1999), *Update 2000* (IOM, 2001), and *Update 2002* (IOM, 2003), describe the details of those studies.

Bodner et al. (2003) compared the mortality in Dow Chemical Company workers with mortality among the NIOSH and IARC cohorts. Study details are in the Dow Chemical Company section of this chapter.

Monsanto

The NIOSH study cohort (Fingerhut et al., 1991) included employees of Monsanto's production facilities. One set of studies was based on an unintentional release that occurred on March 8, 1949, in the TCP production process at the Monsanto plant in Nitro, West Virginia (Collins et al., 1993; Moses et al., 1984; Zack and Suskind, 1980). Others focused on exposure of workers involved in numerous aspects of 2,4,5-T production (Moses et al., 1984; Suskind and Hertzberg, 1984; Zack and Gaffey, 1983). The Monsanto studies are discussed in more detail in *VAO*. No new studies have been published on those subjects.

Dow Chemical Company

Several studies of Dow Chemical Company production workers are summarized in *VAO, Update 1996, Update 1998*, and *Update 2002*. The populations of many of those studies were included in the NIOSH cohort (Fingerhut et al., 1991). Originally, Dow conducted a study of workers engaged in the production of 2,4,5-T (Ott et al., 1980) and one on TCP-manufacturing workers with 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 with 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) and a large-scale cohort mortality study of workers exposed to herbicides in several of its plants (Bloemen et al., 1993; Bond et al., 1988; Burns et al., 2001; Ramlow et al., 1996) also were conducted.

Dow assembled a large cohort at the Midland, Michigan, plant (Bond et al., 1989a; Cook et al., 1986, 1987). Exposure to TCDD was characterized in that cohort on the basis of chloracne diagnosis (Bond et al., 1989b). Within that 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.

Since *Update 2002*, Bodner et al. (2003) have published a 10-year follow-up of the work of Cook et al. (1986), comparing the mortality experience of 2,187 male Dow Chemical Company workers potentially exposed to large amounts of dioxin before 1983 with that of the NIOSH and IARC cohorts. Worker exposures were determined by combining detailed work histories with an analysis of historical plant operations and industrial hygiene monitoring. All of the workers in the analysis are male; 5 female workers were excluded from the analysis. Diagnosis of chloracne resulting from high exposure incidence corroborated many of the

exposures. The IARC international study and the NIOSH Dioxin Registry include most of the Dow workers; Dow workers make up the largest component of those two cohorts. A modified life table program was used to calculate standardized mortality ratio and 95% confidence intervals relative to the US male population. A pool of local workers with no previous work history in the targeted departments and no known dioxin exposures served as the reference population. Internal analyses were stratified by age, calendar year, and hourly salary status.

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*, *Update 1998*, and *Update 2000* summarized studies on those workers, including 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 2000.*

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 registry includes information on mortality and exposure of 18,390 workers—16,863 men and 1,527 women. *Update 1996* described the individual national cohorts included in the registry.

One study of people from 10 countries evaluated cancer mortality from STS and malignant lymphoma (Kogevinas et al., 1992). Two nested case–control studies were undertaken using the IARC cohort to evaluate the relationship between STS and non-Hodgkin's lymphoma (NHL) (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) to study cancer mortality. Vena et al. (1998) studied non-neoplastic mortality in the IARC cohorts. A cohort study of cancer incidence and mortality was conducted among 701 women from 7 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.

Several of the smaller cohorts that make up the IARC cohort have been evaluated apart from the IARC-coordinated efforts. They include Danish produc-

tion workers (Lynge, 1985, 1993); British production workers (Coggon et al., 1986, 1991); Dutch production workers (Bueno de Mesquita et al., 1993); German production workers (Becher et al., 1996; Flesch-Janys, 1997; Flesch-Janys et al., 1995; Manz et al., 1991); factory workers from the Netherlands (Hooiveld et al., 1998); and Austrian production workers (Jäger et al., 1998; Neuberger et al., 1998, 1999). *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 on the smaller constituent cohorts.

Since *Update 2002,* Bodner et al. (2003) published a study comparing the mortality experience of Dow Chemical Company workers with that of the NIOSH and IARC cohorts. Study details can be found in this chapter under the heading Dow Chemical Company.

Other Chemical Plants

Studies have reviewed health outcomes among UK chemical workers exposed to TCDD as a result of an industrial accident in 1968 (Jennings et al., 1988; May, 1982, 1983); 2,4-D production workers in the former Soviet Union (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 pentachlorophenol, lower-chlorinated phenols, and esters of chlorophenoxy acids (Hryhorczuk et al., 1998). The long-term immune-system effects of TCDD were examined 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 were studied 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*.

Agriculture and Forestry Workers

Cohort Studies

Agriculture *VAO*, *Update 1996, Update 1998, Update 2000*, and *Update 2002* detailed cohort studies that examined health outcomes in people involved in agriculture. 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 pesti-

cides in Italy's southern Piedmont (Corrao et al., 1989) and among female gardeners in Denmark (Hansen et al., 1992); sperm abnormalities among Argentinian farmers (Lerda and Rizzi, 1991); cancer and birth defects among the offspring of Norwegian farmers (Kristensen et al., 1997); pregnancy outcomes in couples living on family farms in Ontario, Canada (Arbuckle et al., 1999, 2001; Curtis et al., 1999; Savitz et al., 1997); and immune, neurobehavioral, and lung function of residents from an agricultural area of Saskatchewan, Canada, and immunologic changes in 10 farmers who mixed and applied commercial formulations that contained the chlorophenoxy herbicides (Faustini et al., 1996). The Mortality Study of Canadian Male Farm Operators evaluated the risk to farmers of death and specific health outcomes: NHL (Morrison et al., 1994; Wigle et al., 1990), prostatic 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). Data from the Swedish Cancer Environment Register (which links population census data, including occupation, with the Swedish Cancer Registry) were used in cohort studies that 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 occupational activities (Eriksson et al., 1992). Brain, lymphatic, and hematopoietic cancers in Irish agricultural workers also have been studied (Dean, 1994). No new studies of agricultural workers have been published since *Update 2002*.

Forestry Studies have been conducted among forestry workers potentially exposed to the types of herbicides used in Vietnam. A cohort mortality study examined men employed at a Canadian public utility (Green, 1987, 1991), a Dutch study of forestry workers exposed to 2,4,5-T investigated the prevalence of acne and liver dysfunction (van Houdt et al., 1983), and another study examined mortality and cancer incidence in a cohort of Swedish lumberjacks (Thörn et al., 2000). No new studies of forestry workers have been published since *Update 2002*.

Herbicide and Pesticide Application Several cohort studies have assessed health outcomes among herbicide and pesticide appliers: 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 (Asp et al., 1994; Riihimaki et al., 1982, 1983), reproductive outcomes among male chemical appliers in New Zealand (Smith et al., 1981, 1982), and doctor visits resulting from pesticide exposure (Alavanja et al., 1998) and chemical predictors of wheeze (Hoppin et al., 2002) in Iowa and North Carolina. 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 who spent a portion of their work year applying pesticides (Barthel, 1981), British Columbia sawmill workers potentially exposed to chlorophenate wood preservatives used as a fungicide during spraying and dipping of sawed logs and planed boards (Dimich-Ward et al., 1996; Heacock et al. 1998; Hertzman et al., 1997), and cancer risk among pesticide users in Iceland (Zhong and Rafnsson, 1996). Some of those studies included agriculture and forestry worker cohorts; details of the studies, designs, and results are included in *VAO*, *Update 1996, Update 1998, Update 2000*, and *Update 2002.*

Since *Update 2002*, Alavanja et al. (2003) examined the correlation between exposure to agricultural pesticides, including 2,4-D and 2,4,5-T and prostate cancer incidence among pesticide appliers in Iowa and North Carolina in the Agricultural Health Study. The study cohort consisted of 55,332 male commercial and private pesticide appliers who completed a self-administered enrollment questionnaire between December 1993 and 1997, and who had no history of prostate cancer. The enrollment questionnaire sought information on a variety of issues: pesticide exposures, uses, and practices; dietary and cooking practices; personal medical history; and tobacco and alcohol use. A subset of 24,034 appliers also completed a take-home questionnaire that sought more specific information on similar topics. Members of the cohort were matched to Iowa and North Carolina cancer registry files, and to state and national death registries for vital statistics. Standardized incidence for prostate cancer was computed. Researchers used multivariate and unconditional logistic regression and factor analysis to evaluate and interpret the data.

Flower et al. (2004) examined the correlation between parental pesticide exposure and cancer risk in the children of pesticide appliers in the AHS cohort. That group consisted of 20,625 appliers and spouses who completed enrollment and secondary questionnaires on female and family health. Completed questionnaires identified 21,375 children born during or after 1975. Cancer cases within the group of children were identified both retrospectively and prospectively after enrollment in the study. In Iowa, 50 cases of children diagnosed with cancer between birth and 19 years of age were identified from the questionnaires and verified through cancer registries; childhood cancer cases in North Carolina were excluded from the study because too few were identified. Parental pesticide exposure data collected through the questionnaires included information on the mixing and application of pesticides and the use of protective equipment. Logistic regression was used to compute odds ratios and 95% confidence intervals.

Confounders were analyzed, but were excluded from the final models when the researchers deemed them insignificant.

Swaen et al. (2004) published an updated mortality study of a cohort of 1,341 licensed herbicide appliers in the Netherlands (Swaen et al., 1992), extending the follow-up by 13 years (1988–2001). They reviewed information on the types and amounts of herbicides used in all municipal spraying projects in 1980; those data could not be linked to the work of any individual appliers. Mortality, using International Classification of Disease (ICD) coding, was determined from a cause-of-death registry. SMRs were calculated based on cause-specific mortality rates from the Netherlands.

Case–Control Studies

In 1977, case-series reports in Sweden (Hardell, 1977, 1979) of a potential connection between STS and exposure to phenoxyacetic acids prompted several case–control investigations of a possible association (Eriksson et al., 1979, 1981, 1990; Hardell and Eriksson, 1988; Hardell and Sandstrom, 1979; Wingren et al., 1990). After the initial STS reports (Hardell, 1977, 1979), case–control studies of other cancer outcomes including HD, NHL, and other lymphomas were conducted in Sweden (Hardell and Bengtsson, 1983; Hardell et al., 1980, 1981). Also studied were HD and NHL (Persson et al., 1989, 1993); NHL (Hardell and Eriksson, 1999; Olsson and Brandt, 1988); 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), a set of case–control studies in New Zealand evaluated the association between phenoxy herbicide and chlorophenol exposure and STS incidence and mortality (Smith and Pearce, 1986; Smith et al., 1983, 1984). 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 for other cancers, including NHL (Cantor, 1982; Cantor et al., 1992; Tatham et al., 1997; Zahm et al., 1993); multiple myeloma (Boffetta et al., 1989; Brown et al., 1993; Morris et al., 1986); cancers of the stomach, 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 and Polissar, 1989; Woods et al., 1987).

Other studies outside the United States have examined 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). 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.

Non-cancer endpoints also have been investigated in case–control studies: spontaneous abortion (Carmelli et al., 1981); congenital malformations (García et al., 1998); immunosuppression and subsequent 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 agriculture workers (Nurminen et al., 1994). Those studies are discussed in detail in *VAO, Update 1996,* and *Update 1998*. No new case–control studies of agriculture and forestry 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 can be generated by the bleaching process during the production and treatment of paper and paper products. *VAO* describes studies of pulp and paper

mill workers potentially exposed to TCDD 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 paper mill workers in Finland (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* described studies of cancer risk among workers in the Danish paper industry (Rix et al., 1998) and oral cancer risk among occupationally exposed workers in Sweden (Schildt et al., 1999). No new studies of paper and pulp workers have been published since *Update 2002*.

ENVIRONMENTAL STUDIES

The occurrence of industrial accidents has led to the evaluation of the long-term health outcomes of exposure to the compounds of interest.

Seveso, Italy

Among the largest industrial accidents resulting in environmental exposures to TCDD was one in Seveso, Italy, in July 1976 that resulted from an uncontrolled reaction during trichlorophenol production. TCDD contamination of soil has been the most extensively used of the indicators for estimating individual exposure. Three areas were defined on the basis of soil sampling: zone A, the most heavily contaminated, from which all residents were evacuated within 20 days; zone B, an area of lower contamination that all children and women in the 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, Update 2000*, and *Update 2002* and are summarized here.

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

Other studies examined specific health effects associated with TCDD exposure among Seveso residents: 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 (Bertazzi et al., 1993; Pesatori et al., 1992, 1993); breast cancer (Warner et al.,

2002); 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 non-cancer mortality.

Since *Update 2002,* Eskenazi et al. (2002a,b, 2003, 2004) have published 4 studies that used data from the Seveso Women's Health Study (SWHS) to evaluate the association between individual serum TCDD and reproductive effects in women who resided in Seveso at the time of the accident in 1976. The study group consisted of 981 volunteers who were between infancy and age 40 at the time of the accident, who had resided in zones A or B, and for whom there was adequate stored serum collected shortly after the explosion for TCDD measurements.

Eskenazi et al. (2002a) studied the association between menstrual cycle characteristics and serum TCDD. That study group consisted of 301 women from the original SWHS cohort of 981; women who were over the age of 44, who had surgical or natural menopause, who had Turner syndrome, or who were pregnant, breastfeeding, or had used an intrauterine device or hormonal medicine such as contraceptives within the previous year were excluded from this study. Researchers who were blinded to participants' serum TCDD and zones of residence obtained sociodemographic data, information about personal habits, work histories, and medical and reproductive histories (gynecologic, menstrual, and pregnancy related factors). Logistic regression was used to study the relationship of TCDD to irregular cycle length and heaviness of menstrual flow; multiple linear regression was used to examine TCDD and cycle length and days of flow. Confounders—age at interview, education, parity, smoking, body mass index (BMI), alcohol and coffee consumption, age at menarche, sexual activity, hours of work and physical exercise per week, chronic illness, and abdominal surgeries—were factored into the final results. The final analyses were rerun to exclude women over the age of 40 to eliminate perimenopausal women.

Another study by Eskenazi et al. (2002b) examined the association between endometriosis and serum TCDD concentration. The group consisted of 601 SWHS participants who had consented to participate; women who were 30 years of age or younger at the time of the accident or who were virgins, had Turner syndrome, or refused examinations or ultrasound were excluded. Study participants gave blood samples; received gynecologic and ultrasound examinations; and were interviewed about their sociodemographic information, personal habits, and work and medical history, including gynecologic and reproductive histories. Detailed information about pelvic pain, pain during intercourse, and menstrual

cramps was collected during interviews. Endometriosis was confirmed by laparoscopy, laparotomy, or ultrasound examination. Results were analyzed in association with serum TCDD.

Eskenazi et al. (2003) examined the association between maternal serum TCDD and birth outcome. The researchers identified 888 pregnancies that occurred in 510 women from the original SWHS cohort in the years after the Seveso accident. An analysis of spontaneous abortion identified 769 pregnancies (476 women) that did not end in voluntary abortions or ectopic or molar pregnancies. Study participants provided information about sociodemographic characteristics; personal habits; work history; and detailed medical histories that included gynecologic, menstrual, pregnancy (outcome; date pregnancy ended; length of pregnancy; birth weight, sex, and presence of congenital anomalies or developmental disorders in offspring), and other medical information. Statistical analyses tracked pregnancy outcome in relation to time elapsed since the accident, and logistic regression examined the relationship between serum TCDD and spontaneous abortion, preterm delivery, and the ratio of size to gestational age in offspring. Further analysis evaluated the relationship between serum TCDD and birth weight and gestational age. Confounders, including maternal age at pregnancy, maternal tobacco and alcohol use, and years from pregnancy to interview also were considered.

The most recent study by Eskenazi et al. (2004) examined the relationship between serum TCDD concentration and age at exposure of female Seveso residents. Of the SWHS cohort, 92%—899 of 981 women—had serum TCDD concentrations measured between 1976 and 1977, 54 (5%) serum measurements between 1978 and 1981, and 28 (3%) for whom there was inadequate stored serum had serum measured in 1996. For women whose serum TCDD measured >10 parts per trillion (ppt) after 1977, TCDD exposure was extrapolated back to 1976. Serum TCDD from women in zones A and B was pooled by age to determine concentrations of 22 polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls (PCBs) by high-resolution gas chromatography and mass spectrometry. Multiple linear regression was used to determine associations within zones A and B between serum log TCDD concentrations and exposure-related covariates—reports of chloracne, consuming homegrown produce, finding dead animals on their property, and being indoors versus outdoors at the time of the accident. Additional analysis calculated the mean, standard deviation, and toxic equivalents from pooled samples.

Three studies since 2002 have used 62 randomly chosen subjects from zones A and B matched with 59 control subjects from uncontaminated areas who were matched for age, sex, and tobacco use (Baccarelli et al., 2002, 2004; Landi et al., 2003). Interviewers collected detailed personal medical histories and information about medicine use in the week before the study from all study participants. Internal dose of TCDD and 21 other dioxin or dioxin-like congeners was determined from participants' plasma by high-resolution gas chromatography–high-

resolution mass spectrometric analysis. Baccarelli et al. (2002) studied immunologic effects among the cohort of Seveso residents and compared the results with those in previously published studies. The plasma immunoglobulins IgA, IgG, and IgM, and complement components C3 and C4 were quantified by common nephelometric methods. Plasma protein electrophoresis was used to exclude monoclonal immunoglobulins. Non-parametric tests were used for group comparisons, and simple and multiple regression analyses were used to assess correlations between variables—age, sex, tobacco and alcohol use, BMI, diet, medical history, and medication use—for possible confounding. A comprehensive literature search was conducted to identify any work on associations between TCDD and immunologic parameters that had been published from 1966 through 2001. Baccarelli et al. (2004) examined messenger ribonucleic acid (mRNA) concentrations of the arylhydrocarbon receptor (AhR), arylhydrocarbon receptor nuclear transporter (ARNT), cytochrome P450 1A1 (CYP1A1), and cytochrome P450 1B1 (CYP1B1) genes and 7-ethoxyresorufun-*O*-deethylase (EROD) activity in peripheral blood lymphocytes for the cohort. The population-based study by Landi et al. (2003) evaluated the effect of TCDD-mediated alterations in the AhR-dependent pathway in residents living in zones A and B in Seveso. Peripheral blood lymphocytes, extracted from study participant's plasma, were evaluated to measure the expression of several genes involved in the AhR pathway, specifically AhR, ARNT, and CYP1A1, CYP1B1, and CYP1A1-associated EROD activity. Those AhR pathways were further evaluated in relation to serum TCDD concentrations to determine whether associations existed between them and whether environmental or host factors affected the association.

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, including the Times Beach and Quail Run areas, for dust control. TCDD was a contaminant of the mixtures sprayed, and the contamination was reported by the Environmental Protection Agency. Several studies evaluated health effects attributable to 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* discussed those studies; no further work has been published.

Vietnam

Researchers in Vietnam have studied 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. That article also examined 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, two 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 of reproductive outcomes attendant to 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).

Other studies have focused on different outcomes of environmental exposure: 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); neuropsychological effects in Germany (Peper et al., 1993); early-onset PD 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); immunologic effects of prenatal and postnatal exposure to PCB or TCDD in Dutch infants from birth to 18 months of age (Weisglas-Kuperus et al., 1995); and public health and cytogenetic effects in residents of Chapaevsk, Russia (Revazova et al., 2001; Revich et al., 2001).

Fierens et al. (2003) completed a population-based cross-sectional study in several Belgian towns to assess the association between serum dioxin concentrations and the prevalence of diabetes and endometriosis. A total of 194 adults were recruited from households near likely sources of exposure to dioxins (an iron and steel plant, a waste-dumping site, and a municipal solid-waste incinerator). Employees of the aforementioned worksites were excluded from the study, given its focus on the effects of exposure in the general community environment. In selecting the sample, Fierens and co-workers specifically sought people who had

lived in the neighborhoods for a long time and who regularly consumed local produce. A comparison sample of 63 adults was chosen from a separate area with no known source of exposure to dioxins. Self-administered questionnaires identified 10 cases of endometriosis among the 142 female respondents. The presence of diabetes was based on self-report of physicians' diagnoses. Seventeen 2,3,7,8-polychlorinated dibenzodioxins or dibenzofurans were measured from fasting blood samples; the analyses were based on the sum of all dioxins in toxicity equivalents (TEQs) per gram of fat. (See the section "Exposure to Dioxin-like Compounds" in Chapter 5 for an explanation of how TEQs are used in the toxic equivalency factor method of comparing the relative toxicity of dioxin-like compounds.)

Fukuda et al. (2003) examined the correlation between incinerator dioxin emissions and mortality in 803 municipalities in Japan. Researchers used dioxin measurements, in TEQs, from incinerator emissions data collected by the Ministry of Health and Welfare to establish four dioxin-related municipal indices. Socioeconomic conditions—population and six health-related indicators—were evaluated and compared among the municipalities. Sex-specific and age-adjusted mortality rates were calculated using 1995 population data and mortality rates for all causes and five disease categories (stroke, ischemic heart disease, cancer at all sites, stomach cancer, lung cancer), according to municipality and sex and age categories in 1994–1996, data from a 1985 population model, and the average number of deaths for three consecutive years per municipality and category. Mortality rates were evaluated in relation to the dioxin emission data. Further calculations were done to evaluate the influence of socioeconomic conditions on mortality within the municipalities.

VIETNAM-VETERAN STUDIES

Studies of Vietnam veterans who might have been exposed to herbicides, including Agent Orange, have been conducted in the United States at the national and state levels and in Australia, Korea, and Vietnam. Exposures in those studies have been estimated by various means, and health outcomes have been evaluated with reference to various comparison or control groups. This section is organized primarily by research sponsor because it is more conducive to methodologic presentation of the articles. Exposure measures fall on a crude scale from individual exposures of Ranch Hand personnel, as reflected in serum TCDD measurements, to some statewide studies' use of service in Vietnam as a surrogate for TCDD exposure.

Several comparison groups have been used for veteran cohort studies: Vietnam veterans who were stationed in areas essentially not exposed to active herbicide missions and were unlikely to have been in areas sprayed with herbicides; Vietnam-era veterans who were in the military at the time of the conflict but did not serve in Vietnam; non-Vietnam veterans who served in other wars or con-

flicts such as the Korean War or World War II; and various US male populations (either state or national).

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), *Update 2000*, *Update 2002,* and *Veterans and Agent Orange: Length of Presumptive Period for Association Between Exposure and Respiratory Cancer* (IOM, 2004) discussed reports and papers addressing the cohort in more detail.

A retrospective matched-cohort study design was used to examine morbidity and mortality; follow-up was scheduled to continue until 2002. Records from the National Personnel Records Center and the US Air Force Human Resources Laboratory 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. Control subjects were individually matched for age, type of job (based on Air Force specialty code), and race (white or not white) to control for age-related effects, educational and socioeconomic status, and potential race-related differences in development of chronic disease. To control for many potential confounders related to the physical and psychophysiological effects of combat stress and the Southeast Asia environment, Ranch Hands were matched to control subjects who performed similar combat or combat-related jobs (AFHS, 1982). Rank also was used as a surrogate of exposure. Alcohol use and smoking were included in the analysis when they were known risk factors for the outcome of interest.

Ten matches formed a control set for each exposed subject. For the mortality study, the intent was to follow each exposed subject and a random sample of half of each subject's control set for 20 years in a 1:5 matched design. The morbidity component of follow-up consisted of a 1:1 matched design, with the first control randomized to the mortality ascertainment component of the study. If a control was noncompliant, another control from the matched "pool" was selected; controls who died were not replaced.

The baseline physical examination occurred in 1982; subsequent exams took

place in 1985, 1987, 1992, 1997, and 2002. Morbidity was ascertained through questionnaire and physical examination, which emphasized dermatologic, neurobehavioral, 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 non-exposed; baseline physical examination responses were 87% and 76%, respectively (Wolfe et al., 1990). 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).

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. Exposure could occur while operating spray equipment and through contact with herbicides in the aircraft.

Serum TCDD was measured in 1982 (36 Ranch Hands; Pirkle et al., 1989), 1987 (866 Ranch Hands; AFHS, 1991b), 1992 (455 Ranch Hands; AFHS, 1995), and 1997 (443 Ranch Hands; AFHS, 2000). Serum TCDD analysis of the 1987 follow-up examinations was published in 1991 (AFHS, 1991b).

Results have been published for 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 for the reproductive-outcomes study (AFHS, 1992; Michalek et al., 1998d; Wolfe et al., 1995). 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).

Other Ranch Hand publications have addressed the relationship between serum TCDD and reproductive hormones (Henriksen et al., 1996); diabetes mellitus, glucose, and insulin (Henriksen et al., 1997); skin disorders (Burton et al., 1998); infant death (Michalek et al., 1998a); sex ratios (Michalek et al.,

1998c); skin cancer (Ketchum et al., 1999); insulin, fasting glucose, and sex-hormone-binding globulin (Michalek et al., 1999a); immunologic responses (Michalek et al., 1999b); diabetes mellitus (Longnecker and Michalek, 2000; Steenland et al., 2001); cognitive function (Barrett et al., 2001); hepatic abnormalities (Michalek et al., 2001a); peripheral neuropathy (Michalek et al., 2001b); and hematologic results (Michalek et al., 2001c).

Since *Update 2002*, Akhtar et al. (2004) conducted a follow-up study of the work by Ketchum et al. (1999) comparing cancer incidence among US Air Force Veterans to Vietnam veterans who served in Southeast Asia but did not spray herbicides and US national cancer rates. Researchers used serum dioxin measurements from the 1987, 1992, and 1997 examinations; missing TCDD measurements from either of the later exams were extrapolated back to 1987 (the first exam in which serum TCDD was measured) using a half-life of 7.6 years. Cancer incidence was determined from physicals, interviews, and reviews of medical records. Veterans were categorized by tour date and time spent in Southeast Asia. Internal comparisons were made between Ranch Hand veterans who had existing serum dioxin measurements and who served in Vietnam ($N = 2,965$) and veterans who served in Southeast Asia but did not spray herbicides. External comparisons were made between participants who attended one of the first five physicals and who did not have cancer either before or during their tour ($N = 2,438$), and national cancer incidence and mortality rates; national statistics were analyzed according to anatomical site, sex, race, and 5-year intervals of age and calendar year, covering the period 1950–2000.

Barrett et al. (2003) studied the relationship between serum dioxin measurements and psychological functioning among the cohort of Ranch Hand veterans previously described by Wolfe et al. (1990). TCDD measurements from physical examinations through 1997 were analyzed and used to categorize 1,109 Ranch Hand and 1,493 comparison subjects as "background," "low," or "high," according to their current and initial dioxin concentrations. Veterans whose TCDD measurements were missing or non-quantifiable, and comparison subjects with TCDD concentrations greater than 10 ppt were excluded. As part of the physical exam, study participants completed one of two self-administered questionnaires—the Minnesota Multiphasic Personality Inventory (MMPI) (1982 and 1985 examinations) and the Million Clinical Multiaxial Inventory (MCMI) (1987 and 1992 examinations)—to evaluate personality status and emotional adjustment and basic personality characteristics and clinical disorders, respectively. Researchers analyzed T-scores and posttraumatic stress disorder (PTSD) subscales from the MMPI, and used MCMI results to evaluate personality patterns, pathological personality disorders, and clinical symptom syndromes. Adjustments were made for age, race, military rank, marital status, and combat exposure according to participants' responses relating to their military service.

A recent study by Michalek et al. (2003) used data from a pharmacokinetic study of TCDD in Ranch Hand veterans (Michelak and Tripathi, 1999) to examine

the correlation between Ranch Hand veterans with diabetes and TCDD elimination. The original study analyzed TCDD measurements from 343 Ranch Hand veterans during physical examinations through 1997; some veterans' TCDD measurements were missing because of follow-up medical deferral, broken blood bags, or death. Cases of diabetes mellitus were confirmed at the exams or through medical records, and they were included in the analysis only if diabetes was diagnosed after a veteran's last tour of duty through December 2000. A proportional-hazards model, logistic regression models, and analyses of covariance were used to study the relationships between TCDD serum values and diagnoses of diabetes mellitus in veterans; adjustments were made for age at the 1982 physical examination, logarithm of 1982 TCDD body burden, family history of diabetes, BMI at the end of the tour of duty in Vietnam, percentage change in BMI from end of tour to the 1982 physical examination, and smoking history.

Pavuk et al. (2003) studied the relationship of serum TCDD to thyroid function in the cohort of veterans of Operation Ranch Hand previously described by Wolfe et al. (1990). TCDD measurements from physical examinations through 1997 were analyzed and used to categorize Ranch Hand and comparison subjects as "background," "low," or "high," according to current and initial dioxin concentrations; missing TCDD measurements from 1987 (the first exam in which serum TCDD was measured) were extrapolated back from later exams using a half-life of 8.7 years. Cases of thyroid disease were confirmed at the physical examinations or through medical records; they were included in the analysis only if thyroid disease was diagnosed after service in Southeast Asia through December 1998. Analyses of thyroxine, thyroid-stimulating hormone, triiodothyronin percentage uptake, free thyroxine index, and thyroid diseases were completed for the 1,009 Ranch Hands and 1,429 comparison subjects participating in the study. Veterans without TCDD serum measurements who were diagnosed with thyroid disease before leaving Southeast Asia, who had thyroidectomies, or who also were receiving irradiation of the thyroid were excluded from analysis. Logistic regression was used to evaluate associations between abnormal results of thyroid biochemical parameters or the presence of thyroid disease and serum TCDD measurement. Adjustments were made for age, race, and military occupation.

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 VES data, 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). Another 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 for 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

Numerous cohort and case–control studies are discussed in detail in *VAO*, *Update 1996*, *Update 1998, Update 2000*, and *Update 2002*. Among the earliest was a proportionate-mortality study (Breslin et al., 1988). The 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 VA'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) compared 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) also was conducted (Watanabe and Kang, 1996).

VA also examined the morbidity and mortality experience of a subgroup of

Vietnam veterans from some US Army Chemical Corps units who might have been exposed to high concentrations of herbicides (Thomas and Kang, 1990). In an extension, Dalager and Kang (1997) compared mortality among veterans of the Chemical Corps specialties, including Vietnam veterans and non-Vietnam veterans. Watanabe and Kang (1995) 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).

VA 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), lung cancer (Mahan et al., 1997), and pregnancy outcomes and gynecologic cancers in female veterans (Kang et al., 2000a,b). It also has conducted a co-twin study of self-reported physical health in a series of Vietnam-era monozygotic twins (Eisen et al., 1991). A preliminary long-term health study of US Army Chemical Corps Vietnam veterans began in 2001 (Kang et al., 2001).

VA has examined other outcomes—PTSD (Bullman et al., 1991; True et al., 1988), suicide and motor-vehicle crashes (Farberow et al., 1990), and tobacco use (McKinney et al., 1997)—among Vietnam veterans and has studied cause-specific mortality among veterans with non-lethal (combat and non-combat) wounds sustained during the Vietnam War (Bullman and Kang, 1996). *VAO* and *Update 1998* discuss those studies in detail. Most of those publications do not discuss exposure to Agent Orange; exposure to "combat" is evaluated as the risk factor of interest.

No new VA studies have been published since *Update 2002*.

American Legion

The American Legion conducted a cohort study of the health and well-being of Vietnam veterans who were members of the American Legion, a voluntary service organization for veterans. Studies examined physical health and reproductive outcomes, social–behavioral consequences, and PTSD among veterans who had served in Southeast Asia and elsewhere (Snow et al., 1988; Stellman SD et al., 1988; Stellman JM et al., 1988). No new studies have been published on this cohort.

State Studies

Several states have conducted studies of Vietnam veterans, most of them unpublished in the scientific literature. *VAO* and *Update 1996* reviewed studies from Hawaii (Rellahan, 1985), Iowa (Wendt, 1985), Maine (Deprez et al., 1991), Massachusetts (Clapp, 1997; Clapp et al., 1991; Kogan and Clapp, 1985, 1988; Levy, 1988), Michigan (Visintainer et al., 1995), New Jersey (Fiedler and

Gochfeld, 1992, Kahn et al., 1992a,b,c, 1998), 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 examined 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 for 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: birth anomalies (Donovan et al., 1983, 1984; Evatt, 1985), mortality (Crane et al., 1997a,b; Commonwealth Institute of Health, 1984a,b,c; Evatt, 1985; Fett et al., 1987a,b; Forcier et al., 1987), deaths from all causes (Fett et al., 1987b), cause-specific mortality (Fett et al., 1987a), and morbidity (AIHW, 1999, 2000; CDVA, 1998a,b). A revised morbidity study has been published (AIHW, 2001). An independent study in Tasmania evaluated reproductive and childhood-health problems for associations with paternal service in Vietnam (Field and Kerr, 1988). 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 antinuclear and sperm autoantibodies in Vietnamese veterans who served in a "dioxin-sprayed zone" (Chinh et al., 1996). Available details of this study are presented in *Update 1998*.

Since *Update 2002*, Kim J-S et al. (2003) published a cross-sectional epidemiologic study examining the effect of Agent Orange exposure in 1,224 male Korean Vietnam veterans and in 154 Korean non-Vietnam veterans between the ages of 45 and 64. Standardized comprehensive clinical investigations were conducted to assess health outcomes of study subjects. Examination results were categorized by health outcome and were evaluated based on veteran status and exposure quartile (described below). Multiple logistic regression analysis was

used and adjustments were made for age, tobacco use, BMI, education, and marital status. An earlier report (Kim et al., 2001) described the scoring method used to categorize Agent Orange exposure for that cohort; the committee had this article translated into English from Korean. Study participants responded to questions about their age, service in Vietnam, military rank, and Agent Orange exposure; responses were checked against military records. An Agent Orange net exposure index was calculated for each study participant by calculating a service-exposure matrix that calculated data on annual Agent Orange spraying in Vietnam, troop locations and exposures, and information on individual exposure opportunities (ingestion and dermal exposures). Researchers used net exposure index scores to assign each Vietnam veteran to a quartile. The order of the quartiles was validated using TCDD concentrations from three pooled blood samples for each quartile and one pooled sample for the non-veterans' group, but there were so few measurements that the distinction between quartiles could not actually be demonstrated.

Another study of Korean Vietnam War veterans (Kim H-A et al., 2003) examined the immunotoxicologic effects of Agent Orange exposure. Study participants were divided into three categories based on general health and TCDD exposure: veterans–patient (24 veterans with chronic disease who were exposed to TCDD while serving in Vietnam); veterans–normal (27 veterans with no chronic disease who were exposed to TCDD while serving in Vietnam); or control volunteers (36 age-matched, healthy volunteers with no Vietnam War military service). Researchers conducted a variety of immunologic tests, including blood analysis, determination of cytokine concentration, concentration of plasma IgG subclass, and evaluation of plasma IgE and autoantibody concentrations.

An English abstract described one study (Mo et al., 2002) that examined skin and general disease patterns in 332 Korean veterans who were exposed to dioxin in Vietnam. Researchers used clinical and dermatologic evaluations, physical and pathology examinations, and other medical tests to determine the prevalence of disease among the exposed veterans.

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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. It discusses exposure assessments from selected epidemiologic studies introduced in Chapter 4 and provides background information for the health-outcome chapters that follow; health outcomes are not discussed here. Further discussion of exposure assessment, 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* (*VAO*; IOM, 1994); additional information is in Chapter 5 of *Veterans and Agent Orange: Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), *Update 2000* (IOM, 2001), and *Update 2002* (IOM, 2003a). 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 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 a compound at the site of toxic action in the tissue of an organism. In studies of human populations, however, it generally is not possible to measure those concentrations. Instead, exposure assessments are based on measurements in environmental media or in

biologic specimens. In either case, exposure serves as a surrogate for dose. Exposure assessments based on measurements of environmental contaminants attempt to quantify the amount of the contaminant that contacts a body barrier over a defined period. Exposure can occur through inhalation, skin contact, and ingestion. Exposure also can be assessed by measuring the compounds of interest—or their metabolites—in human tissues. Such biologic markers of exposure integrate absorption from all routes. The evaluation of those markers can be complex, because most are not stable for long periods. Knowledge of pharmacokinetics is essential to the linkage of measurements at the time of sampling with past exposures. Similarly, the assessment of markers that could be markers of effect—such as DNA adducts—shows promise, but does not necessarily provide accurate measurements of past exposure; that is, there is little evidence that currently measured DNA adducts are related to occupational or environmental exposures experienced years before.

Because quantitative assessments based on environmental or biologic samples are not always available for epidemiologic studies, investigators rely on a mixture of qualitative and quantitative information to derive estimates. There are a few basic approaches to exposure assessment for epidemiology (Armstrong et al., 1994; Checkoway et al., 1989). The simplest compares the members of a presumably exposed group with the general population or with a non-exposed group. That approach offers simplicity and ease of interpretation. If, however, only a small fraction of the group is exposed to the agent, the increased risk posed by exposure might not be detectable when the risk of the entire group is assessed.

A more refined method assigns each study subject to an exposure category, typically high, medium, low, or no exposure. Disease risk for each group is calculated separately and compared with a reference or non-exposed group. That method can identify the presence or absence of a dose–response trend. In some cases, more-detailed information is available for use in quantitative exposure estimates, which are sometimes called exposure metrics. They 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, these refined estimates reduce errors associated with misclassification and thereby increase the power of statistical analysis to identify true associations between exposure and disease.

The temporal relationship between exposure and disease is complex and often difficult to define in epidemiologic investigations. Many diseases do not appear immediately following exposure. In the case of cancer, for example, the disease may not appear for many years after the exposure. The time between an exposure and the occurrence of disease is often referred to as a latency period (IOM, 2004). Exposures can be brief (sometimes referred to as acute exposures) or protracted (sometimes referred to as chronic exposures). At one extreme the exposure can be the result of a single insult, as in an accidental poisoning. At the other, an individual exposed to a chemical that is stored in the body may continue

to experience "internal exposure" for years, even if exposure from the environment has ceased. The definition of the proper time frame for exposure duration represents a challenging aspect of exposure assessment and epidemiology.

Occupational–exposure studies use work histories, job titles, and workplace measurements of contaminant concentration; those data are combined to create a job–exposure matrix that assigns a quantitative exposure estimate to each job or task, and the time spent on each job or task is calculated. Those metrics 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) used those methods.

Many environmental-exposure studies use proximity to the source of a contaminant to classify exposure. If an industrial facility emits a contaminant, investigators might identify geographic zones around the facility and assign exposure categories to people on the basis of residence. That approach was used to analyze data from the industrial accident in Seveso, Italy, that contaminated nearby areas with TCDD. Assessments often are refined to include exposure pathways (how chemicals move from the source through the environment) and personal behavior; they sometimes include measurements of contaminants in environmental samples, such as soil.

Biologic markers of exposure can provide important information for use in occupational and environmental studies; a quantitative exposure estimates can be assigned to each person in the study group. The most important marker 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 past 20 years. In the late 1980s, the Centers for Disease Control and Prevention (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 exposure in Vietnam veterans and other people.

Exposure to Dioxin-like Compounds

A major focus of the work of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Fifth Biennial Update) has been the analysis of studies involving exposure to a single compound: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD. The committee recognizes that there are hundreds of similar compounds to which humans might be exposed, among them the polychlorinated biphenyls (PCBs), other polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polycyclic aromatic hydrocarbons. The literature on those compounds was often not considered in this evaluation, for several reasons. The exposure of Vietnam veterans to significant amounts (relative to TCDD) was considered unlikely or had not been docu-

mented. Several of them might act by different mechanisms in addition to their ability to bind to and activate the aryl hydrocarbon receptor (AhR). Those mechanisms are sometimes related to the ability of the compounds to be metabolized to chemicals that might induce greater biologic activity. In addition, the exposure of human populations, for example occupationally, to those compounds occurs most often along with exposures to other compounds. It is difficult to determine whether the toxic effects should be attributed to the dioxin-like compound or to some other compound that has a different mechanism of action.

The toxic equivalency factor (TEF) method of comparing the relative toxicity of dioxin-like compounds has come into common use by agencies of governments around the world. Although it is considered among the best of the approaches for assessing the relative risk posed by exposure to complex mixtures of the contaminants, it presents several uncertainties. TEFs are determined through inspection of the available congener-specific biologic and biochemical data on a compound and then assignment of a relative toxicity for that compound in comparison with TCDD. TEF values are by no means precise; they are the result of scientific judgment and expert opinion considering all available data. The quantity and quality of those data might vary considerably, and the values might differ by several orders of magnitude, depending on the different biologic endpoints chosen for a particular compound. Thus, there is considerable unquantifiable, uncertainty about their use. Although the World Health Organization 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. Nevertheless, most agencies in the United States, including the Environmental Protection Agency, support the basic approach as providing a "reasonable estimate" of relative toxicity. Many countries and international organizations have adopted it although, again, the accepted values might differ.

The TEF concept is based on the premise that the toxic and biologic responses of a particular group of compounds are mediated through the AhR. Although all the available data support that idea, the set of data on individual compounds within the group considered to be dioxin-like is incomplete. One limitation is that use of TEF values does not consider synergistic or antagonistic interactions among the compounds. It also does not consider possible actions or interactions of compounds that are not mediated by the AhR. Indeed, little research has been done on this. For some mixtures, another limitation is that the risk posed by non-dioxin-like chemicals (non-coplanar PCBs) is not assessed, and some non-coplanar PCBs can act as AhR antagonists (Safe, 1997–1998). The kinetics and metabolism of each dioxin-like compound might differ considerably from the others, and complete data on tissue concentrations often are unavailable.

Extrapolation to a meaningful dose might add considerable uncertainty to calculation of the TCDD toxicity equivalent (TEQ) to which a person was exposed. There also is exposure to dietary flavonoids and other phytochemicals that bind the AhR that is not considered by the TEQ method (Ashida et al., 2000; Ciolino

et al., 1999; Quadri et al., 2000). Considering the many difficulties of interpretation relative to the exposure of veterans to Agent Orange and other herbicides in Vietnam, some published literature on humans exposed either occupationally or environmentally to several other dioxin-like compounds was not evaluated. However, when such exposures were considered relevant to Vietnam veterans, the data were critically evaluated.

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 could 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 workers in chemical plants that 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 extensive set of data on chemical production workers potentially contaminated with TCDD has been compiled by NIOSH. More than 5,000 TCDD-exposed workers in 12 companies were identified from personnel and payroll records. Exposure status was determined initially through a review of process operating conditions; employee duties; and analytical 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 the 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 efficient = 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: <1 year, 1 to <5 years, 5 to <15 years, and 15+ years. In some cases, information was not available for duration of exposure, so a separate metric, called duration of employment, was defined as the total time each worker was employed at the study plant.

The NIOSH cohort study was updated in 1999 (Steenland et al., 1999), and a more refined exposure assessment was conducted. Workers whose records were inadequate to determine duration of exposure were excluded. The final analysis was restricted to 8 plants because 4 plants (with 591 workers) had no records on the degree of TCDD contamination of work processes or lacked the detailed work histories required to estimate TCDD exposure by job. Another 38 workers at the remaining 8 plants were eliminated because they worked in processes in which TCDD contamination could not be estimated. Finally, 727 workers with exposure to both pentachlorophenol (PCP) and TCDD were eliminated to avoid possible confounding of any TCDD effects by PCP effects. 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) that 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 (through exclusion of non-exposed workers) and uncertainty (through exclusion of workers with incomplete information) and improved accuracy (through more detailed information on daily exposure).

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 was identified with serum TCDD greater than 10 parts per trillion (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 for each worker. Results of the analysis were used to estimate serum TCDD over time that was attributable to occupational exposure for all 3,538 workers in the subcohort defined in 1999.

Crump et al. (2003) conducted a meta-analysis of dioxin–cancer, dose–response studies for three occupational cohorts: the NIOSH cohort (Fingerhut et al., 1991); the Hamburg cohort (Flesch-Janys et al., 1998); and the BASF cohort

(Ott and Zober, 1996). That analysis incorporated recent exposure data for the NIOSH cohort generated by Steenland et al. (2001). No other reports on the cohort have been published since *Update 2002*. An update of the Dow Chemical Company worker cohort (Bodner et al., 2003), which is part of the NIOSH cohort, is discussed below.

International Agency for Research on Cancer Cohort Studies

A multisite study by the International Agency for Research on Cancer (IARC) 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 manufacturers of chlorophenoxy herbicides or chlorinated phenols and for spraying cohorts. Surveys were completed with the assistance of industrial hygienists, workers, and factory personnel. Industry and production records also were used. Job histories were examined when available. Workers were classified as exposed, probably exposed, exposure unknown, or non-exposed. The exposed-workers group ($N = 13,482$) consisted of 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. Workers with no exposure information ($N = 541$) were classified as "exposure unknown." Non-exposed workers ($N = 3,951$) were those who had never been employed in parts of factories that produced chlorophenoxy herbicides or chlorinated phenols and those who had never sprayed chlorophenoxy herbicides. Review of the later analysis indicated that the lack of detailed occupational exposure information prevented meaningful classification beyond exposed and non-exposed.

An expanded and updated version of that cohort study was published in 1997 (Kogevinas et al., 1997). The researchers added herbicide production workers from 12 plants in the United States (the NIOSH cohort) and from 4 plants in Germany. Exposure was reconstructed from 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 of exposure to TCDD or higher-chlorinated dioxins: those exposed ($N = 13,831$), those not exposed ($N = 7,553$), and those with unknown exposure ($N = 479$). 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 studied various subgroups of the IARC cohort. Flesch-Janys et al. (1995) updated 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 by mean of measurements of serum or adipose concentrations of PCDD/F. The authors regressed the estimated PCDD/F exposure of those workers at the end of their exposure against the length of time they worked in each production department in the plant. The authors estimated the contribution of the time worked in each production department to PCDD/F exposure. The working-time "weights" were then used with work histories for the remainder of the cohort to estimate PCDD/F exposure for each member at the end of that person's exposure. The epidemiologic analysis used the estimated TCDD doses.

Becher et al. (1996) reported on analysis of several German cohorts, including the Boehringer–Ingelheim cohort described above (Kogevinas et al., 1997), a cohort from the BASF Ludwigshafen plant that did not include those involved in a 1953 accident, and a cohort from a Bayer plant in Uerdingen and a Bayer plant in Dormagen. All of the plants were involved in production of phenoxy herbicides or chlorophenols. Exposure assessment involved estimation of duration of employment from the start of work in a department where exposure was possible until the end of employment at the plant; a period that could include some time without exposure. Analysis was based on time since first exposure.

Hooiveld et al. (1998) reported on an update of a mortality study of workers (production workers who had known exposure to dioxins, workers in herbicide production, non-exposed production workers, and workers known to be exposed as a result of an accident that occurred in 1963) from two chemical factories in the Netherlands. Assuming first-order TCDD elimination with an estimated half-life of 7.1 years, measured TCDD levels were extrapolated to the time of maximum exposure ($TCDD_{max}$) for a group of 47 workers. A regression model then estimated the effect on estimated $TCDD_{max}$ for each cohort member attributable to exposure as a result of the accident, duration of employment in the main production department, and time of first exposure before (or after) 1970.

No new report for that cohort has been published since *Update 2002*. An update of the Dow Chemical Company worker cohort (Bodner et al., 2003), which is part of the IARC cohort, is discussed below.

Dow Cohort Studies

Workers at Dow Chemical Company facilities where 2,4-D was manufactured, formulated, or packaged 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. That matrix was merged with employee work histories to assign an exposure magnitude to each 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 in the job and then adding those products for all jobs. The 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 the facilities does not appear to have been considered in the exposure estimates. It is not clear to what extent the use of air measurements alone can provide 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 apparently it was 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 those studies modified the exposure assessment procedures of the original study. Since *Update 2002*, new cancer risk estimates for that cohort have been reported (Bodner et al., 2003). The exposure assessment procedures were unchanged from previous studies.

Dow also has conducted a cohort study of manufacturing workers exposed to PCP (Ramlow et al., 1996). Exposure assessment for that cohort was based on consideration of the available industrial-hygiene and process data, including process and job description information obtained from 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 PCP in the flaking–prilling–packaging area; the industrial-hygiene data suggest about a 3-fold difference between the areas of highest to lowest potential exposure. 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 the 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 adding the products across all exposed jobs.

Other Production Worker Studies

Several other occupational studies for chemical production plants have relied on job titles as recorded on individual work histories and company personnel records to classify exposure (Coggon et al., 1986, 1991; Cook et al., 1986; Ott et al., 1980; Zack and Gaffey, 1983; 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 (Bueno de Mesquita et al., 1993; Garaj-Vrhovac and Zeljezic 2002; Manz et al., 1991).

Agriculture, Forestry, and Other Outdoor Work

Occupational studies for agricultural workers have had various methods to estimate exposure to herbicides or TCDD. The simplest method derives occupational data from death certificates, cancer registries, or hospital records (Burmeister, 1981). Although such information is relatively easy to obtain, it cannot be used to estimate duration or intensity of exposure or to determine the specific exposure. Some studies of agricultural workers have attempted to investigate differences in occupational practices, allowing identification of subsets of workers who were likely to have had higher exposures (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 have used county of residence as a surrogate for exposure, relying on agricultural censuses of farm production and chemical use to characterize exposure in individual countries (Blair and White, 1985; Cantor, 1982; Gordon and Shy, 1981). Still others have 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 pesticide sales to estimate exposure or estimating acreage sprayed to determine the amount used (Morrison et al., 1992; Wigle et al., 1990). Some studies used self-reported information on exposure that recounted direct handling of a herbicide, whether it was applied by tractor or hand-held sprayer, and what type of protective equipment or safety precautions were used (Hoar et al., 1986; Zahm et al., 1990). Other studies have validated self-reported information through written records, signed statements, or telephone interviews with co-workers or former employers (Carmelli et al., 1981; Woods and Polissar, 1989).

Forestry and other outdoor workers, such as highway maintenance workers, are likely to have been exposed to herbicides and other compounds (see Table A-1 in Appendix A for a summary of studies). Exposure for those groups has been classified by approaches similar to those noted above for agricultural workers, for example, by the number of years employed, job category, and occupational title.

The Ontario Farm Family Health Study has produced several reports that are 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-reports of mixing or application of 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, and their exposure classifications were based on activities in 3-month segments of time. The exposure classification was refined

through answers to 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 biologic marker 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 in 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 was the urine analysis), the questionnaire's prediction of exposure, when compared with the urine 2,4-D concentrations, had a sensitivity of 57% and a specificity of 86%. In multivariate models, the variables for 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.

The Agricultural Health Study in the United States enrolled approximately 58,000 commercial and private pesticide applicators in two states (Iowa and North Carolina) between 1993 and 1997 (Alavanja et al., 1994). Exposure assessment in this study is based primarily on questionnaire data collected at the time of enrollment and in periodic follow-up. Recently, Dosemeci et al. (2002) published an algorithm designed to better characterize personal exposures for that population. Weighting factors for key exposure variables were developed from the literature on pesticide exposure. That new quantitative approach is likely to improve the accuracy of exposure classification for the cohort.

Herbicide Spraying

Studies of herbicide applicators are relevant because they can be presumed to have had more sustained exposure to herbicides. However, because they also are likely to be exposed to a variety of compounds, assessment of individual or group exposure to specific phenoxy herbicides or TCDD is complicated. Some studies have attempted to measure applicators' exposure on the basis of information from work records on acreage sprayed or on the number of days of spraying. Employ-

ment records also can be used to extract information on which compounds are sprayed.

One surrogate indicator of herbicide exposure is the receipt of a license to spray. Several studies have specifically identified licensed or registered pesticide and herbicide applicators (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 applicators were known to have applied many kinds of herbicides, pesticides, and other substances. In addition, herbicide spraying is generally a seasonal occupation, and information is not always available on possible exposure-related activities during the rest of the year.

One study provided information on serum TCDD concentrations in herbicide applicators. Smith et al. (1992) analyzed blood from nine professional spray applicators 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: type of exposure, routes of entry, and routes of excretion (Ferry et al., 1982; Frank et al., 1985; Kolmodin-Hedman and Erne, 1980; Kolmodin-Hedman et al., 1983; Lavy et al., 1980a,b; Libich et al., 1984). Those studies appear to show 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 in ground crews was 0.1–5 mg/day; for air crews the value was lower.

A study of Canadian farmers examined pesticide exposures of men (McDuffie et al., 2001). Data were collected by questionnaires that included 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 purchases. Investigators reported an "excellent concordance" between the two sources, but they 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 from 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.

Other studies of the 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; Chiu et al., 2004;

Duell et al., 2001; Garry et al., 2003; Gorell et al., 2004; Hanke et al., 2003; Van Wijngaarden et al., 2003).

Pulp and Paper Mill Work

Pulp and paper mill workers are likely to be exposed to TCDD and chlorinated phenols during the bleaching process. Pulp and paper production workers also are likely to be exposed to other compounds, depending on 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). One study of a cohort of Danish paper mill workers (Rix et al., 1998) presented no direct measures of occupational exposure, and the qualitative assessment of compounds used by each 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

In the past, workers in sawmills might have been exposed to pentachlorophenates, which are contaminated with higher-chlorinated PCDDs (Cl_6–Cl_8), or to 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

A large industrial accident involving environmental exposure to TCDD occurred in Seveso in July 1976 as the result of an uncontrolled reaction during trichlorophenol production. Various indicators, including TCDD measurements in soil, have been used as indicators of individual exposure. Three areas were defined around the release point on the basis of soil sampling for TCDD (Bertazzi et al., 1989). Zone A was the most heavily contaminated; all residents were

evacuated within 20 days. Zone B was less contaminated; women in the first trimester and all children were urged to avoid it during daytime. Zone R had some contamination; 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 by CDC (1988a). In those with severe chloracne ($N = 10$), TCDD was 828–56,000 ppt of lipid weight. Those without chloracne ($N = 10$) had TCDD 1,770–10,400 ppt. TCDD was undetectable in all control subjects 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 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. Adults seem much less likely than children to develop chloracne after acute exposure, but surveillance bias could have affected that 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 between 1976 and 1980 from 601 residents (97 from zone A; 504 from zone B). A questionnaire the women completed between 1996 and 1998 included 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 the models.

Several new studies of the Seveso population have been published since *Update 2002* (Baccarelli et al., 2002; Eskenazi et al., 2002a,b, 2003, 2004; Landi et al., 2003). All of the studies used lipid-adjusted serum TCDD concentrations as the primary exposure metric. Fattore et al. (2003) measured current air concentrations of PCDDs in zones A and B, and compared them with measurements from a control area near Milan. The authors concluded that release from PCDD-contaminated soil does not add appreciably to air concentrations in the Seveso study zone. Finally, Weiss et al. (2003) collected breast milk from 12 mothers in Seveso to compare TCDD concentrations with those from a control population near Milan. The investigators reported that the TCDD concentrations in human milk from mothers in Seveso were two times higher than were those in controls.

The authors concluded that breast-fed children in the Seveso area are likely to have higher body burdens of TCDD than are children from other areas.

Times Beach, Missouri

Several reports have provided information on environmental exposure to TCDD 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. Among 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 that 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 128 non-exposed control subjects with comparison concentrations from 51 exposed persons who had ridden or cared for horses at arenas sprayed with TCDD-contaminated oil; who lived in areas where the oil had been sprayed; who were involved in trichlorophenol (TCP) production; or who were involved in TCP non-production activities, such as laboratory or maintenance work. Persons were considered exposed if they lived near, worked with, or had other contact for at least 2 years with soil contaminated with TCDD at 20–100 parts per billion (ppb) or for 6 months or more with soil contaminated with TCDD above 100 ppb. Of the exposed-population samples, 87% had adipose tissue TCDD concentrations below 200 ppt; however, TCDD concentrations in 7 of the 51 exposed persons were 250–750 ppt. In non-exposed persons, adipose tissue TCDD ranged from undetectable to 20 ppt, with a median of 6 ppt. On the basis of a 7-year half-life, it is calculated that 2 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. Dioxin emissions from the incinerator were measured in international toxicity equivalent (I-TEQ) units at 16.3 nanograms (ng) I-TEQ per

cubic meter (m^3), far in excess of the European Union (EU) standard of 0.1 ng I-TEQ/m^3. TCDD concentrations in cows' milk measured at three farms near the incinerator were well below the EU guideline of 6 ng I-TEQ/kg of fat, but the concentrations were highest at the farm closest to the incinerator. No new studies have been published since *Update 2000*.

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). From 1967 to 1987 the plant produced hexachlorocyclohexane (lindane) and its derivatives. Since then, the plant has produced various crop protection products. Dioxins have been detected in air, soil, drinking water, and cows' milk. However, the researchers do not describe air-, soil-, or water-sampling methods. The number of samples analyzed was small for some media (2 drinking-water samples, 7 breast-milk samples pooled from 40 women, and 14 blood samples) and unreported for others (air, soil, and vegetables). Results from the samples suggested elevated concentrations of dioxin around the center of Chapaevsk compared with those from outlying areas. That conclusion was based primarily on concentrations measured in soil: 141 ng TEQ/kg soil less than 2 km from the plant, compared with 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 measurements from Chapaevsk with those from other Russian cities with industrial facilities. The data presented do not allow direct comparison of dioxin concentrations in soil 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 were the maximum concentrations reported by 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 or with occupational or lifestyle factors that might have influenced TCDD exposure.

One additional article has been published on the Chapaevsk population since *Update 2002*. Akhmedkhanov et al. (2002) sampled 24 volunteers for lipid-adjusted serum dioxin concentrations. Residents living near the plant (<5 km) had higher concentrations than did those who lived farther from the plant. It is not clear whether the analysis included adjustments for age, body mass index, or education, all of which are significant predictors of dioxin concentrations.

Other Studies

Several epidemiologic studies have been conducted in association with industrial-facility emissions, or in regions with documented differences in dioxin

exposures. Combustion records for 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 exposed 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.

A recent study of dioxin exposure pathways in Belgium focused on long-time residents in the vicinity of two municipal-waste incinerators (Fierens et al., 2003a). Residents near a rural incinerator had significantly higher serum dioxin concentrations than did a control group (38 vs. 24 picograms [pg] TEQ/g fat). Concentrations in residents living near the incinerators increased proportionately with intake of local-animal fat. A second study (Fierens et al., 2003b) measured dioxin body burden in 257 people who had been environmentally exposed. The object was to identify an association for dioxin and PCB exposures with diabetes and endometriosis (Fierens et al., 2003b). No difference in body burden was found between women with endometriosis and women in a control group, but the risk of diabetes was significantly higher for those with higher body burdens of dioxin-like compounds and PCBs. Another study of the correlation between dioxin-like compounds in Italian and Belgian women and the risk of endometriosis used measurements of TCDD and other dioxins in blood (De Felip et al., 2004). There was no difference in body burden among women with endometriosis and a control group, but dioxin concentrations were substantially higher in the control groups of women from Belgium than in a similar group from Italy (45 vs. 18 pg TEQ/g, lipid-adjusted, respectively).

Studies in Vietnam

Studies of exposure to herbicides among the residents of South Vietnam (Constable and Hatch, 1985) have compared unexposed residents of the South with residents of the North. Other studies have attempted to identify wives from 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, village residents were considered exposed if a herbicide mission had 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 the number of times the area reportedly had 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 during surgery or autopsy in

South Vietnam during 1984. The concentrations in the positive samples were 3–103 ppt. TCDD was not detected in 9 samples from residents of North Vietnam who had never been to South Vietnam; detection sensitivity was 2–3 ppt. Analysis of 3 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.

In a more recent study, 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 those residents, compared with an average of 2 ppt in residents of Hanoi. The study also indicated that TCDD exposure of the population was continuing, presumably through consumption of fish and other foods.

Dwernychuk et al. (2002) collected environmental and food samples, human blood, and breast milk from residents of the Aluoi Valley of central Vietnam. The investigators identified locations where relatively high dioxin concentrations remain in soil or water systems. Dioxin soil concentrations were particularly high around former airfields and military bases where herbicides were handled. Fish harvested from ponds in these areas were found to contain elevated dioxin concentrations. The study is not directly relevant to this Institute of Medicine (IOM) committee's task, but it could prove useful in future epidemiologic studies of the Vietnamese population.

MILITARY USE OF HERBICIDES IN VIETNAM

Military use of herbicides in Vietnam began in 1962, expanded in 1965 and 1966, and reached a peak between 1967 and 1969. The herbicides were primarily 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. In 1974, a National Academy of Sciences committee estimated the amount of herbicides sprayed from helicopters and other aircraft using Operation Ranch Hand records gathered from August 1965 to February 1971 (NAS, 1974). The committee calculated that about 17.6 million gallons (~66.5 million liters [L]) of herbicide were sprayed over about 3.6 million acres (~1.5 million hectares) in Vietnam in that time period. Soldiers also sprayed herbicides on the ground to defoliate the perimeters of base camps and fire bases; that spraying was performed from the rear of trucks and from backpack spray units carried by soldiers. Navy boats also sprayed herbicides along river banks.

A new analysis of spray activities and exposure potential of troops emerged from a recent study overseen by a committee of the IOM (Stellman and Stellman, 2003). Location data for military units assigned to Vietnam were compiled into a database developed from five primary and secondary sources: the Unit Identification Code list (a reference list of units serving in Vietnam created

and used by the Army); a command post list (data on division level of the command locations for army personnel); Army Post Office lists (compilations of locations down to and including battalion size and other selected units that were updated on a monthly basis); troop strength reports (data assembled by the US Military Assistance Command on troop allocations, updated on a monthly basis and generally collected on the battalion level); and order of battle information (data on command post, arrival and departure dates, and authorized strength of many but not all units). For units that served in the III Corps Tactical Zone between 1966 and 1969, battalion tracking data were also available. These are data on the grid coordinate locations of battalion-sized units derived from Daily Journals, which recorded the company locations over 24-hour periods.

"Mobility factor" analysis, a new concept for studying troop movement, was developed for use in reconstructing herbicide exposure histories. The analysis is a three-part classification system for characterizing the location and movement of military units in Vietnam. It comprises a mobility designation (stable, mobile, or elements mobile), a distance designation (usually in a range of kilometers) to indicate how far the unit might travel in a day, and a notation of the modes of travel available to the unit (air; ground—truck, tank, or armored personnel carrier; or water). A mobility factor was assigned to every unit that served in Vietnam.

The same work also yielded new estimates of the use of military herbicides in Vietnam from 1961 to 1971 (IOM, 2003b,c; Stellman et al., 2003a). Investigators reviewed the original data used in the 1970s to make estimates and identified inconsistencies, data gaps, and typographical errors. They determined the amounts of herbicide applied but not recorded on the data tapes (the so-called HERBS tapes) compiled in the 1970s and clarified data on missions that presumably "dumped" herbicide loads over very short periods before returning to base. The new analyses led to a revision in estimates of the amounts of the agents applied (Table 5-1). Previous *VAO* reports estimated that a total of ~67.8 million L of military herbicides were applied from 1961 to 1971. The new research effort estimated that ~77 million L was applied; a difference of more than 9 million L.

Four compounds were used in the herbicide formulations: 2,4-D, 2,4,5-T, picloram, and cacodylic acid. The chlorinated phenoxy acids (2,4-D and 2,4,5-T) persist in soil only for 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.

Herbicides were identified by the color of a band on 55-gal containers and called Agents Pink, Green, Purple, Orange, White, and Blue (Table 5-1). Agent Green and Agent Pink were used in 1961 and 1965; Agent Purple was used from 1962 through 1965. Agent Orange was used from 1965 through 1970, and a slightly different formulation (Agent Orange II) probably was used after 1968. Agent White was used from 1966 through 1971. Agent Blue was used in powder form from 1962 through 1964 and as a liquid from 1964 through 1971. Agents Pink, Green, Purple, Orange, and Orange II all contained 2,4,5-T, and were

TABLE 5-1 Military Use of Herbicides in Vietnam (1961–1971)

Code Name	Chemical Constituents [a]	Concentration of Active Ingredient [a]	Years Used[a]	Amount Sprayed	
				VAO Estimate[b]	Revised Estimate[a]
Pink	60%–40% *n*-butyl, isobutyl ester of 2,4,5-T	961–1,081 g/L acid equivalent	1961, 1965	464,817 L (122,792 gal)	50,312 L sprayed; 413,852 L additional on procurement records
Green	*n*-butyl ester 2,4,5-T	—	—	31,071 L (8,208 gal)	31,026 L shown on procurement records
Purple	50% *n*-butyl ester 2,4-D, 30% *n*-butyl ester 2,4,5-T, 20% isobutyl ester 2,4,5-T	1,033 g/L acid equivalent	1962–1965	548,883 L (145,000 gal)	1,892,733 L
Orange	50% *n*-butyl ester 2,4-D, 50% *n*-butyl ester 2,4,5-T	1,033 g/L acid equivalent	1965–1970	42,629,013 L (11,261,429 gal)	45,677,937 L (could include Agent Orange II)
Orange II	50% *n*-butyl ester 2,4-D, 50% isooctyl ester 2,4,5-T	910 g/L acid equivalent	Post–1968 (?)	—	Unknown, but at least 3,591,000 L shipped
White	Acid weight basis: 21.2% triisopropanolamine salts of 2,4-D and 5.7% picloram	By acid weight: 240 g/L 2,4-D and 65 g/L picloram	1966–1971	19,860,108 L (5,246,502 gal)	20,556,525 L
Blue powder	Cacodylic acid (dimethylarinic acid) and sodium cacodylate	Acid: 65% active ingredient; salt: 70% active ingredient	1962–1964	—	25,650 L
Blue Aqueous Solution	21% Sodium cacodylate + cacodylic acid to yield at least 26% total acid equivalent by weight	Acid weight: 360 g/L	1964–1971	4,255,952 L (1,124,307 gal)	4,715,731 L
Total, all formulations				67,789,844 L (17,908,238 gal)	76,954,766 L (including procured)

[a] Based on Stellman et al. (2003a).
[b] Based on data from MRI (1967), NAS (1974), and Young and Reggiani (1988).

contaminated to some extent with TCDD. Agent White contained 2,4-D and picloram. Agent Blue (powder and liquid) contained cacodylic acid. More details on the herbicides used are presented in the earlier reports (IOM, 1994, 1996, 1999, 2001, 2003a).

In addition to the four major compounds, Dinoxol, Trinoxol, and diquat were applied to native grasses and bamboo (Brown, 1962). Soil-applied herbicides also were reportedly used around base camp perimeters, minefields, ammunition storage areas, and other sites where it was necessary to control grasses and woody vegetation (Darrow et al., 1969). Other accounts discuss the use of other herbicides, fungicides, insecticides, insect repellents, wetting agents, and wood preservatives (Gonzales, 1992). There are no data on the number of military personnel potentially exposed to those substances.

Air and Ground Spraying of Herbicides

The number of US military personnel who directly handled (mixed, loaded, or applied) 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. The US Army Chemical Corps, using hand-operated equipment and helicopters, conducted smaller operations, including defoliation around special forces camps; clearing the 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 also were likely to have handled or sprayed herbicides around bases or lines of communication. Navy river patrols were reported to have used herbicides to clear inland waterways, and engineering personnel used herbicides to remove 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. The precautions that were prescribed were consistent with those applied in the domestic use of herbicides before the Vietnam conflict (US GAO, 1979).

Recent work conducted under contract with the IOM (IOM, 1997, 2003b,c) has produced a geographic information system for characterizing exposure of ground troops to Agent Orange and other herbicides used in Vietnam (Stellman and Stellman, 2003; Stellman et al., 2003b). The method integrated extensive data resources on the dispersal of herbicides, locations of military units and bases, dynamic movement of combat troops in Vietnam, and locations of civilian population centers. It has been used to generate exposure opportunity models for Agent Orange, dioxin, and other herbicides used in Vietnam (Stellman and Stellman, 2004).

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).

New analyses resulted from the Department of Veterans Affairs (VA)-sponsored exposure characterization have proposed 366 kg of TCDD as a plausible estimate of the total amount of TCDD applied in Vietnam between 1961 and 1971; the true amount is thought to be higher (Stellman et al., 2003a). Before those estimates, data from Young and Gough were used to estimate the amount of TCDD in the various herbicide formulations (Gough, 1986; Young, 1992; Young et al., 1978). Young et al. (1978) estimated that Agents Green, Pink, and Purple used early in the program (through 1965) contained 16 times the mean TCDD content of formulations used between 1965 and 1970. 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 at 32.8 ppm. In Agents Pink and Green, it was estimated at 65.6 ppm (Young et al., 1978). Gough (1986) estimated that ~368 lb (~167 kg) of TCDD was sprayed in Vietnam over a 6-year period.

EXPOSURE ASSESSMENT IN STUDIES OF VIETNAM VETERANS

Different approaches have been used to estimate the exposure of Vietnam veterans, including self-reports, record-based exposure estimates, and assessments of biologic markers of TCDD exposure. Each approach has a limited ability to ascribe 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 a proxy for exposure to herbicides. Studies of that type include the CDC Vietnam Experience Study and Selected Cancers Study, VA mortality studies, and most studies of veterans conducted by the states. The approach almost surely underestimates the health effects of expo-

sure to herbicides; many members of the cohorts presumed to have been exposed to herbicides might, 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 responsible for the aerial spraying of herbicides. Biologic marker studies of 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, 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 the number of days of skin exposure, percentage of skin exposed, and a factor for the concentration of TCDD in the herbicide. A fourth index that used no information gathered from individual subjects was calculated by multiplying the volume of herbicide sprayed during a person's tour of duty by the concentration of TCDD in herbicides sprayed in that period and then dividing that 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 concentrations. 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).

Four new articles on the Ranch Hand cohort have appeared since *Update 2002*: Akhtar et al. (2004), Barrett et al. (2003), Michalek et al. (2003), and Pavuk et al. (2003). All of those studies used serum dioxin concentrations as the primary exposure metric for epidemiologic classification.

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. That 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 by Kang et al. (2001). That study recruited 565 veterans: 284 Vietnam veterans and 281 non-Vietnam-veteran control subjects. Blood samples were collected in 1996 from 50 Vietnam veterans and 50 control veterans, and 95 of the samples met CDC standards for quality assurance and quality. Comparison of the entire Vietnam cohort with the entire non-Vietnam cohort showed that the geometric mean TCDD concentrations did not differ significantly ($p = 0.6$). Of the 50 Vietnam veterans sampled, analysis of questionnaire responses indicated that those who reported spraying herbicides had higher TCDD concentrations than did those who reported no spraying activities. The authors concluded that Agent Orange exposure was a likely contributor to TCDD concentrations in Vietnam veterans who had a history of spraying herbicides. The main study of 5,000 Vietnam veterans, including analysis of an additional 900 blood specimens, continues.

No new studies have been published related to the Army Chemical Corps since *Update 2002*.

Korean Vietnam Veterans

Military personnel from the Republic of Korea served in Vietnam between 1964 and 1973. Kim et al. (2001) evaluated the validity of an exposure index by comparing group exposure estimates with pooled serum dioxin concentrations. The study involved 720 veterans who served in Vietnam, and 25 veterans who did not serve in Vietnam. The exposure index was based on Agent Orange spray patterns across military regions in which Korean personnel served, time–location data for the military units stationed in Vietnam, and an exposure score derived from self-reported activities during service. A total of 13 blood samples were submitted to CDC for serum dioxin analysis. One sample was prepared from the 25 veterans who did not serve in Vietnam; the remaining 12 blood samples were created by pooling blood samples from 60 veterans into 12 exposure categories. The 12 categories ultimately were reduced to 4 exposure groups, each group containing 3 exposure categories and representing quartiles of veterans with Vietnam service.

The paper by Kim et al. (2001) reported highly significant Pearson correlation coefficients and multiple regression analysis results. The statistical analyses apparently were based on the assignment of the pooled-serum-dioxin value to each individual in the exposure group, thereby inflating the true sample size. The multiple regression analysis evaluated such variables as age, body-mass index, and consumption of tobacco or alcohol. In a subsequent report for the same exposure groups and serum dioxin data, the authors corrected their analysis (Kim et al., 2003). A correlation was observed between serum dioxin concentrations and ordinal exposure categories, but the correlation was not statistically significant. The authors attributed the lack of statistical significance to the small sample size, and they noted that the data exhibited a distinct monotone upward trend (average serum dioxin concentrations of 0.3, 0.6, 0.62, 0.78, and 0.87 pg/g [lipid adjusted] for exposure categories 0–4, respectively). The decision to pool blood samples from a large number of persons within each exposure set (Kim et al., 2001) greatly reduced the power of the validation study. Instead of 180 samples for each of the final exposure categories, the pooled analysis produced only 3 samples for each category. The lipid-adjusted serum TCDD concentrations from the 12 pooled samples for Vietnam veterans ranged from 0.25 to 1.2 pg/g, whereas the single sample from the non-Vietnam veterans contained 0.3 pg/g. The narrow range of results puts into question the biologic relevance of any differences.

Thus, it appears that there was not a clear separation between Vietnam veterans and non-Vietnam veterans. Furthermore, the range of mean values for the four Vietnam-veteran exposure categories was narrow, and all concentrations were relatively low (<1 pg/g). The relatively low serum dioxin concentrations observed in the 1990s in those individuals are the residual of substantially higher initial concentrations, as has been seen with other Vietnam-veteran groups. However, the concentrations reported in the Korean veterans' study are significantly lower than those reported for American Vietnam veterans in the 1988 CDC Agent Orange Validation Study, which was nonetheless unable to distinguish Vietnam veterans from non-Vietnam veterans on the basis of serum dioxin assay (CDC, 1988b). The authors were able to construct plausible exposure categories based on military records and self-reports, but they were unable to validate those categories with serum dioxin measurements.

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 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' service-related exposures to herbicides. 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 in Vietnam-era veterans who did not serve in Vietnam (CDC, 1988b). Vietnam veterans were selected for further study on the basis of the 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 consisted of 298 veterans, the "medium" exposure group had 157 veterans, and the "high" exposure group had 191 veterans. Blood samples were obtained from 66% of Vietnam veterans ($N = 646$) and from 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 in 1987 was 4 ppt, with a range of <1–45 ppt; 2 veterans had concentrations above 20 ppt. The distribution of the measurements was nearly identical to that for a control group of 97 non-Vietnam veterans. The CDC validation study reported 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 report 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.

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

In 1997, a committee convened by IOM issued a request for proposals (RFP) seeking individuals and organizations to develop historical exposure reconstruction approaches suitable for epidemiologic studies of herbicide exposure among US veterans during the Vietnam War (IOM, 1997). The RFP resulted in the project "Characterizing Exposure of Veterans to Agent Orange and Other Herbicides in Vietnam", carried out under contract by a team of researchers from Columbia University's Mailman School of Public Health. The project, which began in 1998, created a geographic information system (GIS) for Vietnam with

a grid resolution of 0.01° latitude and 0.01° longitude. Herbicide-spraying records were integrated into the GIS and linked with data on military unit locations to permit estimation of exposure opportunity scores for individuals. The results are the subject of reports by the contractor (Stellman and Stellman, 2003) and the committee (IOM, 2003b,c). A summary of the findings regarding the extent and pattern of herbicide spraying (Stellman et al., 2003a), a description of the GIS for characterizing exposure to Agent Orange and other herbicides in Vietnam (Stellman et al., 2003b), and an explanation of the exposure opportunity models based on that work (Stellman and Stellman, 2004) have been published in peer-reviewed journals. Those publications demonstrate the feasibility of epidemiologic investigations of veterans who served as ground troops during the Vietnam War.

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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 main cause of death in the United States (US Census, 1999). In 2004, about 563,700 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, 2004a).

In this chapter, the *Veterans and Agent Orange: Update 2004* committee summarizes and reaches conclusions about the strength of the evidence from epidemiologic studies regarding associations between exposure to the compounds of interest (2,4-dichlorophenoxyacetic acid [2,4-D]; 2,4,5-trichlorophenoxyacetic acid [2,4,5-T] or its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]; picloram; cacodylic acid) and each type of cancer under consideration in the report. For any new study that reports on just a single type of cancer and that does not revisit a previously studied population, its design information is summarized here with its results; design information for all other new studies can be found in Chapter 4, and tables that summarize the major studies are in Appendix A. 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 the classification used to code and classify mortality data from death certificates. ICD-9 CM (clinical modification) is used to code and classify morbidity data from medical records, hospital records, and surveillance surveys. Appendix C lists ICD-9 codes (and corresponding ICD-10 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 connection between herbicide exposure and risk of cancer, one important issue is the magnitude of exposure for the people included in a study. As noted in Chapter 5, there is a great variety in detail and accuracy of exposure assessment among the studies the committee reviewed. A small number used biologic markers of exposure, such as the presence of a compound in serum or tissues; some developed an index of exposure from employment or activity records; others used surrogate measures 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 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]; *Veterans and Agent Orange: Update 2000*, hereafter*, Update 2000* [IOM, 2001]; and *Veterans and Agent Orange: Update 2002*, hereafter*, Update 2002* [IOM, 2003]), 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, service in Vietnam). Each section ends with the committee's conclusion regarding the strength of the evidence from epidemiologic studies, biologic plausibility, and evidence regarding epidemiology and Vietnam veterans.

Cancer incidence data for the general US population are included in the background sections to provide a context for consideration of cancer risk in Vietnam veterans. Incidences are reported for people 50–64 years old because most Vietnam-era veterans are in this age group. The data, which were collected for the Surveillance, Epidemiology, and End Results (SEER) Program of the National Institutes of Health—National Cancer Institute, are categorized by sex, age, and race, all of which can have a profound effect on risk. Prostate cancer incidence, for example, is approximately 4.4 times higher in men between the ages of 60 and 64 than it is in men 50–54 years old; it is approximately twice as high in blacks 50–64 years old as it is in whites in the same age group (NCI, 2004). The figures presented for each cancer are estimates for the entire US population, not predictions for the Vietnam-veteran cohort. Many factors can influence incidence, among them personal behavior (tobacco use, diet), genetic predisposition, and medical history. Those factors can make someone more or less likely than average to contract a given cancer. Incidence data are reported for all races and also separately for blacks and whites. The data reported are for 1997–2001, the most recent data set available to the committee.

Incidence figures given here are not directly comparable to the figures listed in earlier *Update*s. Earlier reports used 1990 US Census data; this report used data from the 2000 Census, so some of the differences in incidence estimates

resulted from changes in demographics rather than from changes in the factors that determine cancer rates.

There is still considerable uncertainty about the magnitude of potential risk posed by exposure to the compounds of interest as shown by the occupational, environmental, and veterans' studies reviewed by the committee. Many of those studies provided inadequate controls for important confounders, and there is not enough information to extrapolate from exposure as presented in those studies to that of individual Vietnam veterans. The committee therefore cannot measure the likely risk to Vietnam veterans that is attributable to exposure to the compounds of interest in Vietnam. Where the data permit, qualitative observations are offered.

Information about biologic mechanisms that could contribute to carcinogenic activity by any of the agents of interest is summarized in the Biologic Plausibility section at the conclusion of this chapter. It distills toxicologic information concerning how any of the chemicals of interest impact general mechanisms of carcinogenesis, which is presented in detail in Chapter 3. Such information, of course, applies to all the cancer sites discussed individually in this chapter. When biologic plausibility is addressed for a particular site, the generic information is implicit, and only toxicologic information specific to carcinogenesis at the site in question is presented.

GASTROINTESTINAL TRACT CANCERS

Gastrointestinal tract tumors are among the most common of cancers. The committee reviewed data on esophageal cancer (ICD-9 150.0–150.9), stomach cancer (ICD-9 151.0–151.9), pancreatic cancer (ICD-9 157.0–157.9), colon cancer (ICD-9 153.0–153.9), and rectal cancer (ICD-9 154.0–154.9). According to American Cancer Society (ACS) estimates, about 255,640 people will be diagnosed with those cancers in the United States in 2004, and 134,840 people will die from them (ACS, 2004a). Colon cancer accounts for about 40% of those diagnoses and deaths. Collectively, gastrointestinal tract tumors are expected to account for 19% of new diagnoses and 24% of cancer deaths in 2004. Colorectal cancer is the third most common form of cancer in men and in women, excluding basal- and squamous-cell skin cancers. The average annual incidences for gastrointestinal cancers are shown in Table 6-1.

Carcinoma of the esophagus has great geographic variation. The region of the world extending from Iran through the steps of Central Asia, Mongolia, and northern portion of China has cancer frequencies that are 10 times those of the rest of the world. In northern China, the incidence is 160 cases per 100,000, compared with 4–8 per 100,000 in North America, Europe, Southeast Asia, and Japan. In addition to a different disease incidence, there is a difference in the histopathologic type of cancer; squamous-cell carcinoma is predominant in the high-endemic areas, adenocarcinoma makes up approximately 50% of cases in the low-incidence areas of the United States, Europe, Southeast Asia, and Japan.

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

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Stomach									
Males	9.7	8.7	17.3	16.4	14.9	20.9	27.1	22.7	47.4
Females	4.6	3.5	8.8	6.8	5.8	9.3	10.8	8.6	19.0
Esophagus									
Males	9.7	9.0	20.6	16.8	16.1	31.2	24.8	24.7	35.5
Females	1.5	1.2	5.1	3.3	2.7	9.0	5.8	5.0	16.4
Colon (excluding the rectum)									
Males	33.3	31.1	53.6	59.4	58.7	82.4	105.7	102.8	148.5
Females	27.5	25.4	41.0	44.6	41.6	74.5	78.2	77.5	111.4
Rectum and rectosigmoid junction									
Males	23.3	22.2	24.1	36.9	36.8	34.7	55.1	54.2	58.7
Females	14.0	13.3	17.5	22.0	21.2	30.6	29.6	30.3	35.0
Pancreas									
Males	12.7	12.1	22.5	20.3	18.8	34.7	33.7	33.4	48.4
Females	7.7	7.5	11.3	13.6	12.7	21.3	24.0	22.5	38.0

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

The incidences of stomach, colon, rectal, and pancreatic cancers increase with age in people 50–64 years old. In general, incidence is higher in men than it is in women, and is higher in blacks than in whites. Other 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. Tobacco use is a risk factor for pancreatic cancer that might also increase the risk of stomach cancer (Miller et al., 1996). Infection with the bacterium *Helicobacter pylori* increases the risk of stomach cancer. Type 2 diabetes is associated with an increased risk of cancers of the colon and pancreas (ACS, 2004a).

Summary of *VAO, Update 1996, Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of *no* association between exposure to the compounds of interest and gastrointestinal tumors. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000,* and *Update 2002* did not change that finding. Tables 6-2, 6-3, 6-4, 6-5, and 6-6 summarize the results of the relevant studies.

TABLE 6-2 Selected Epidemiologic Studies—Stomach Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Bodner et al., 2003	Dow chemical production workers—mortality	—	1.5 (0.7–2.7)
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	3	0.4 (0.1–1.3)[b]
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers—cancer of the digestive organs—mortality	16	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

continues

TABLE 6-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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 (0.0–2.7)[b]
Fingerhut et al., 1991	NIOSH cohort	10	1.0 (0.5–1.9)
Manz et al., 1991	German production workers	12	1.2 (0.6–2.1)
Saracci et al., 1991	IARC cohort	40	0.9 (0.6–1.2)
Wigle et al., 1990	Canadian farmers	246	0.9 (0.8–1.0)
Zober et al., 1990	BASF production workers—basic cohort	3	3.0 (0.8–11.8)
Alavanja et al., 1989	USDA forest 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	Iowa Farmers	338	1.1 ($p < 0.01$)
Axelson et al., 1980	Swedish railroad workers—total exposure	3	2.2 (*)
ENVIRONMENTAL			
New Studies			
Fukuda et al., 2003	Residents of municipalities in Japan with or without waste incineration plants		
	Age-adjusted mortality (100,000) in males		38.2 ± 7.8 vs 39.0 ± 8.8 ($p = 0.28$)
	Age-adjusted mortality (100,000) in females		20.7 ± 5.0 vs 20.7 ± 5.8 ($p = 0.92$)
Studies Reviewed in *Update 2002*			
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]

TABLE 6-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	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)
	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)

continues

TABLE 6-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
VIETNAM VETERANS			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans—cancer of the digestive system		
	All Ranch Hand veterans		
	Incidence (SIR)	16	0.6 (0.4–1.0)
	Mortality (SMR)	6	0.4 (0.2–0.9)
	Veterans, tours 1966–1970—incidence	14	0.6 ((0.4–1.1)
	White Air Force comparison veterans—cancer of the digestive system		
	All comparison veterans		
	Incidence (SIR)	31	0.9 (0.6–1.2)
	Mortality (SMR)	14	0.7 (0.4–1.1)
	Veterans, tours 1966–1970—incidence	24	0.9 (0.6–1.3)
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.
— Information denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; CI, confidence interval; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; USDA, US Department of Agriculture.

TABLE 6-3 Selected Epidemiologic Studies—Esophageal 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		
	Esophagus	28	1.0 (0.7–1.4)
Studies Reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide applicators—incidence	3	1.6 (0.3–4.6)
	Finnish herbicide applicators—mortality	2	1.3 (0.2–4.7)
Studies Reviewed in *VAO*			
Ronco et al., 1992	Danish male self-employed—incidence		
	farmworkers	32	0.4 (NS)
Saracci et al., 1991	IARC cohort	8	0.6 (0.3–1.2)
Coggon et al., 1986	British MCPA production workers	8	0.9 (0.6–1.3)
Wiklund, 1983	Swedish agricultural workers	169	0.6 (0.5–0.7)
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) in males		4.1 in Chapaevsk; 4.0 in Samara Region[b]
	Age-adjusted incidence (100,000) in females		0.0 in Chapaevsk; 1.4 in Samara Region[b]
VIETNAM VETERANS			
Studies Reviewed in *Update 1998*			
Crane et al. 1997a	Australian military Vietnam veterans		
	Esophagus	23	1.2 (0.7–1.8)
Crane et al. 1997b	Australian national service Vietnam veterans		
	Esophagus	1	1.3 (0.0–10)

[a] Given when available.

[b] Incidence rates provided in absence of information on exposed cases or estimated relative risk for morbidity.

ABBREVIATION: CI, confidence interval; IARC, International Agency for Research on Cancer; ICD-9, *International Classification of Diseases*, Ninth Edition; MCPA, methyl-4-chlorophenoxyacetic acid; NS, not significant.

TABLE 6-4 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 (or higher-chlorinated dioxins)	52	1.0 (0.8–1.3)
	Workers not exposed to TCDD (or higher-chlorinated dioxins)	33	1.2 (0.8–1.6)
	Workers exposed to any phenoxy herbicide or chlorophenol	86	1.1 (0.8–1.3)
Becher et al., 1996	German chemical production workers		
	Plant I	2	0.4 (0.0–1.4)
	Plant II	0	
	Plant III	1	2.2 (0–12)
	Plant IV	0	
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*			
Swaen et al., 1992	Dutch herbicide applicators	4	2.6 (0.7–6.5)
Ronco et al., 1992	Danish male self-employed farm workers	277	0.7 ($p < 0.05$)
Fingerhut et al., 1991	NIOSH cohort	25	1.2 (0.8–1.8)
Manz et al., 1991	German production workers	8	0.9 (0.4–1.8)
Saracci et al., 1991	IARC cohort	41	1.1 (0.8–1.5)
Zober et al., 1990[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	Pulp and paper workers	9	1.0 (0.5–2.0)
Solet et al., 1989	Pulp and paper 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 6-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relatve 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	Pulp and paper workers	7	0.4 (0.2–0.9)
Lynge, 1985	Male Danish production workers	10	1.0 (*)
Blair et al., 1983	Florida pesticide applicators	5	0.8 (*)
Wiklund, 1983	Swedish agricultural workers	1,332	0.8 (0.7–0.8)[c]
Thiess et al., 1982	BASF production workers	1	0.4 (*)
Burmeister, 1981	Iowa farmers	1,064	1.0 (NS)
Hardell, 1981	Sweden residents		
	Exposed to phenoxy acids	11	1.3 (0.6–2.8)
	Exposed to chlorophenols	6	1.8 (0.6–5.3)
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
Revich et al., 2001	Residents of Chapaevsk, Russia		22.7 in Chapaevsk; 21.7 in Samara region[d]
	Age-adjusted incidence (100,000) in males		
	Age-adjusted incidence (100,000) 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 6-4 *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 6-4 *Continued*

[a] Given when available.
[b] Colon and rectal cancer results combined.
[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.
— Information 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; CI, confidence interval; 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 6-5 Selected Epidemiologic Studies—Rectal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	5	2.1 (0.6–4.8)
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 6-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) in males		15.3 in Chapaevsk; 17.1 in Samara region[c]
	Age-adjusted incidence (100,000) 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 6-5 *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.
— Information 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; CI, confidence interval; 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 6-6 Selected Epidemiologic Studies—Pancreatic Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	5	1.1 (0.4–2.7)
Studies Reviewed in *Update 2000*			
Ojajärvi et al., 2000	Meta-analysis of 161 populations		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 (*)

continues

TABLE 6-6 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Coggon et al., 1986	British MCPA production workers	9	0.7 (0.3–1.4)
Robinson et al., 1986	Paper and pulp workers	4	0.3 (0.1–1.1)
Lynge, 1985	Danish male production workers	3	0.6 (*)
Blair et al., 1983	Florida pesticide appliers	4	1.0 (*)
Wiklund, 1983	Swedish agricultural workers	777	0.8 (0.8–0.9)[b]
Burmeister, 1981	Iowa Farmers	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 (*)

TABLE 6-6 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Studies Reviewed in *Update 1996*			
Dalager et al., 1995	Female US Vietnam Veterans	7	2.8 (0.8–10.2)
	Nurses	7	5.7 (1.2–27.0)
Visintainer et al., 1995	Michigan Vietnam veterans	14	1.0 (0.6–1.7)
Studies Reviewed in *VAO*			
Thomas et al., 1991	Female US Vietnam veterans	5	2.7 (0.9–6.2)
Breslin et al., 1988	Army Vietnam veterans	82	0.9 (0.6–1.2)
	Marine Vietnam veterans	18	1.6 (0.5–5.8)
Anderson et al., 1986a	Wisconsin Vietnam veterans	6	5.5 (2.8–10.9)
Anderson et al., 1986b	Wisconsin Vietnam veterans	4	—

[a] Given when available.
[b] 99% CI.
* Information not provided by study authors.
— Information denoted by a dash in the original study.
ABBREVIATIONS: CI, confidence interval; 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.

Update of the Scientific Literature

Occupational Studies

Bodner et al. (2003) completed a 1940–1995 mortality assessment of male chemical production workers previously exposed to substantial concentrations of dioxin. No increased risk of death from gastric cancer was reported.

Swaen et al. (2004) updated the follow-up of 1,341 licensed herbicide appliers in the Netherlands. A lower-than-expected number of deaths from esophagus and stomach cancer was reported.

Environmental Studies

Fukuda et al. (2003) conducted an ecological study of 590 municipalities in Japan to examine the relationship between several indexes of dioxin emissions from incineration plants and cause-specific mortality among residents of those municipalities. Municipalities with and without incineration plants had similar age-adjusted mortality from stomach cancer ($p = 0.29$ and 0.92 for men and women, respectively). In analyses restricted to municipalities with incineration plants, there was a positive and statistically significant ($p < 0.05$) correlation in men for stomach cancer and one dioxin index (the amount of dioxins released per

unit of population), but a statistically significant ($p < 0.05$) negative correlation for three dioxin indexes (concentration of dioxins released from incinerator plants, cumulative amount of dioxins released from incinerator plants, and cumulative amount released from incinerator plants per unit of land area). There were positive and statistically significant ($p < 0.05$) correlations in women for stomach cancer and all dioxin indexes.

Vietnam-Veteran Studies

Akhtar et al. (2004) reported on cancer incidence and mortality in Air Force veterans of the Vietnam War. Index cases were Operation Ranch Hand veterans who sprayed dioxin-contaminated herbicides in Vietnam. Comparison subjects served in Southeast Asia during the same period but did not spray herbicides. The group reported that the incidence of cancer of the digestive system was significantly lower than expected (compared with national incidence rates) in white Ranch Hand veterans (standardized incidence ratio [SIR], 0.61; 95% confidence interval [CI], 0.36–0.96). Among the cohort's white "comparison" veterans, digestive system cancer incidence also was lower than expected (SIR, 0.85; 95% CI, 0.59–1.19). There were insufficient numbers of other Ranch Hand veterans to make estimates.

Synthesis

With only rare exceptions, studies on exposure to the compounds of interest in production, in agricultural use, from environmental sources, and among veteran populations reported that the estimated relative risk (RR) was close to 1.0, providing evidence of no increased risk for gastrointestinal cancers. The updated analysis of the Ranch Hand cohort (Akhtar et al., 2004) showed a lower-than-expected incidence rate of digestive system cancers. The occupational studies by Bodner et al. (2003) and Swaen et al. (2004) are consistent with other reported exposure analysis and show no or decreased risk for esophageal and gastric cancer.

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 compounds of interest and cancer of the esophagus, stomach, pancreas, rectum, or colon. The evidence 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 here and in previous *VAO* reports, the

committee finds that there is still limited or suggestive evidence of *no* association between exposure to the compounds of interest and gastrointestinal cancers.

Biologic Plausibility

No animal studies have reported an increased incidence of gastrointestinal cancer after exposure to the compounds of interest. However, recent investigations noted an increased incidence of stomach tumors in mice expressing a constitutively active aryl hydrocarbon receptor (AhR), as might occur with constant increased exposure to TCDD.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The evidence suggesting that there is no association between exposure to the chemicals of interest and gastrointestinal tumors also implies that Vietnam veterans are not at increased risk of gastrointestinal tumors as a result of any exposures they might have had to the chemicals of interest.

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, 12,580 men and 6,340 women will be diagnosed with liver or intrahepatic bile duct cancer in the United States in 2004, and 9,450 men and 4,820 women will die from those cancers (ACS, 2004a).

In the United States, liver cancers account for about 1.4% of new cancer cases and 2.5% of cancer deaths. Misclassification of metastatic cancers as primary liver cancer can lead to overestimating the number of deaths attributable to liver cancer (Percy et al., 1990). In developing countries, especially those in 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 50–64, 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 6-7.

Summary of *VAO, Update 1996, Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D,

TABLE 6-7 Average Annual Incidence (per 100,000) of Liver and Intrahepatic Bile Duct Cancers in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	7.5	4.9	16.1	11.3	8.2	20.3	16.2	12.2	28.1
Females	2.4	1.7	3.9	3.3	2.6	5.1	5.4	4.2	6.8

[a]SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

2,4,5-T, TCDD, picloram, or cacodylic acid and hepatobiliary cancers. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Table 6-8 summarizes the results of the relevant studies.

Update of the Scientific Literature

An occupational study by Swaen et al. (2004) examined cancer mortality in 1,341 licensed herbicide appliers in the Netherlands. No deaths from liver or biliary cancer were observed in the cohort.

No other relevant environmental or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

The evidence from epidemiologic studies is inadequate to link the compounds of interest with hepatobiliary cancer; no new published information was found to change this opinion.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the compounds of interest 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. Al-

TABLE 6-8 Selected Epidemiologic Studies—Hepatobiliary Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators	0	—
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 weight	1	2.8 (0.1–15.5)
	TCDD 0.1–0.99 μg/kg of body weight	0	
	TCDD >1 μg/kg of body weight	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 6-8 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
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)
Hoffman et al., 1986	Residents of Quail Run Mobile Home Park	0	—

TABLE 6-8 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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.
— Information 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; CI, confidence interval; IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

though several of those studies involve sizable cohorts, hepatobiliary cancers are rare, and the power of these studies to detect an increase in incidence is still likely to be low.

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. A recent study by the National Toxicology Program (NTP, 2004) also reported an increase in hepatocellular adenoma in female rats treated orally with 100 ng TCDD/kg for 5 days/week for 104 weeks. There also were dose-related increases in cholangiocarcinoma in rats treated with 22 nanograms (ng) per kilogram (kg) of body weight or more. However, those groups also exhibited increased incidence of hepatic necrosis, oval cell hyperplasia, and bile duct hyperplasia. Cacodylic acid administered to laboratory animals has been shown to induce hepatic neoplasms.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and hepatobiliary cancers, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

ORAL, NASAL, AND PHARYNGEAL CANCER

Oral, nasal, and pharyngeal cancers are found in many anatomic subsites, including the structures of the mouth (inside lining of the lips, cheeks, gums, tongue, and the hard and soft palate) (ICD-9 140–145), oropharynx (ICD-9 146), nasal cavity and paranasal sinuses (ICD-9 160), hypopharynx (ICD-9 148), and nasopharynx (ICD-9 147). Although those sites are anatomically diverse, cancers that occur in the nasal cavity, oral cavity, and pharynx are for the most part similar in descriptive epidemiology and risk factors. The exception is cancer of the nasopharynx, which has a different epidemiologic profile.

ACS estimates that about 30,000 men and women will be diagnosed with oral, nasal, and pharyngeal cancers in the United States in 2004 (ACS, 2004a). Almost 95% of those cancers originate in the oral cavity or oropharynx. Most of the oral, nasal, and pharyngeal cancers are squamous-cell carcinomas. Nasopharyngeal carcinoma (NPC) is the most common malignant tumor of the nasopharynx. There are three types of NPC: keratinizing squamous-cell carcinoma, nonkeratinizing carcinoma, and undifferentiated carcinoma.

The average annual incidences reported in Table 6-9 show that men are at a greater risk than are women for those cancers and that the incidences increase with age, although the small number of cases indicates that care should be exercised in interpreting the numbers. Tobacco and alcohol use are established risk factors for oral and pharyngeal cancers. 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).

Nasopharyngeal cancer is relatively rare in the United States. It accounts for about 0.25% of all cancers, and generally occurs two to three times more often in men than in women. NPC is rare in most parts of the world, although incidence is higher in some parts of China, among Eskimos in the Artic region, and among some indigenous people of Southeast Asia. Studies of NPC have reported associations with the consumption of salt-preserved foods (Miller et al., 1996) and with Epstein-Barr virus (Mueller, 1995).

TABLE 6-9 Average Annual Incidence (per 100,000) of Nasal, Nasopharyngeal Oral Cavity and Pharynx, and Oropharynx Cancers in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Nose, Nasal Cavity, and Middle Ear									
Males	1.4	1.3	1.4	1.8	1.8	1.2	2.0	1.9	3.2
Females	0.6	0.6	0.9	0.9	1.0	0.7	1.0	1.1	0.9
Nasopharynx									
Males	1.9	1.1	1.9	2.3	1.8	0.4	2.9	1.4	2.2
Females	0.8	0.4	0.7	0.5	0.2	0.3	0.9	0.7	[b]
Oral Cavity and Pharynx									
Males	28.7	27.6	46.6	37.9	37.5	51.3	47.8	46.9	66.2
Females	9.4	9.1	9.9	13.3	13.7	15.3	17.3	18.2	15.6
Oropharynx									
Males	0.9	0.8	2.7	1.2	1.2	2.4	1.9	1.7	5.9
Females	0.1	0.2	[b]	0.5	0.6	0.7	0.3	0.3	0.4

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.
[b] Insufficient data to provide a meaningful incidence estimate.

Summary of *VAO*, *Update 1996*, *Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and oral, nasal, and pharyngeal cancers. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000,* and *Update 2002* did not change that finding. Studies evaluated previously and in this report are summarized in Table 6-10.

Update of the Scientific Literature

Occupational Studies

An occupational study by Swaen et al. (2004) examined cancer mortality in 1,341 licensed herbicide appliers in the Netherlands. No deaths from nasal, oral, or pharynx cancers were observed in that cohort.

TABLE 6-10 Selected Epidemiologic Studies—Oral, Nasal, and Pharyngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators		
	Nose	0	—
	Mouth and pharynx	0	—
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			
Studies Reviewed in *Update 2002*			
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)

TABLE 6-10 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
VIETNAM VETERANS			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans—buccal cavity cancer—incidence		
	all Ranch Hand veterans	6	0.9 (0.4–1.9)
	veterans with tours 1966–1970	6	1.1 (0.5–2.3)
	White Air Force comparison veterans—buccal cavity cancer—incidence		
	all comparison veterans	5	0.6 (0.2–1.2)
	veterans with tours 1966–1970	4	0.6 (0.2–1.4)
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	9	1.0 (0.4–2.8)
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.
— Information denoted by a dash in the original study.
ABBREVIATIONS: AFHS, Air Force Health Study; CDC, Centers for Disease Control and Prevention; CI, confidence interval; IARC, International Agency for Research on Cancer; ICD-9, *International Classification of Diseases*, Ninth Edition; MCPA, methyl-4-chlorophenoxyacetic acid; NS, not significant.

Environmental Studies

No relevant environmental studies have been published since *Update 2002*.

Vietnam-Veteran Studies

Akhtar et al. (2004) examined cancers of the buccal cavity—the cavity between the jaw and the cheek—as part of their investigation of health outcomes in Operation Ranch Hand veterans who were involved in the aerial spraying of herbicides. They reported no significant difference between expected and observed incidence of the cancer in either the Ranch Hand veterans or in a

comparison group of veterans who did not spray herbicides. No other oral, nasal, or pharyngeal cancers were examined.

Synthesis

Studies from previous *Updates* have not shown an association between oral, nasal, and pharyngeal cancers and the compounds of interest. The study by Akhtar et al. (2004) reviewed here also showed no increased risk of cancer of the buccal cavity. The committee affirms that there is inadequate or insufficient evidence of an association.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *Veterans and Agent Orange* reports, the committee concludes that there is still inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and oral, nasal, and pharyngeal cancers.

Biologic Plausibility

A recent NTP study (2004) reported an increase in the incidence of gingival squamous-cell carcinoma in female rats treated orally (by gavage) with 100 ng TCDD/kg for 5 days/week for 104 weeks. The incidences of gingival squamous hyperplasia were significantly increased in all other groups treated with 3–46 ng/kg. This is the first time this type of tumor has been reported in animals.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and oral, nasal, and pharyngeal cancers, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

LARYNGEAL CANCER

According to ACS estimates, 8,060 men and 2,210 women will be diagnosed with cancer of the larynx (ICD-9 161.0–161.9) in the United States in 2004, and 3,010 men and 820 women will die from it (ACS, 2004a). Those numbers represent a little less than 1% of new cancer diagnoses and deaths. Cancer of the larynx

TABLE 6-11 Average Annual Incidence (per 100,000) of Laryngeal Cancer in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	10.0	9.6	19.8	16.9	16.2	35.1	24.4	24.7	39.8
Females	2.5	2.3	5.5	3.5	3.4	6.3	6.5	6.7	10.8

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

is more common in men than in women, with a ratio in the United States of about 4:1 among persons 50–64 years old. Incidence increases with age in that group. The average annual incidence for laryngeal cancer is shown in Table 6-11.

Established risk factors for laryngeal cancer are tobacco and alcohol use, which are independent risk factors and act as synergistic risk factors. Occupational exposures—long and intense exposures to wood dust, paint fumes, and to some compounds used in the metalworking, petroleum, plastics, and textile industries—also could elevate risk (ACS, 2004b). Infection with human papilloma virus (HPV) also might raise the risk of laryngeal cancer (Hobbs and Burchall, 2004).

Summary of *VAO, Update 1996, Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of an association between exposure to at least one of the compounds of interest and laryngeal cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000*, and *Update 2002* did not change that finding. Table 6-12 summarizes the results of the relevant studies.

Update of the Scientific Literature

Occupational Studies

Swaen et al. (2004) published an updated mortality study of a cohort of 1,341 licensed herbicide appliers in the Netherlands. The study was a 13-year follow-up of a mortality study of the same cohort (Swaen et al., 1992). Standardized mortality ratios (SMRs) were calculated based on the age and calendar-year cause-specific mortality rates of the general population of the Netherlands. One

TABLE 6-12 Selected Epidemiologic Studies—Laryngeal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	1	1.0 (0.01–5.1)
Studies Reviewed in *Update 2002*			
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			
Studies Reviewed in *Update 2002*			
Revich et al., 2001	Residents of Chapaevsk, Russia		
	Age-adjusted incidence (100,000) in males		18.0 in Chapaevsk; 11.3 in all of Russia[b]
	Age-adjusted incidence (100,000) 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)

TABLE 6-12 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
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; CI, confidence interval; 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.

death from cancer of the larynx was reported. Only one death was expected, leading to an SMR of 1.0 (95% CI, 0.01–5.1). The study was limited by the small number of cases and by potential confounders that could not be evaluated.

Environmental Studies

No relevant environmental studies have been published since *Update 2002.*

Vietnam-Veteran Studies

Akhtar et al. (2004) described cancer incidence and mortality in a prospective cohort study of Air Force Ranch Hand veterans who engaged in aerial spraying of herbicides while serving in Southeast Asia and in a comparison population of veterans who served but did not spray herbicides. Because the study does not distinguish between laryngeal and other respiratory cancers, the risk of laryngeal

cancer in the cohort cannot be described from the data presented. Information on respiratory cancer incidence and mortality in the cohorts is presented in the lung cancer discussion.

Synthesis

No studies published since *Update 2002* provide 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 compounds of interest is not challenged by the few data available since *Update 2002*.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one compound of interest and laryngeal cancer.

Biologic Plausibility

No animal studies have identified an increased incidence of laryngeal cancer associated with exposure to the compounds of interest. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and laryngeal cancer, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

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, 93,110 men and 80,660 women will be diagnosed with lung cancer in the United States in 2004, and about 91,930 men and 68,510 women will die from it (ACS, 2004a). Those numbers represent roughly 14% of new cancer diagnoses and 29% of cancer deaths in 2004. The principal types of lung neoplasms are identified collectively as bronchogenic carcinoma (the bronchi are the two main

TABLE 6-13 Average Annual Incidence (per 100,000) of Lung and Bronchial Cancer in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	64.2	59.0	132.1	132.6	125.0	244.0	246.7	242.0	387.0
Females	48.7	50.0	63.8	100.9	103.8	130.0	164.0	174.0	176.2

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

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 greatly beginning about the age of 40. The incidence in people 50–54 years old is double that in people 45–49 years old, and it doubles again in those between the ages of 55 and 59. 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 6-13.

ACS estimates that more than 90% of lung cancers in males are attributable to tobacco use (ACS, 1998), but there are other risk factors: occupational exposure to asbestos, uranium, vinyl chloride, nickel chromates, coal products, mustard gas, chloromethyl ethers, gasoline, diesel exhaust, and inorganic arsenic. Important environmental risk factors include exposure to tobacco smoke and radon (ACS, 2004c).

Summary of *VAO, Update 1996*, *Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of an association between exposure to at least one compound of interest and lung cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000*, and *Update 2002* did not change that finding. Table 6-14 summarizes the results of the relevant studies.

Update of the Scientific Literature

Occupational Studies

Bodner et al. (2003) provide an update of cancer mortality among 2,187 male Dow Chemical Company employees who worked in production areas where there was a potential for dioxin exposure. There were 168 cancer deaths reported in that cohort over the years 1940–1994. RR and SMR were not elevated for lung

TABLE 6-14 Selected Epidemiologic Studies—Lung and Bronchus Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Bodner et al., 2003	Dow chemical production workers—mortality	54	0.8 (0.6–1.1)
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	27	0.7 (0.5–1.0)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4–D production workers—mortality	31 0.9	(0.6–1.3)
Thörn et al., 2000	Swedish lumberjack workers exposed to phenoxyacetic herbicides		
	Foremen—incidence	1	4.2 (0.1–23.2)
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)

TABLE 6-14 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
McDuffie et al., 1990	Saskatchewan farmers applying herbicides	103	0.6 NS
Zober et al., 1990	TCDD reactor accident workers	6	1.6 (*)
	High exposure	4	2.0 (0.6–5.2)
	Chloracne	6	1.8 (0.7–4.0)
Wiklund et al., 1989a	Pesticide appliers in Sweden	38	0.5 (0.4–0.7)
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			
Fukuda et al., 2003	Residents of municipalities in Japan with vs without waste incineration plants		
	Age-adjusted mortality (100,000), lung cancer in males		39.0 ± 6.7 vs 41.6 ± 9.1 (p = 0.001)
	Age-adjusted mortality (100,000), lung cancer in females		13.7 ± 3.8 vs 14.3 ± 4.6 (p = 0.11)
Studies Reviewed in *Update 2002*			
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]

continues

TABLE 6-14 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	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)
	Zone R females	29	1.0 (0.7–1.6)
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			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans, respiratory-system cancer		
	All Ranch Hand veterans		
	Incidence (SIR)	33	1.1 (0.8–1.6)
	Mortality (SMR)	21	0.9 (0.6–1.3)
	Veterans, tours, 1966–1970—incidence	26	1.1 (0.7–1.6)
	White Air Force comparison veterans, respiratory-system cancer		
	All comparison veterans		
	Incidence (SIR)	48	1.2 (0.9–1.6)
	Mortality (SMR)	38	1.1 (0.8–1.5)
	Veterans, tours 1966–1970—incidence	37	1.2 (0.9–1.6)

TABLE 6-14 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
	Non-Vietnam	77	0.9 (*)
Watanabe and Kang, 1995	Marines Vietnam service 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; AIHW, Australian Institute of Health and Welfare; CDVA, Commonwealth Department of Veterans' Affairs; CI, confidence interval; MCPA, methyl-4-chlorophenoxyacetic acid; NS, not signficant; PCDD, polychlorinated dibenzodioxin; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

cancer among all exposure categories (54 observed; 62.5 expected), and the risk did not increase with higher exposure potential (as estimated by job title). There was not an excess number of lung cancers among workers who were diagnosed with chloracne (SMR, 0.3; 95% CI, 0.0–1.1), which is a crude marker for dioxin exposure.

Swaen et al. (2004) published an updated mortality study of a cohort of 1,341 licensed herbicide applicators in the Netherlands. That study is a follow-up of an earlier mortality study of the same cohort (Swaen et al., 1992), extending the period for 13 years. SMRs were calculated based on the Netherlands' cause-specific mortality rates. Swaen et al. (2004) reported no significant increase in cancer of the trachea and lung (SMR, 0.71; 95% CI, 0.47–1.04), but the study was limited because it did not evaluate potential confounders, such as tobacco use.

Environmental Studies

Fukuda et al. (2003) conducted an ecological study examining the association between mortality and dioxin emissions from incinerators in 590 Japanese municipalities. The researchers developed dioxin-related municipal index for the concentration of dioxins, the amount of dioxins per unit of population, the cumulative amount of dioxins, and the cumulative amount of dioxins per unit area of land. Sex-specific and age-adjusted mortality rates were calculated for specific health outcomes, including lung cancer. There was significantly lower mortality from lung cancer in male residents of municipalities with incinerators than among male residents of municipalities without incinerators (39.0 cases/100,000 vs 41.6 cases/100,000; $p = 0.001$). Regression analysis showed no significant association between lung cancer and the dioxin-related municipal indexes after controlling for socioeconomic factors.

The study was limited by its ecological design: Because there were no individual measures of dioxin exposure, only group averages, it would be difficult to determine whether those who developed lung cancer also had higher exposures to dioxins. The authors did not report on migration patterns in the municipalities or on the period in which the incinerators operated. Thus it is unclear whether there would be sufficient latency for lung cancer to develop. The study also was limited because the authors did not control for important potential confounders, such as tobacco use.

Vietnam-Veteran Studies

Akhtar et al. (2004) describe cancer incidence and mortality in a prospective cohort study of Operation Ranch Hand veterans who participated in aerial herbicide spraying during the Vietnam War. There was no difference in the incidence of cancer of the respiratory tract in all Ranch Hand veterans (SIR, 1.13; 95% CI, 0.79–1.57) from that in others who served during the time of heaviest use of Agent Orange (1966–1970) (SIR, 1.08; 95% CI, 0.72–1.55) when compared with national incidence rates. No difference in mortality attributable to respiratory tract cancer was noted (SMR, 0.87; 95% CI, 0.55–1.31). Cancer of the lung was not differentiated from other cancers of the respiratory tract.

Synthesis

Evidence remains inconclusive but suggestive of an association between exposure to at least one compound of interest and lung cancer. The best evidence comes from studies of heavily exposed occupational cohorts. Bodner et al. (2003) did not identify an increased risk of lung cancer among chemical company employees, which is consistent with results of previous studies of that cohort. But the results must be weighed against results for previously reviewed occupational cohorts that did show evidence of an association (Becher et al., 1996; Otto and

Zober, 1996; Steenland et al., 1999). Also supportive of an association are the numerous lines of mechanistic evidence, discussed in the section on biologic plausibility, that provide further support for the conclusion of limited or suggestive evidence.

The evidence of an association is less conclusive from environmental exposures and studies of Vietnam veterans. Many studies lack sufficient information on potential confounders, such as history of tobacco use; adjusting for those factors cannot be done, and thus interpretation of the results is limited.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one compound of interest and carcinomas of the lung and bronchus.

Biologic Plausibility

As noted in *Update 2002*, there is evidence of increased incidence of squamous-cell carcinoma of the lung in rats exposed to high concentrations of TCDD. A more recent study reported a significant increase in cystic keratinizing epitheliomas in rats exposed to TCDD for 2 years. Rats administrated cacodylic acid showed 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 provide evidence that TCDD promotes the carcinogenic process. Lung tissue has been found to have high concentrations of the AhR, which mediates the effects of TCDD, and recent data have shown that CYP1A1 and CYP1A2 are 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 synergizes the carcinogenic effects of a variety of other compounds to which human lung tissue is exposed.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and lung cancer, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

BONE AND JOINT CANCER

ACS (2004a) reports that about 1,230 men and 1,210 women will be diagnosed with bone or joint cancer (ICD-9 170.0–170.9) in the United States in 2004 and that 720 men and 580 women will die from bone or joint cancers this year. 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 cancer is considered here. The average annual incidence of bone and joint cancer is shown in Table 6-15.

Bone cancer is more common in teenagers than it is in adults. Bone cancer is quite rare among people in the age groups of most Vietnam veterans (50–64 years of age). 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 non-cancer bone diseases, including Paget's disease.

Summary of *VAO, Update 1996, Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and bone and joint cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000*, and *Update 2002* did not change that finding. Table 6-16 summarizes the results of the relevant studies.

Update of the Scientific Literature

An occupational study by Swaen et al. (2004) examined cancer mortality in 1,341 licensed herbicide appliers in the Netherlands. No deaths from bone cancers were observed in the cohort.

TABLE 6-15 Average Annual Incidence (per 100,000) of Bone and Joint Cancer in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	0.9	1.0	1.1	1.4	1.5	1.2	1.7	1.9	[b]
Females	0.9	1.1	0.5	1.0	1.1	0.3	0.9	0.9	[b]

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.
[b] Insufficient data to provide meaningful incidence.

TABLE 6-16 Selected Epidemiologic Studies—Bone and Joint Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators	0	—
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	Iowa Farmers	56	1.1 (NS)
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
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)

continues

TABLE 6-16 *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.
— Information denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; CI, confidence interval; 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.

No other relevant environmental or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

There are no new data in the one new study (Swaen et al., 2004) to indicate an increase in bone cancer. Therefore, there is no evidence to support a change from the earlier conclusion that there is inadequate or insufficient evidence to determine an association between bone cancer and exposure to the compounds of interest.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the compounds of interest and bone cancer. The conclusion is based on

occupational and environmental studies in which the subjects were exposed to a variety of herbicides and herbicide compounds.

Biologic Plausibility

No animal studies reported an increased incidence of bone cancer after exposure to the compounds of interest. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and bone and joint cancer, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

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 4,760 men and 3,920 women will be diagnosed with STS in the United States in 2004 and that about 2,020 men and 1,640 women will die from it (ACS, 2004a). 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 6-17.

Among the risk factors for STS are exposure to ionizing radiation during

TABLE 6-17 Average Annual Incidence (per 100,000) of Soft-Tissue Sarcoma (Including Malignant Neoplasms of Heart) in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	4.1	4.2	4.3	4.4	4.2	5.9	6.3	6.7	4.3
Females	2.8	3.0	2.8	4.3	4.1	6.7	5.0	4.7	7.3

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

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*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was sufficient information to determine an association between exposure to at least one of the compounds of interest and STS. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. (Table 6-18 summarizes the relevant studies.)

Update of the Scientific Literature

Occupational Studies

Bodner et al. (2003) completed a mortality assessment (1940–1994) of male chemical production workers who worked in areas in which there was a potential for dioxin exposure. Although not statistically significant, the incidence of STS in that cohort was considerably greater than expected (SMR, 2.4; 95% CI, 0.3–8.6; 2 cases); the broad confidence interval resulting from the small number of cases makes it difficult to draw a conclusion from the data. Both cases were in workers whose job titles were associated with a "very high" potential for exposure.

Environmental Studies

Comba et al. (2003) performed a case–control study of STS in a population living near an industrial-waste incinerator in Mantua, Italy. A paper by Costani et al. (2000), reviewed in *Update 2002*, assesses the same population. Dioxins are among the contaminants produced by the combustion of such wastes. Cases (17 male; 20 female) were identified from pathology registries in 4 adjoining municipalities; control subjects were age- and gender-matched from population registries for the region. There was a significant increase in risk of STS associated with residence within 2 km of the incinerator (odds ratio [OR] 31.4; 95% CI, 5.6–176.1; 5 cases); no association was found for STS in residents outside that area. The study was considerably weakened by lack of data on TCDD concentrations in the environment or in tissue samples and by the absence of pathologic review of cases.

Tuomisto et al. (2004) performed a case–control study of STS in a population in Southern Finland. Cases (110) were location- and age-matched with control subjects (227) who had had appendectomies. Fish consumption—the putative source of dioxin exposure—was similar among both groups. TCDD concentrations were measured in subcutaneous fat samples taken from the subjects. The

TABLE 6-18 Selected Epidemiologic Studies—Soft-Tissue Sarcoma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Bodner et al., 2003	Dow chemical production workers—Mortality	2	2.4 (0.3–8.6)
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)
	Latency 20 years, exposure 1 year	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)

continues

TABLE 6-18 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
Woods et al., 1987	Male residents of Washington State		
	High phenoxy exposure	*	0.9 (0.4–1.9)
	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		
	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	13	5.5 (2.2–13.8)
Eriksson et al., 1979, 1981	Swedish workers	25	(2.2–10.2) 5.1 matched
ENVIRONMENTAL			
New Studies			
Comba et al., 2003	Residents near an industrial-waste incinerator in Mantua, Italy		
	Residence within 2 km of incinerator	5	31.4 (5.6–176.1)
Tuomisto et al., 2004	STS patients and controls—southern Finland	110	
	Quintile 2 (median tissue concentration 20 ng/kg WHO-TEQ)	*	0.4 (0.2–1.1)
	Quintile 5 (median tissue concentration ~60 ng/kg WHO-TEQ)	*	0.7 (0.2–2.0)
Studies Reviewed in *Update 2002*			
Costani et al., 2000	Residents near a chemical plant in Mantua, Italy—mortality	20	2.3 (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	(*)

TABLE 6-18 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
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	0–9	<1
Crane et al., 1997b	Australian national service Vietnam veterans	4	0.7
	Comparison group	2	—
AFHS, 1996	Ranch Hand veterans	1	—
	Comparison group	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)

continues

TABLE 6-18 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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	(*)
	Comparison group	1	(*)
Breslin et al., 1988	Army Vietnam veterans	30	1.0
Fett et al., 1987	Australian Vietnam veterans	1	1.3 mortality
	Comparison group	1	rate, age-adjusted (0.1–20.0)
Anderson et al., 1986a,b	Wisconsin Vietnam veterans	5	1.5 (0.6–3.5)
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 CIs include 1.
* Information not provided by study authors.
— Information 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; CI, confidence interval; 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; STS, soft-tissue sarcoma; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; WHO TEQ, Toxicity Equivalent as defined by the World Health Organization.

researchers did not observe an association between STS and either 2,3,7,8-TCDD or World Health Organization (WHO) toxicity equivalent (TEQ) dioxin concentrations in the human tissue. The OR for the highest quartile of WHO TEQ was 0.7 (95% CI, 0.2–2.0); for 2,3,7,8-TCDD it was 0.5 (95% CI, 0.2–1.4).

No relevant Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

The environmental-exposure report from Mantua, Italy (Comba et al., 2003) lacks exposure data, tissue concentrations for dioxin, and independent pathologic review of diagnosed cases. The case report study from Finland (Tuomisto et al., 2004) was a carefully conducted study in men and women who have a low but

life-long exposure to dioxin in the food chain (Baltic fish). Individual biologic samples were used to estimate dioxin exposure in STS cases and controls; there was no positive association between STS risk and dioxin concentrations. In fact, there was a suggestion for increased risk of STS at the lowest dioxin exposure.

Findings from occupational, environmental, and Vietnam-veteran studies show sufficient evidence to link herbicide exposure to STS. That evidence is supported by long-term follow-up studies of dioxin-exposed chemical workers. The reports by Bodner et al. (2003), Comba et al. (2003), and Tuomisto et al. (2004) do not change the conclusions from those of previous committees.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is sufficient evidence of an association between exposure to at least one of the compounds of interest and STS.

Biologic Plausibility

No increased incidence of STS has been reported from animal studies. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and STS, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

SKIN CANCER—MELANOMA

Skin cancers are generally divided into two broad categories: neoplasms that develop from melanocytes (malignant melanoma [MM]) and neoplasms that do not. Non-melanocytic 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 6-19. Beginning with *Update 1998,* the committee chose to address MM studies separately from those of non-melanocytic cancers. Because non-melanocytic cancers are highly treatable, their discussion is divided further into studies on mortality and studies on incidence. Many researchers report results by combining all types of skin cancer; often, they do not specify the

TABLE 6-19 Average Annual Cancer Incidence (per 100,000) of Skin Cancers (Excluding Basal and Squamous-Cell Cancers) in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Melanomas of the Skin									
Males	33.0	38.8	0.8	43.1	49.6	1.6	54.1	63.9	4.3
Females	23.7	28.3	0.5	27.0	32.0	2.7	27.9	33.9	1.3
Other Non-Epithelial Skin Cancers									
Males	3.3	3.3	4.3	2.7	2.9	1.6	4.2	4.3	4.8
Females	1.8	1.7	3.0	1.8	1.8	2.0	2.5	2.8	1.3

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001. SEER incidence data are not available for nonmelanocytic skin cancer.

types assessed. In the interest of completeness, mortality and morbidity studies are listed in Tables 6-20 and 6-21.

According to ACS estimates, about 29,900 men and 25,200 women will be diagnosed with cutaneous melanoma (ICD-9 172.0–172.9) in the United States in 2004, and 5,050 men and 2,860 women will die from it (ACS, 2004a). More than 1 million cases of non-melanocytic skin cancer (ICD-9 173.0–173.9), primarily basal-cell and squamous-cell carcinomas, are diagnosed in the United States each year (ACS, 2004a). Because it is not required to report those cancers to registries, the numbers of cases are not as precise as for other cancers. ACS reports that although melanoma accounts for only about 4% of skin cancer cases it is responsible for about 79% of skin cancer deaths (2004a). ACS estimates that 1,000–2,000 people die each year from non-melanocytic skin cancer.

Melanomas occur more frequently 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 include the presence of some moles on the skin, 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 that is the result of genetic factors or attributable to similarities in skin type and sun exposure patterns.

Excessive exposure to UV radiation is the most important risk factor for non-melanocytic skin cancer, although some skin diseases and chemical exposures also have been identified as potential risk factors. Exposure to inorganic arsenic

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

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	5	3.6 (1.2–8.3)
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	Iowa Farmers	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: CI, Confidence Interval; IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; NS, not significant; USDA, US Department of Agriculture.

is a risk factor for skin cancer, and cacodylic acid is a metabolite of inorganic-arsenic.

Summary of *VAO, Update 1996*, *Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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 MM separately from that of non-melanocytic skin cancers. It found that there was inadequate or insufficient information to determine association between the compounds of interest and MM. The *Update 2000* and *Update 2002* committees concurred with the findings of the *Update 1998* committee. (Tables 6-22 and 6-23, respectively, summarize the relevant melanoma mortality and morbidity studies.)

TABLE 6-21 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 RH 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.

Update of the Scientific Literature

Occupational Studies

Swaen et al. (2004) presented results for a 21 year follow-up—from January 1980 to January 2001—of mortality in a cohort of 1,341 licensed herbicide applicators working for government agencies in the Netherlands. Information was available on the types and amounts of herbicides used in all municipal spraying projects in 1980, but those data could not be linked to the work of any individual applicators. No data were available on any potential risk factors other than age. SMRs were calculated based on age and calendar-year, cause-specific mortality rates of the general population of the Netherlands. Five deaths from skin cancer were recorded for the cohort (one melanoma, two squamous-cell carcinoma, two

TABLE 6-22 Selected Epidemiologic Studies—Melanoma Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers	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 6-22 *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; CI, confidence interval; IARC, International Agency for Research on Cancer; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

unknown). Only 1.4 deaths would have been expected, leading to an SMR of 3.6 for deaths attributable to all skin cancers (95% CI, 1.2–8.3).

Environmental Studies

No environmental studies of melanoma have been published since those reviewed in *Update 2002*.

Vietnam-Veteran Studies

Akhtar et al. (2004) reported on the incidence of cancer in veterans of Operation Ranch Hand and a comparison cohort of other Air Force veterans who served in Southeast Asia during the same period but who were not involved in herbicide spraying. The cancer occurrence was ascertained for the period between each veteran's departure from Southeast Asia and December 31, 1999. Information on cancer was derived from study examinations (1982, 1985, 1987, 1992, 1997), medical records, and death certificates. SIRs were calculated to compare the observed number of cancers with the number expected based on sex, race, age, and calendar-year-specific incidence rates from SEER data. In all, 1,189 Ranch Hand veterans and 1,776 comparison Air Force veterans were included in the analyses.

The analyses of melanoma were restricted to white veterans (89% of the study population). Melanoma was more common among Ranch Hand veterans

TABLE 6-23 Selected Epidemiologic Studies—Melanoma Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2002*			
Thörn et al., 2000	Swedish lumberjack workers exposed to phenoxyacetic herbicides		
	Female	1	3.5 (0.1–19.2)
	Male	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 ($p < 0.05$)
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
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			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand (RH) veterans—incidence		
	All RH veterans	17	2.3 (1.4–3.7)
	RH veterans w/ tours between 1966–1970	15	2.6 (1.5–4.1)
	White Air Force comparison veterans—incidence		
	All comparison veterans	15	1.5 (0.9–2.4)
	Comparison veterans w/ tours between 1966–1970	12	1.5 (0.8–2.6)
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force RH veterans	16	1.8 (0.8–3.8)
AIHW, 1999	Australian Vietnam veterans—male	483	380 expected (342–418)

continues

TABLE 6-23 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Ketchum et al., 1999	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)
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 RH 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; CI, confidence interval.

than would have been predicted from the general population (17 observed, 7.33 expected; SIR, 2.3; 95% CI, 1.4–3.7). The comparison cohort of Air Force veterans had a more modest excess of melanoma (15 observed, 10.24 expected; SIR, 1.46; 95% CI, 0.85–2.36). The probability value for the statistical test of difference between the SIRs (2.33 and 1.46) was 0.19. The SIR for Ranch Hand veterans was fairly constant when the analyses with SEER data were repeated with restriction to veterans whose tours ended between 1966 and 1970 (SIR, 2.57), veterans who spent no more than 2 years in Southeast Asia (SIR, 2.36), and veterans who were in Vietnam for 100% of their service in Southeast Asia (SIR, 3.05).

The incidence of melanoma also was analyzed by a categorical measure of dioxin concentration. The comparison Air Force veterans served as the referent group; the Ranch Hand veterans were divided into background (≤10 parts per trillion [ppt]); low exposure (>10 ppt, dioxin at end of service in Vietnam estimated at ≤118.5 ppt); and high exposure (>10 ppt; dioxin at end of service in Vietnam estimated at >118.5 ppt). The analyses used proportional hazards models to adjust for age at tour, military occupation, skin reaction to sun exposure, and eye color. When the analyses were restricted to veterans who had served no more than 2 years in Southeast Asia, elevated incidence rates were observed for each

Ranch Hand category: background, RR, 2.99; 95% CI, 0.53–16.8; low, RR, 7.42; 95% CI, 1.34–41.04; high, RR, 7.51; 95% CI, 1.12–50.21. There was no statistically significant association between exposure and incidence of melanoma among veterans with more than 2 years of service in Southeast Asia (data not shown). Similar rate ratios were observed when Ranch Hand veterans with 100% of their service in Vietnam were contrasted with comparison veterans who had served elsewhere in Southeast Asia. There was no difference in the incidence of melanoma in the analysis of the remaining veterans (Ranch Hand veterans with some service outside of Vietnam and comparison veterans with some service in Vietnam).

Synthesis

The study by Swaen et al. (2004) reported that mortality from all skin cancers combined is greater among herbicide applicators than would have been expected in the general population of the Netherlands during the same 21-year study period. The authors recognize that herbicide applicators are likely to have significant exposure to UV radiation, a well-established, important risk factor for skin cancer. Because of the study design (comparison based on the general population), it is impossible to separate the effect of the two occupationally related exposures (sunlight and herbicides).

Akhtar et al. (2004) assessed the incidence of melanoma in Ranch Hand veterans relative to a sample of the general population (studied by the SEER Program) and to a comparison cohort of Air Force veterans. Melanoma was more common in both groups of veterans than in the general population, particularly in the Ranch Hand veterans. The authors acknowledge an important limitation in this contrast—detection of melanoma in study participants could be elevated compared with that in the general population because of the better detection made possible by the study's schedule of periodic physical examinations. In addition, comparisons with the SEER data cannot be adjusted for such confounders as sun exposure. For those reasons, the "internal" comparison (between groups of veterans) assumes added importance.

In the analyses limited to Ranch Hand and comparison Air Force veterans, the associations with melanoma are restricted to the stratum of veterans with no more than 2 years of service in Southeast Asia and to a stratum created by the subset of Ranch Hand veterans who served only in Vietnam and comparison veterans who served elsewhere in Southeast Asia. If those categories somehow capture a confounding factor, the appropriate analysis would combine information from each stratum (more than 2 years of service and 2 years or less) to produce an adjusted RR. No satisfactory rationale is given to support the notion that a valid analysis of melanoma must be limited to veterans with less than 2 years of service or to a definition that completely confounds Ranch Hand status with service in Vietnam. In the absence of such a rationale, the overall associa-

tion between exposure to herbicides and the incidence of melanoma in this study is not definitive.

Although previously reviewed studies of US and Australian veterans have reported a higher incidence of melanoma among male, non-black 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 (Ketcham et al., 1999) was observed in veterans in the low-TCDD category; that would not be expected if there were an association between exposure and melanoma. Comparison of the results with those from the report by Akhtar et al. (2004) is not possible because Akhtar et al. (2004) limited analysis to a subset of their study population. 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.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and melanoma. The evidence regarding association is drawn from occupational, environmental, and veterans' studies in which subjects were exposed to herbicides and herbicide components.

Biologic Plausibility

Mice were treated topically (on the 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 provide evidence that TCDD promotes the carcinogenic process. Furthermore, recent data have shown the ability of TCDD to repress the expression of several tumor suppressor genes and immortalize human keratinocytes.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and melanoma, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

SKIN CANCER—BASAL-CELL AND SQUAMOUS-CELL (NON-MELANOMA)

The preceding section presents background information on skin cancer.

Summary of *VAO, Update 1996*, *Update 1998, Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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 non-melanocytic skin cancers separately from that for malignant melanoma. It found that there was inadequate or insufficient information to determine an association between exposure to the compounds of interest and basal-cell or squamous-cell cancers. The *Update 2000* and *Update 2002* committees concurred with that finding. (Tables 6-24 and 6-25 summarize the relevant studies.)

Update of the Scientific Literature

The only new study is an occupational cohort study with data on non-melanoma skin cancer (Swaen et al., 2004) summarized in the previous section of this chapter. In that study of cancer mortality in a cohort of herbicide applicators, the analysis of skin cancer is based on five deaths (one melanoma, two squamous-cell carcinoma, two unknown type), and the SMR is computed for all skin cancers combined. The results and interpretation of the study are stated in the update of the scientific literature on melanoma.

No relevant environmental or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

In the study by Swaen et al. (2004), the mortality from all skin cancers combined is greater among herbicide applicators than would have been expected from the general population of the Netherlands during the same 21-year period. The authors recognize that herbicide applicators are also more likely than are those in the general population to be exposed to sunlight, a well-established,

TABLE 6-24 Selected Epidemiologic Studies—Other Non-melanoma (Basal-Cell and Squamous-Cell) Skin Cancer—Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licensed herbicide appliers		
	Melanoma, squamous cell carcinoma, and unknown skin cancer	5	3.6 (1.2–8.3)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers		
	Non-melanoma skin cancer	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.
— Information denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; CI, confidence interval; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

important risk factor for skin cancer. The study design (with comparison based on the general population) makes it impossible to distinguish the effects of the two occupationally related exposures (sunlight and herbicides).

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or

TABLE 6-25 Selected Epidemiologic Studies—Other Non-melanoma (Basal-Cell and Squamous-Cell) Skin Cancer—Morbidity

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2002*			
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			
Studies Reviewed in *Update 2002*			
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 6-25 *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; CI, confidence interval.

insufficient evidence to determine an association between exposure to the compounds of interest and basal-cell or squamous-cell cancers.

Biologic Plausibility

Fibrosarcomas occurred in the integumentary system of female mice treated with TCDD topically (on the skin surface) for 2 years (Huff et al., 1991). 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 provide evidence that TCDD promotes the carcinogenic process. Furthermore, recent data have shown the ability of TCDD to repress the expression of several tumor suppressor genes and immortalize human keratinocytes.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and non-melanoma skin cancers, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

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. ACS estimates that 215,990 women will be diagnosed with breast cancer in the United States in 2004 and that 40,110 will die from it (ACS, 2004a). Overall, those numbers represent about 33% of the incidence of new cancers and 15% of cancer deaths among women. Incidence data on breast cancer are presented in Table 6-26.

Breast cancer incidence generally increases with age. In the age groups of most Vietnam veterans, the incidence in whites is higher than is 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. A pooled analysis of six large-scale prospective studies of invasive breast cancer showed that alcohol consumption was associated with a linear increase in incidence in women over the range of consumption reported by most

TABLE 6-26 Average Annual Incidence (per 100,000) of Breast Cancer in Females in United States[a]

50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
All Races	White	Black	All Races	White	Black	All Races	White	Black
258.3	268.7	226.5	334.1	349.3	273.3	389.0	407.9	326.1

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

women (Smith-Warner et al., 1998). The potential of other personal behavioral and environmental factors (including exogenous hormones) to affect breast cancer incidence is being studied extensively.

Most female Vietnam veterans who were potentially exposed to herbicides in Vietnam are approaching or have recently reached menopause. Given the high incidence of breast cancer among older and post-menopausal women in general, based on demographics alone it is expected that the breast cancer burden among female Vietnam veterans will increase in the near future.

Breast cancer occurs primarily in women, but occasionally does occur in men (ACS, 2004a). The vast majority of breast cancer epidemiology studies, however, involve women. The committee, therefore, makes its conclusions based on the studies in women.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and breast cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000*, and *Update 2002* did not change that finding. Table 6-27 summarizes the relevant research.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

No studies published since *Update 2002* have investigated breast cancer. Previously-published studies support the conclusion that the evidence is inadequate or insufficient to determine an association between exposure to the compounds of interest and breast cancer.

Conclusions

Strength of Evidence from Epidemiologic Studies

The committee maintained the conclusion that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and risk of breast cancer.

TABLE 6-27 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	Female farm workers and residents in North Carolina		
	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			
Studies Reviewed in *Update 2002*			
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)

continues

TABLE 6-27 *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)

TABLE 6-27 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
VIETNAM VETERANS			
Studies Reviewed in *Update 2002*			
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; CI, confidence interval; IARC, International Agency for Research on Cancer; NS, not significant; PCB, polychlorinated biphenyl; RR, relative risk; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Biologic Plausibility

All experimental evidence indicates that 2,4-D, 2,4,5-T, and TCDD are weakly genotoxic if anything. However, TCDD is a demonstrated carcinogen in animals and is classified as a human carcinogen because of its ability to act as a strong tumor promoter. The promoting activity could occur by several 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 that tissue. One study showed 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 that compound. 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, which could 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 could have antiestrogenic properties. TCDD also can induce the expression of a cytochrome P450, CYP1B1, that metabolizes estradiol to 4-hydroxyestradiol. That metabolite is directly genotoxic and can undergo redox cycling to form genotoxic reactive oxygen species; it could be involved in carcinogenesis in the breast (Jefcoate et al., 2000). Some studies suggest that TCDD exposure facilitates the transition of breast cancer cells from estrogen dependence to estrogen independence; that has been demonstrated to be one important step in the progression of breast cancer. Some studies also have shown that prenatal exposure to TCDD increases the number of mammary tumors induced by other chemicals. Thus, experimental data indicate biologic plausibility for an association between exposure to TCDD and TCDD-containing herbicides and breast cancer.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and breast cancer, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

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 ovary (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 2004 are presented in Table 6-28 (ACS, 2004a). Genital system cancers represent roughly 12% of new cancer cases and 11% of cancer deaths in women.

TABLE 6-28 Estimates of New Cases and Deaths in 2004 in United States for Selected Cancers of the Female Reproductive System[a]

Site	New Cases	Deaths
Cervix	10,520	3,900
Endometrium	40,320	7,090
Ovary	25,580	16,090
Other female genital	6,130	1,640

[a] ACS (American Cancer Society), 2004.

TABLE 6-29 Average Annual Incidence (per 100,000) of Female Genital System Cancers in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
All genital sites	91.0	95.4	58.5	120.6	127.1	86.1	150.9	156.2	139.9
Cervix	12.9	11.5	15.2	13.4	12.2	21.9	15.5	13.3	26.4
Endometrium	47.9	51.4	24.0	68.3	73.1	37.2	88.0	93.7	71.7
Ovary	25.5	27.5	15.0	33.3	35.7	21.3	38.9	40.5	31.5
Other genital organs	1.1	1.1	0.5	1.4	1.6	0.3	1.8	2.1	1.3

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

Incidence patterns of and risk factors for those diseases vary (Table 6-29). 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; older women are at greater risk. Obesity increases the risk of endometrial cancer by 2–5 times, depending on how obese the subject is. HPV infection is the most important risk factor for cervical cancer; HPV types 16 and 18 account for about half of those cancers. 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*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to the compounds of interest and female reproductive cancers. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000*, and *Update 2002* did not change that finding. Tables 6-30, 6-31, and 6-32 summarize the results of the relevant studies.

Update of the Scientific Literature

No occupational, environmental, or Vietnam-veteran studies of cancer of the female reproductive system have been published since *Update 2002*.

TABLE 6-30 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			
Studies Reviewed in *Update 2002*			
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			
Studies Reviewed in *Update 2002*			
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[d]	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; CI, confidence interval; IARC, International Agency for Research on Cancer.

TABLE 6-31 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			
Studies Reviewed in *Update 2002*			
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; CI, confidence interval; IARC, International Agency for Research on Cancer.

TABLE 6-32 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			
Studies Reviewed in *Update 2002*			
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; CI, confidence interval; IARC, International Agency for Research on Cancer.

Synthesis

No studies published since *Update 2002* have investigated female reproductive cancers. Previously-published studies support the conclusion that the evidence is inadequate or insufficient to determine an association between exposure to the compounds of interest and female reproductive cancers.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and uterine, ovarian, or cervical cancer.

Biologic Plausibility

No animal studies have reported an increased incidence of female reproductive cancer after exposure to the compounds of interest. One study (Kociba et al., 1978), however, showed 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 the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and female reproductive cancer, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

PROSTATE CANCER

According to ACS estimates, 230,110 new cases of prostate cancer (ICD-9 185) will be diagnosed in the United States in 2004, and 29,900 men will die from the disease (ACS, 2004a). That makes prostate cancer the second most common cancer among men (after non-melanocytic skin cancers); it is expected to account for about 33% of new cancer diagnoses and 10% of cancer deaths in 2004. The average annual incidence of prostate cancer is shown in Table 6-33.

Incidence varies dramatically with age and race. The risk more than doubles between the ages of 50–54 years and 55–59 years, and it nearly doubles again between the ages of 55–59 years and 60–64 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 twice that of whites in the United States, 5 times that of Alaska 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.

TABLE 6-33 Average Annual Incidence (per 100,000) of Prostate Cancer in United States[a]

50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
All Races	White	Black	All Races	White	Black	All Races	White	Black
137.6	132.6	259.4	331.8	321.5	580.0	600.6	587.3	1,009.6

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

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 have led to improved detection and thus to reports of increased incidence in the United States. The long-term influence of better screening on incidence and mortality, however, is difficult to predict for any country or population, and it 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 PSA use could cause more variability in the results.

Prostate cancer tends not to be fatal, so mortality studies might miss 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 treatment that would increase the likelihood of death.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was limited or suggestive evidence to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and prostatic cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Table 6-34 summarizes results of the relevant studies, and it includes morbidity and mortality studies. The type of study must be considered in the interpretation and weighing of evidence. Simply examining all the estimated risks in the table together will not provide a good assessment of the risks.

TABLE 6-34 Selected Epidemiologic Studies—Prostate Cancer Morbidity and Mortality

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Alavanja et al., 2003	Pesticide appliers from Iowa and North Carolina—Incidence	566	1.14 (1.05–1.24)
Bodner et al., 2003	Dow chemical production workers—Mortality	—	1.7 (1.0–2.6)
Swaen et al., 2004	Dutch licenced herbicide applicators—Mortality	6	1.0 (0.4–2.2)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers—Mortality	7	1.3 (0.5–2.8)
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides		
	Foremen—Incidence	2	4.7 (*)
	Male lumberjacks—Incidence	3	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)
	Incidence (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)

continues

TABLE 6-34 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
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			
Studies Reviewed in *Update 2002*			
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)

TABLE 6-34 *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—Incidence		
	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)
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			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans		
	All Ranch Hand veterans		
	Incidence (SIR)	36	1.5 (1.0–2.0)
	Mortality (SMR)	2	0.7 (0.1–2.3)
	Veterans, tours 1966–1970—Incidence	34	1.7 (1.2–2.3)
	White Air Force comparison veterans		
	All comparison veterans		
	Incidence (SIR)	54	1.6 (1.2–2.1)
	Mortality	3	0.8 (0.2–2.1)
	Veterans, tours 1966–1970—Incidence	42	1.6 (1.2–2.2)
Giri et al., 2004	Veterans using the DVA Medical Center in Ann Arbor, MI		
	All cases	11	OR 2.1 (0.8–5.2)
	Cases in white veterans only	*	OR 2.7 (0.9–8.2)
Studies Reviewed in *Update 2000*			
AFHS, 2000	Air Force Ranch Hand veterans	26	0.7 (0.4–1.3)
AIHW, 1999	Australian Vietnam veterans	212	147 expected (123–171)
CDVA, 1998a	Australian Vietnam veterans	428[d]	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 (*)

continues

TABLE 6-34 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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.
— Information 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; CI, confidence interval; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NIOSH, National Institute for Occupational Safety and Health; OR, odds ratio; USDA, US Department of Agriculture.

Update of the Scientific Literature

Occupational Studies

Alavanja et al. (2003) conducted a prospective cohort study of 55,332 male agricultural pesticide applicators from Iowa and North Carolina. 2,4-D and 2,4,5-T were among the herbicides for which data were collected; no data on dioxin were reported. Exposure, health, and demographic information were collected via questionnaire, with matching to state cancer registries for cases. The overall prostate cancer SIR was 1.14 (95% CI, 1.05–1.24) in an analysis that controlled for age, race, state of residence, years of education, type of applicator license, tobacco use, and family history of prostate cancer. In the factor analysis, use of chlorinated pesticides, ever having used chlorinated phenoxy-herbicides 2,4,5-T and 2,4,5-TP, and farmer–applicator over age 50 were correlated with prostate cancer risk. However, the incidence ratios for 2,4-D and 2,4,5-T use were not reported "because they did not demonstrate a significant exposure–response association with prostate cancer."

Environmental Studies

No relevant environmental studies have been published since *Update 2002*.

Vietnam-Veteran Studies

Akhtar et al. (2004) analyzed cancer incidence and mortality in Air Force veterans of the Vietnam War. Statistically significant SIRs for prostate cancer were noted for Operation Ranch Hand (1.46; 95% CI, 1.04–2.00) and comparison (1.62; 95% CI, 1.23–2.10) veterans. When the analysis was restricted to veterans whose tours ended in 1966–1970, the period of heaviest herbicide spraying, the estimate increased in the Ranch Hand cohort (SIR, 1.68; 95% CI, 1.19–2.33) but did not materially change in the comparison cohort (SIR, 1.64; 95% CI, 1.20–2.20).

In a pilot case–control study of veterans using a Department of Veterans Affairs medical center in Ann Arbor, Michigan, Giri et al. (2004) identified cases by searching computerized pathology records for a diagnosis of prostate cancer, and limited cases to those in the same age groups that characterize service in Vietnam. Controls were randomly selected from males within the medical center who were born in the appropriate years and who had not had a diagnosis of prostate cancer. Exposure to Agent Orange was determined by reviewing administrative records within the veterans' medical records. Veterans were asked about exposure to Agent Orange. Of the 47 case subjects and 142 control subjects questioned, 7 cases (14.9%) and 19 controls (13.3%) said they did not know whether they had been exposed; they were excluded from the analyses. After adjusting for age and race, men with prostate cancer were shown to be twice as likely to report previous exposure to Agent Orange, but the 95% CI included 1 (OR, 2.1; 95% CI, 0.8–5.2). In a smaller analysis, of white veterans only, the OR was 2.7 (95% CI, 0.8–8.2).

Synthesis

The occupational study (Alavanja et al., 2003) does not contain an adequate breakdown of the farmer–applicators' exposure to herbicides, so a statement about prostate cancer risk in that population is not possible. The pilot study by Giri et al. (2004) produced results that are consistent with other studies', indicating an increased risk of prostate cancer after exposure to the compounds of interest. Because of its limitations, however, that study does not provide much weight of evidence. Although the ORs are elevated, the confidence intervals include 1—a possible result of the small sample size. The use of self-reported exposure assessments also could have biased the results. The study of Air Force veterans (Akhtar et al., 2004) reports an increased risk of prostate cancer in Ranch Hand men compared with national incidence rates (SIR, 1.46; 95% CI,

1.04–2.0; $p = 0.03$). The investigators also compared prostate cancer incidence in Ranch Hands with Air Force veterans who were stationed in Southeast Asia but not in Vietnam; there was no significant increase in prostate cancer (SIR, 1.68 vs SIR, 1.64; $p = 0.92$). The RR of prostate cancer in Ranch Hands correlated with putative exposure to Agent Orange (high 6.04 vs low 2.17 vs background 1.5; $p = 0.01$). Those data support the committee's prior conclusion that there is limited or suggestive evidence of an association between exposure to Agent Orange and prostatic cancer.

Prostatic cancer is a common condition in older men, so it is likely that multiple factors are responsible and unlikely that herbicide exposure is a major cause. Still, even a small relative risk can mean a large number of cases. Therefore, an observed increase of 13% in incidence among Swedish pesticide appliers attributable solely to exposure to the pesticides or TCDD could translate into many cases. Generally speaking, for common conditions, such as prostatic cancer incidence and cardiovascular disease, RRs are not expected to be high for any particular causative factor because the background rates are already high; that situation is in contrast with rare diseases, which tend to exhibit higher relative ratios.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one of the compounds of interest and prostatic cancer. Although the associations are not large, several studies provide evidence that suggests a small increase in morbidity or mortality. 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 reported an increased incidence of prostatic cancer after exposure to the compounds 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, that it 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. (1997/1998) examined 281 workers at two production facilities included in the National Institute for Occupational Safety and Health (NIOSH) cohort and reported a trend toward higher serum follicle-stimulating hormone and luteiniz-

ing hormone (LH) and a trend toward lower testosterone, according to the serum concentration of lipid-adjusted TCDD. Those results were seen in models adjusted for age, alcohol and tobacco use, and diabetes mellitus; the models for LH and testosterone also were adjusted for body mass index. The data suggest that exposures of workers to TCDD, particularly above 20 pg/g serum lipids, are associated with alterations in male reproductive hormone concentrations. That the prostate could be a target organ for hormonally active xenobiotics lends biologic plausibility to an association with TCDD exposure. In addition, several studies have shown enzyme induction in human prostate cells to be directly responsive to TCDD.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and prostate cancer, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

TESTICULAR CANCER

ACS estimates that 8,980 men will be diagnosed with testicular cancer (ICD-9 186.0–186.9) in the United States in 2004 and that 360 men will die from it (ACS, 2004a). The average annual incidence of testicular cancer is shown in Table 6-35.

Testicular cancer occurs more often in men younger than 40 than it does 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 be a risk factor. Several other hereditary and environmental factors have been suggested, but the results of research are inconsistent (Bosl and Motzer, 1997).

TABLE 6-35 Average Annual Incidence (per 100,000) of Testicular Cancer in United States[a]

50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
All Races	White	Black	All Races	White	Black	All Races	White	Black
4.1	4.7	1.4	2.1	2.3	1.2	1.6	1.8	0.5

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and testicular cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Table 6-36 summarizes the results of the relevant studies.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

The evidence from epidemiologic studies is inadequate to link herbicide exposure and testicular carcinoma; no new published information was found to change that determination.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and testicular cancer.

Biologic Plausibility

No animal studies have reported an increased incidence of testicular cancer after exposure to the compounds of interest. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and testicular cancer, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

TABLE 6-36 Selected Epidemiologic Studies—Testicular Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2002*			
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)

continues

TABLE 6-36 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
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	31	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.
— Information 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; CI, confidence interval; IARC, International Agency for Research on Cancer; MCPA, methyl-4-chlorophenoxyacetic acid; NS, not significant.

URINARY BLADDER CANCER

Urinary bladder cancer (ICD-9 188.0–188.9) is the most common of the urinary tract cancers. According to ACS estimates, 44,640 men and 15,600 women will be diagnosed with this cancer in the United States in 2004, and 8,780 men and 3,930 women will die from it (ACS, 2004a). In males, in whom this cancer is about 3 times as likely as it is in females, those numbers represent about 6% of new cancer diagnoses and 3% of deaths. Overall, bladder cancer is sixth on

TABLE 6-37 Average Annual Incidence (per 100,000) of Urinary Bladder Cancer in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	25.3	27.8	14.1	48.6	53.8	23.3	84.7	93.4	43.6
Females	8.3	9.3	5.1	13.9	15.5	9.6	24.2	27.3	16.4

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

the cancer list in the United States. The average annual incidence of urinary bladder cancer is shown in Table 6-37.

Among men in the age groups that characterize most Vietnam veterans, bladder cancer incidence is about twice as high in whites as in blacks, and almost three times as high in males as in females. Bladder cancer incidence increases greatly after the age of 40. For men in the age groups shown in Table 6-37, the incidence in each 5-year group is almost double that in the age group before it.

The most important known risk factor for bladder cancer is tobacco use, which accounts for about half of bladder cancers in men and one-third in women (Miller et al., 1996). Occupational exposure to aromatic amines (also called arylamines); polycyclic aromatic hydrocarbons (PAHs); and some other organic compounds 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 hæmatobium* have been implicated as risk factors. Exposure to inorganic arsenic is also a risk factor for bladder cancer; cacodylic acid is a metabolite of inorganic arsenic. As discussed in Chapter 3, however, the data are 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*, *Update 2000*, and *Update 2002*

The committees responsible for *VAO* and *Update 1996* concluded that there was limited or suggestive evidence of *no* association between exposure to 2,4-D, 2,4,5-T, 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. The *Update 2000* and *Update 2002* committees did not change that finding. Table 6-38 summarizes the results of the relevant studies.

TABLE 6-38 Selected Epidemiologic Studies—Urinary Bladder Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Bodner et al., 2003	Dow chemical production workers—mortality	—	0.7 (0.1–2.0)
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	2	0.7 (0.1–2.4)
Studies Reviewed in *Update 2002*			
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	Iowa Farmers	274	0.9 (NS)

TABLE 6-38 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
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 6-38 *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			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans—urinary system cancer		
	All Ranch Hand veterans		
	Incidence	14	1.1 (0.6–1.7)
	Mortality	1	0.9 (—*)
	Veterans, tours between 1966–1970—Incidence	14	1.3 (0.7–2.1)
	White Air Force comparison veterans—urinary system cancer		
	All comparison veterans		
	Incidence	8	0.4 (0.2–0.8)
	Mortality	1	0.6 (—*)
	Veterans, tours between 1966–1970—Incidence	4	0.3 (0.1–0.7)
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.
— Information denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; CI, confidence interval; 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.

Update of Scientific Literature

Akhtar et al. (2004) investigated the incidence of urinary bladder cancer in Vietnam veterans who had participated in the Operation Ranch Hand aerial herbicide spraying operation and in a cohort of comparison veterans. They found no significant difference between the expected and observed incidence of the cancer in the Ranch Hand veterans, but they noted that comparison veterans were significantly less likely than expected (SIR, 0.4; 95% CI, 0.2–0.8) to experience the outcome.

No relevant occupational or environmental studies have been published since *Update 2002*.

Synthesis

The new evidence presented by Akhtar et al. (2004) does not change the committee's previous findings, which placed urinary bladder cancer in the inadequate or insufficient category.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine whether an association exists between exposure to the compounds of interest and urinary bladder cancer.

Biologic Plausibility

No studies have reported an increased incidence of urinary bladder cancer in TCDD-treated animals. Cacodylic acid administered to laboratory animals induced neoplasms of the urinary bladder. Chen et al. (2003) studied the mechanism of arsenic detoxification and related it to human bladder cancer. They reasoned that arsenic methylation ability (the ratio of the primary methylation index to the secondary methylation index) could modify the association between cumulative arsenic exposure and the risk of bladder cancer. Patients with newly diagnosed bladder cancer (49) were compared with 224 fracture and cataract patients in Taiwan. Urinary arsenic species were measured in the urine. Patients with the highest cumulative exposure exhibited an increased risk for bladder cancer (OR, 4.33; 95% CI, 1.12–16.01) in the setting of a low secondary methylation index.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and urinary bladder cancer, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

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 could 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 22,080 men and 13,630 women will be diagnosed with renal cancers (ICD-9 189.0, 189.1) in the United States in 2004 and that 7,870 men and 4,610 women will die from those diseases (ACS, 2004a). Those figures represent 2–3% of all new cancer diagnoses and deaths. The average annual incidence of renal cancer is shown in Table 6-39.

Renal cancer is twice as common in men as it is in women. In the age groups that represent most Vietnam veterans, black men have a higher incidence than do white men. With the exception of Wilms' tumor (which is more likely to occur in children), renal cancer is more common in people over the age of 50.

Tobacco use is a well-established risk factor for renal cancer. 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, cadmium, and organic solvents. Firefighters, who are routinely exposed to numerous pyrolysis products, are in a known higher-risk group.

TABLE 6-39 Average Annual Incidence (per 100,000) of Kidney and Renal Pelvis Cancer in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	22.0	22.0	30.3	35.1	35.5	41.8	48.2	48.9	54.9
Females	9.8	9.9	13.6	17.0	17.3	23.3	23.4	23.7	29.4

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and renal cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that conclusion. Table 6-40 summarizes the results of the relevant studies.

Update of the Scientific Literature

Having extended their follow-up by 13 years (1988–2001), Swaen et al. (2004) presented results on a total of 21 years of follow-up on the mortality experience of an established cohort of 1,341 Dutch licensed herbicide appliers (Swaen et al., 1992). SMRs were calculated based on age and on calendar-year, cause-specific mortality rates of the general population of the Netherlands, without adjustment for possible confounders. Four deaths from kidney cancer were reported. Only 3 deaths were expected, leading to an SMR of 1.3 (95% CI, 0.4–3.4).

No relevant environmental or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

The new finding on renal cancer among Dutch herbicide applicators, with unknown individual histories for exposure to specific herbicides, was based on only 4 deaths. This result does not modify the committee's previous determination that the "insufficient or inadequate" categorization is appropriate for renal cancer.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and renal cancer.

Biologic Plausibility

No studies have reported increased incidence of renal cancer in TCDD-treated animals. Cacodylic acid administered to laboratory animals induced neoplasms in the kidney. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

TABLE 6-40 Selected Epidemiologic Studies—Renal Cancer

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	4	1.3 (0.4–3.4)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers	2	0.9 (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	Iowa Farmers	178	1.1 (NS)

TABLE 6-40 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
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—Incidence		
	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)

continues

TABLE 6-40 *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.
— Information denoted by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; CI, confidence interval; 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.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and renal cancer, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

BRAIN TUMORS

Tumors of the brain and central nervous system include tumors of the cranial nerves, cerebral meninges (the outer covering of the brain), spinal cord, and spinal meninges. Any of the cell types within those sites can give rise to tumors. Tumors that originate in other parts of the body, such as the lung or breast, can metastasize to the brain. Although metastatic brain tumors are more common than are primary brain tumors, they are not considered in this section.

Benign and malignant tumors of the brain, cranial nerves, and cranial meninges account for about 95% of tumors of the brain and central nervous system. According to ACS, about 10,540 men and 7,860 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 2004, and 7,200 men and 5,490 women will die from those cancers (ACS, 2004a). The numbers represent about 1.3% of new cancer diagnoses and 2.3% of all cancer deaths. In adults older

TABLE 6-41 Average Annual Incidence (per 100,000) of Brain and Other Nervous System Cancers in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	10.0	11.0	6.2	12.9	14.1	9.1	16.9	18.8	9.7
Females	6.4	7.1	4.2	9.3	10.0	8.0	10.5	11.9	4.3

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

than 45, approximately 90% of tumors that originate in the brain are gliomas—astrocytomas, glioblastoma multiforme, ependymomas, and oligodendrogliomas; astrocytomas are the most common. Meningiomas (tumors of the covering of the brain and spinal cord) can account for 20–40% of tumors of the brain and meninges. They tend to occur in middle age and more commonly in women. Most meningiomas are benign and can be removed surgically. The average annual incidence of brain and other nervous system cancers is shown in Table 6-41.

The descriptive epidemiology of these tumors has been difficult to study because of the wide variation in specific tumors included in published reports. That inconsistency is caused mostly by the inclusion or exclusion of benign tumors. Other problems derive from inconsistencies in the inclusion of subsite tumors (pituitary or eye tumors) and in diagnostic efficiency.

The only well-established environmental risk factor for brain tumors is exposure to high doses of ionizing radiation (ACS, 2004a; Wrensch et al., 2002). Other environmental exposures, to vinyl chloride, petroleum products, and electromagnetic fields, for example, are unproven as risk factors. The causes of most cancers of the brain and nervous system are not known.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of *no* association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and brain cancer. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that conclusion. Table 6-42 summarizes the results of the relevant studies.

TABLE 6-42 Selected Epidemiologic Studies—Brain Tumors

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Bodner et al., 2003	Dow chemical production workers, both brain and other CNS tissues—Mortality	*	0.6 (0.1–1.8)
Swaen et al., 2004	Dutch licenced herbicide applicators—Mortality	4	1.6 (0.4–4.1)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers—Mortality	3	1.1 (0.2–3.2)
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)

TABLE 6-42 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
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	Iowa Farmers	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)

continues

TABLE 6-42 *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 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)
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			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans—cancer of the brain and nervous system		
	All Ranch Hand veterans—		
	Incidence	5	1.8 (0.7–4.1)
	Mortality	3†	1.3 (0.3–3.6)
	Veterans, tours between 1966–1970—		
	Incidence	5	2.2 (0.8–4.8)
	White Air Force comparison veterans—cancer of the brain and nervous system		
	All comparison veterans—		
	Incidence	2	0.5 (0.1–1.8)
	Mortality	1†	0.3 (*)
	Veterans, tours between 1966–1970—		
	Incidence	2	0.7 (0.1–2.3)
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)

TABLE 6-42 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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.
† For central nervous system cancers
— Information was by a dash in the original study.
ABBREVIATIONS: 2,4-D, 2,4-dichlorophenoxyacetic acid; AFHS, Air Force Health Study; CI, confidence interval; CNS, central nervous system; 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.

Update of Scientific Literature

Occupational Studies

Bodner et al. (2003) updated cancer mortality among 2,187 Dow Chemical Company workers who were likely to have been exposed to high concentrations of dioxins. There were 168 cancer deaths reported in the cohort. Cancers of the brain and nervous system were not elevated (SMR, 0.6, 95% CI, 0.1–1.8).

Swaen et al. (2004) published an updated mortality study of a cohort of 1,341 licensed herbicide applicators in the Netherlands as a follow-up to a previous mortality study of the same cohort (Swaen et al., 1992). SMRs were calculated based on national cause-specific mortality rates. The 2004 study showed a non-significant increase in brain cancer (SMR, 1.60; 95% CI, 0.43–4.05), but the study was limited by the small number of cases and by potential confounders that could not be evaluated.

Environmental Studies

No relevant environmental studies have been published since *Update 2002*.

Vietnam-Veterans Studies

Akhtar et al. (2004) describe cancer incidence and mortality in a prospective cohort study of Air Force Operation Ranch Hand veterans who sprayed Agent Orange while serving in Southeast Asia. Cancer incidence and mortality in the Ranch Hand cohort was compared with incidence and mortality for veterans who

did not serve in Southeast Asia and with US national cancer rates. There was a non-significant increase in the incidence of cancer of the brain and nervous system compared with national rates (SIR, 1.84; 95% CI, 0.68–4.08) and a non-significant increase in Ranch Hand veterans who served during the time of heaviest use of Agent Orange (SIR, 2.18; 95% CI, 0.80–4.84). There was no increase in mortality attributable to cancer of the brain and nervous system (SMR, 1.33; 95% CI, 0.34–3.62). The strength of the study is that most cases of brain cancer were likely detected in this cohort; however, the study was limited by the small number of cases.

Synthesis

No studies published since *Update 2002* provide strong evidence of an association between the exposures of interest and cancer of the brain and nervous system. Two new studies (Akhtar et al., 2004; Swaen et al., 2004) showed non-significant increases in brain cancer. However, the small number of cases in those studies do not negate the large number of studies from previous *Updates* that showed evidence of no association. Therefore, the conclusion remains that there is limited or suggestive evidence of *no* association for exposure to the compounds of interest and brain or central nervous system cancer.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VOA* reports, the committee concludes that there is limited or suggestive evidence of *no* association between exposure to the compounds of interest and brain cancer and other nervous system cancers.

Biologic Plausibility

No new studies suggest that TCDD exposure induces cancers of the brain. The evidence that exposure to 2,4-D in animals causes brain tumors remains questionable. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and brain tumors and other nervous system tumors, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

NON-HODGKIN'S LYMPHOMA

Non-Hodgkin's lymphoma (NHL, ICD-9 200.0–200.8, 202.0–202.2, 202.8–202.9) is the more common of the two primary types of cancer of the lymphatic system. ACS estimates that 28,850 men and 25,520 women will be diagnosed with NHL in the United States in 2004 and that 10,390 men and 9,020 women will die from it (ACS, 2004a). Collectively, lymphomas (which also include Hodgkin's disease) are the fifth most common form of cancer in the United States. The average annual incidence is shown in Table 6-43.

NHL incidence is uniformly higher in males than in females and, typically, higher in whites than in blacks. In the cohorts that characterize most Vietnam veterans, rates increase with age.

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 increased incidence in people with HIV, human T-cell lymphotropic virus, Epstein-Barr virus, and gastric *Helicobacter pylori* infections. Behavioral, occupational, and environmental risk factors also have been proposed (Blair et al., 1997).

Chronic lymphocytic leukemia (CLL) and hairy-cell leukemia share many traits with NHL (immunohistochemical traits, B-cell origin, and progression to an acute aggressive form of NHL). CLL is discussed separately after the general section on leukemia.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was sufficient information to determine an association between exposure to at least one of the compounds of interest and NHL. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that conclusion. Table 6-44 summarizes the results of the relevant studies.

TABLE 6-43 Average Annual Incidence (per 100,000) of Non-Hodgkin's Lymphoma in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	26.2	27.1	26.3	36.6	38.5	31.2	52.2	55.5	35.5
Females	18.1	18.4	17.3	28.1	30.2	20.6	36.1	39.3	28.1

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

TABLE 6-44 Selected Epidemiologic Studies—Non-Hodgkin's Lymphoma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Miligi et al., 2003	11 areas of Italy (NHL and CLL combined)		
	Phenoxy acid herbicides exposure		
	Men	18	1.0 (0.5–2.0)
	Women	11	1.3 (0.5–3.7)
	2,4–D exposure		
	Men	6	0.7 (0.3–0.19)
	Women	7	1.5 (0.4–5.7)
Bodner et al., 2003	Dow chemical production workers—mortality	—	1.4 (0.6–2.7)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers—mortality	3	1.0
Thörn et al., 2000	Swedish lumberjacks exposed to phenoxyacetic herbicides—incidence	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)

TABLE 6-44 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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—exposure 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)
Zahm et al., 1993	Females in eastern Nebraska farms	119	1.0 (0.7–1.4)
Kogevinas et al., 1992	IARC cohort		
	Expose 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	Male residents of selected contaminated areas in Italian provinces	*	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 (Sweden) Hospital		
	Exposure to phenoxy acids	6	4.9 (1.0–27.0)
Wiklund et al., 1989b	Swedish pesticide appliers	27	1.1 (0.7–1.6)

continues

TABLE 6-44 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Alavanja et al., 1988	USDA extension agents	*	1.2 (0.7–2.3)
Dubrow et al., 1988	Ohio residents	15	1.6 (0.8–3.4)
Olsson and Brandt, 1988	Lund Hospital patients		
	Exposure to herbicides	*	1.3 (0.8–2.1)
	Exposure 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)
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		
	Exposure to phenoxy acids	41	4.8 (2.9–8.1)[d]
	Exposure to chlorophenols	50	4.3 (2.7–6.9)[d]
ENVIRONMENTAL			
New Studies			
Floret et al., 2003	Residents near a French municipal solid-waste incinerator		
	High exposure category	31	2.3 (1.4–3.8)
Studies Reviewed in *Update 2002*			
Hardell et al., 2001	Case control study of NHL—TEQ >27.8 and EA >80	8	2.8 (0.5–1.8)

TABLE 6-44 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
McDuffie et al., 2001	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)
	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—Incidence		
	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)

continues

TABLE 6-44 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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			
New Studies			
Akhtar et al., 2004	White Air Force Vietnam veterans lymphopoetic leukemia		
	Ranch Hand—Incidence	10	0.85 (0.4–1.5)
	Comparison—Incidence	9	0.55 (0.3–1.0)
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)
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 (*)
	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)
	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)

TABLE 6-44 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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 (—)
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.
— Information 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; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, toxin equivalents; USDA, US Department of Agriculture.

Update of the Scientific Literature

Occupational Studies

Bodner et al. (2003) provided an update of cancer mortality among 2,187 male Dow Chemical Company employees who worked in production areas where there was a potential for dioxin exposure. Between 1940 and 1994, 168 cancer deaths were reported for the cohort. During the 10 years of follow-up evaluated in this study, there were 2 additional NHL cases; 2.9 would have been expected. After stratification by age, calendar year, and pay status, the resulting overall SMR for NHL was 1.4 (95% CI, 0.6–2.7). That was a reduction from the SMR of 2.1 reported in an earlier study. When stratified by exposure (internal and external comparisons), no trend was observed for NHL, but the number of cases in each stratum was small (1–2). Nevertheless, the risk ratio estimates in almost all exposure categories were above 1.0. No cases of NHL were found among the 245 cases of chloracne, but given the small number, only 0.7 would have been expected.

Miligi et al. (2003) conducted a population-based case–control study in 11 areas of Italy, 5 of which had a high prevalence of agricultural work. Questionnaires designed by industrial hygienists and agronomists were used to estimate exposure to various compounds, including specific pesticides. All cases of NHL and leukemia newly diagnosed during 1990–1993 were included, and the control group was selected randomly from the general population aged 20–74. Data were reported on "all the malignancies of the hemato-lymphopoietic system," including leukemia (ICD-9 204–208) and NHL (200, 202). In some analyses all were combined; in others, NHL was combined with CLL (ICD-9 204.1) and then all the leukemia cases were reported separately. For NHL and CLL combined, ORs of 1.0 (95% CI, 0.5–2.0; based on 18 exposed cases) and 1.5 (95% CI, 0.5–3.7; based on 11 exposed cases) for men and women, respectively, were reported for exposure to phenoxy acid herbicides as a class. ORs of 0.7 (95% CI 0.3–0.19, based on 6 exposed cases) for men and 1.5 (95% CI, 0.4–5.7, based on 7 exposed cases) for women were reported specifically for 2,4-D. The usefulness of that study for the committee was limited by failure to specify the number of cases of particular cancer types, specifically NHL and CLL.

Environmental Studies

A case–control study (Floret et al., 2003) investigated the rates of NHL in a population of Bensaçon, France, residing near a municipal solid-waste incinerator. The plant had historically documented high emission of dioxin: In 1997, the concentrations in exhaust gas were 16.3 ng I-TEQ/m^3 (compared with the European Union guideline of 0.1). Cases were identified from a cancer registry of people diagnosed with NHL between 1980 and 1995. Almost all of the cases

were histologically confirmed. Data on each case included date of birth, sex, age at diagnosis, and address at the time of diagnosis. Control subjects were selected from the population census, and because of confidentiality laws and requirements, the only data available to investigators were the age categories (0–19, 29–39, 40–59, 60–74, 75+), sex, and residence in specific blocks. Controls were selected randomly from census lists, according to a 10-to-1 matching that was based on sex and age group.

Exposure was based on geocoding the distance of each study participant's residence from the plant. Dispersion modeling was used to account for meteorologic effects. The exposure assessment took advantage of an earlier study, conducted in 1999, which developed a model to predict dioxin emissions from the solid-waste incinerator. No other industrial sources of dioxin exposure were found in the area. The study region was divided into 4 areas of increasing dioxin concentrations, from <0.0001 pg/m^3 in the low exposure or reference group, to 0.0004–0.0016 pg/m^3 in the highest exposure category.

Overall, the 225 NHL cases resulted in an incidence rate of 14.9 for the study region, compared with 7.8 for France as a whole. The case–control analysis showed OR, 2.3; 95% CI, 1.4–3.8 for the highest dioxin group, but no elevations were found for the low and intermediate categories (compared with the reference group). The authors hypothesize that a threshold effect could have been responsible. Although the exposure assessment relied on sophisticated methods for modeling emissions, because there was insufficient information on residential history and time–activity patterns, the duration of exposure could not be included in the analysis.

Vietnam-Veteran Studies

Akhtar et al. (2004) describe cancer incidence and mortality in a prospective cohort study of Air Force Operation Ranch Hand Vietnam veterans who sprayed Agent Orange during their service in Southeast Asia. Cancer incidence and mortality in the Ranch Hand cohort were compared with incidence and mortality in veterans who did not serve in Southeast Asia and with US national cancer rates. Because of the small number of site-specific cancers among the veterans, all leukemias were combined with multiple myeloma and the lymphomas to form the category of lymphopoietic cancers. The analyses of those cancers were restricted to white veterans (89% of the study population). No excess of lymphopoietic cancers was noted for the Ranch Hand veterans (10 observed, 11.8 expected; SIR, 0.85; 95% CI, 0.4–1.5). The comparison cohort of Air Force veterans actually had a deficit of the cancers (9 observed, 16.5 expected; SIR, 0.55; 95% CI, 0.3–1.0). This pattern did not change when the analyses were restricted to veterans whose tours of duty ended between 1966 and 1970, the years when Agent Orange was the predominant herbicide in use in Vietnam. The

small number of cancers precluded a more detailed analysis by concentration of serum dioxin among the Ranch Hand veterans.

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, but not herbicide, exposure do not have increased rates of NHL. The occupational and environmental studies reviewed for this report are supportive of the previous committee's findings. The Vietnam-veteran study reviewed does not provide information on specific types of lympho-hematopoietic cancer.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is sufficient evidence to conclude that an association exists between exposure to at least one of the compounds of interest and NHL. That evidence 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 shown an increase in lymphoma in TCDD-exposed animals.

A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and NHL, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

HODGKIN'S DISEASE

Hodgkin's disease (HD) (ICD-9 201.0–201.9) is distinct from NHL in its cell of origin, its demographics, and its genetics. According to ACS estimates, 4,330 men and 3,550 women will be diagnosed with the disease in the United States in 2004, and 700 men and 620 women will die from it (ACS, 2004a). Average annual incidence is shown in Table 6-45.

HD is less common in people in the age groups that characterize most Vietnam veterans than it is in younger or older people. Among people over 40, the incidence in males generally exceeds that in females, and the incidence in whites exceeds that in blacks. However, the very small number of cases indicates that care should be exercised in interpreting the figures.

The possibility that HD has an infectious etiology 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*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* determined that there was sufficient information to conclude that an association exists between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and HD. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Table 6-46 summarizes the results of the relevant studies.

TABLE 6-45 Average Annual Incidence (per 100,000) of Hodgkin's Disease in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	2.8	3.0	2.4	3.0	3.1	3.9	3.4	3.9	[b]
Females	1.8	1.8	2.8	1.9	2.2	0.7	2.3	2.4	1.3

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.
[b] Insufficient data to provide a meaningful incidence estimate.

TABLE 6-46 Selected Epidemiologic Studies—Hodgkin's Disease

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators	0	—
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers—		
	Mortality	1	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)

TABLE 6-46 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
Persson et al., 1989	Orebro (Sweden) Hospital patients		
	Farming	6	1.2 (0.4–3.5)
	Exposure 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	Iowa Farmers	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)

continues

TABLE 6-46 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
	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—Incidence		
	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			
New Studies			
Akhtar et al., 2004	White Air Force Vietnam veterans lymphopoietic leukemia		
	Ranch Hand—Incidence	10	0.9 (0.43–1.51)
	Comparison—Incidence	9	0.6 (0.3–1.0)
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)

TABLE 6-46 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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	—
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.
— Information 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; CI, confidence interval; 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.

Update of the Scientific Literature

Occupational Studies

Swaen et al. (2004) presented results for a 21-year follow-up of mortality in a cohort of 1,341 Dutch licensed herbicide appliers (Swaen et al., 1992), extending the follow-up by 13 years from 1988 to 2001. SMRs were calculated based on age and on calendar-year, cause-specific mortality rates for the general population of the Netherlands. No HD deaths were reported, 0.5 deaths were expected, and no SMR or CI was provided.

Environmental Studies

No relevant environmental studies have been published since *Update 2002*.

Vietnam-Veteran Studies

Akhtar et al. (2004) describe cancer incidence and mortality in a prospective cohort study of Air Force Operation Ranch Hand Vietnam veterans who sprayed Agent Orange during their service in Southeast Asia. Cancer incidence and mortality in the Ranch Hand cohort were compared with rates for veterans who did not serve in Southeast Asia and with US national cancer rates. Because of the small number of site-specific cancers among the veterans, all leukemias were combined with MM and the lymphomas to form the category of lymphopoietic cancers. The analyses of those cancers were restricted to white veterans (89% of the study population). No excess of lymphopoietic cancers was noted for the Ranch Hand veterans (10 observed, 11.8 expected; SIR, 0.85; 95% CI, 0.43–1.51). The comparison cohort of Air Force veterans actually had a deficit of those cancers (9 observed, 16.5 expected; SIR, 0.55; 95% CI, 0.27–1.00). This pattern of results did not change when the analyses were restricted to veterans whose tours of duty ended between 1966 and 1970, the years when Agent Orange was the predominant herbicide in use in Vietnam. The small number of cancers precluded a more detailed analysis by serum dioxin concentration among the Ranch Hand veterans.

Synthesis

The relatively low incidence of HD complicates the evaluation of epidemiologic studies addressing this lymphoreticular tumor. However, earlier studies carried out in Sweden (for example, the work of Hardell and colleagues) were well conducted. All were based on good exposure characterization, and none has been contradicted by later work. The committee believes that the small amount of additional data reviewed in this report is consistent with the pattern of increased mortality and morbidity risk noted by previous *VAO* committees. Although it has

not been demonstrated as clearly as for NHL, a positive association between the chemicals of interest and the development of HD is biologically plausible because of their common lymphoreticular origin and common risk factors.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is sufficient evidence to state that an association exists between exposure to at least one of the compounds of interest and HD.

Biologic Plausibility

No animal studies have shown an increased incidence of HD after exposure to the compounds of interest. HD's lymphoreticular origin and the risk factors it shares with NHL add to its biological plausibility. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and HD, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

MULTIPLE MYELOMA

Multiple myeloma (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 in the production of excess abnormal proteins, usually fragments of immunoglobulins. ACS estimates that 8,090 men and 7,180 women in the United States will be diagnosed with MM in 2004 and that 5,430 men and 5,640 women will die from it (ACS, 2004a). The average annual incidence of MM is shown in Table 6-47.

MM incidence is highly age dependent, with a relatively low rate in people under the age of 40; most cases occur in people between the ages of 55 and 70. Rates in blacks are at approximately twice those in whites. Incidence in males is slightly higher than in females, with the difference becoming 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

TABLE 6-47 Average Annual Incidence (per 100,000) of Multiple Myeloma in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	7.5	7.2	13.0	11.3	10.0	28.4	17.0	16.2	31.2
Females	4.9	4.2	12.2	8.7	7.7	19.0	12.4	11.2	25.9

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–200.1

exposure to rubber, leather, paint, and petroleum (Riedel et al., 1991). People with high exposure to ionizing radiation and those who suffer from other plasma-cell diseases such as monoclonal gammopathy of unknown significance or solitary plasmacytoma are also at greater risk.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and MM. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Table 6-48 summarizes the results of the relevant studies.

Update of the Scientific Literature

Occupational Studies

Swaen et al. (2004) presented results for a 21-year follow-up of mortality in a cohort of 1,341 licensed herbicide applicators working for government agencies in the Netherlands. The extended follow-up was primarily motivated by an earlier finding (after 8 years of follow-up) of increased mortality (based on 3 deaths, 0.37 expected). The expanded period was from January 1980 to January 2001. Information was available on the types and amounts of herbicides used in all municipal spraying projects in 1980, but those data could not be linked to the work of any individual applicators. No data were available on any potential risk factors other than age. SMRs were calculated based on age and on calendar-year, cause-specific mortality rates of the general population of the Netherlands. No additional deaths were identified in the 13-year follow-up; 1.4 was expected for the full 21-year follow-up period (3 observed, 1.4 expected; SMR, 2.1; 95% CI, 0.4–6.1)

TABLE 6-48 Selected Epidemiologic Studies—Multiple Myeloma

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Swaen et al., 2004	Dutch licenced herbicide applicators—Mortality	3	2.1 (0.4–6.1)
Studies Reviewed in *Update 2002*			
Burns et al., 2001	Dow 2,4-D production workers—Mortality	1	0.8 (0.0–4.5)
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)

continues

TABLE 6-48 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
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)

TABLE 6-48 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—Incidence		
	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			
New Studies			
Akhtar et al., 2004	White Air Force Vietnam Veterans		
	Lymphopoetic leukemia		
	Ranch Hand Veterans—Incidence	10	0.9 (0.4–1.5)
	Comparison Air Force Veterans—Incidence	9	0.6 (0.3–1.0)
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.

— Information 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; CI, confidence interval; IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; SEER, Surveillance, Epidemiology, and End Results Program; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USDA, US Department of Agriculture.

Environmental Studies

No environmental studies have been published since those reviewed in *Update 2002*.

Vietnam-Veteran Studies

Akhtar et al. (2004) reported on the incidence of cancer in veterans of Operation Ranch Hand and in a comparison cohort of other Air Force veterans who served in Southeast Asia during the same time period but who were not involved in the spraying of herbicides. The occurrence of cancer was ascertained for the period between each veteran's departure from Southeast Asia and December 31, 1999. Information on cancer was derived from study examinations (in 1982, 1985, 1987, 1992, and 1997), medical records, and death certificates. SIRs were calculated to compare the observed number of cancers with an expected number based on sex, race, age, and calendar-year-specific incidence rates from SEER Program data. The study group consisted of 1,189 Ranch Hand veterans; 1,776 comparison Air Force veterans were included in the analyses using the SEER data.

Because of the small number of site-specific cancers among the veterans, MM was combined with lymphomas and leukemias to form the category of lymphopoietic cancers. The analyses of those cancers were restricted to white veterans (89% of the study population). No excess of lymphopoietic cancers was noted for the Ranch Hand veterans (10 observed, 11.8 expected; SIR, 0.85; 95% CI, 0.43–1.51), and the comparison cohort of Air Force veterans actually exhibited a deficit (9 observed, 16.5 expected; SIR, 0.55; 95% CI, 0.27–1.00). That pattern did not change when analyses were restricted to veterans whose tours of duty ended between 1966 and 1970, the years when Agent Orange was the predominant herbicide in use in Vietnam. The small number of cancers precluded a more detailed analysis by serum dioxin concentrations among the Ranch Hand veterans.

Synthesis

The study by Swaen et al. (2004) gives additional data on a cohort study that was included in *VAO*. In the first report on the cohort, the SMR was significantly elevated (8.2; 95% CI; 1.6–23.8), but it was based on 3 deaths. Despite more than doubling the amount of follow-up time, the new report for the cohort does not include any additional cases. The confidence interval for the updated SMR now includes the null value, but its range does not exclude the possibility of an association between herbicide exposure and MM.

The only other new study published since *Update 2002* is an update of cancer incidence in the Ranch Hand veterans and the comparison cohort of Air Force veterans. As in the earlier reports from the Ranch Hand study, the small number of cases did not permit an adequate analysis of MM alone. Given that the

results of Akhtar et al. (2004) are based on a combination of MM, lymphomas, and leukemias, it is not possible to draw separate conclusions about any of the specific cancers. Accordingly, these conclusions should not be interpreted as contradicting earlier assessments of the limited or suggestive evidence for an association between exposure to herbicides and risk of MM.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one of the compounds of interest 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 reported an increased incidence of MM after exposure to the compounds of interest. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and multiple myeloma, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their 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. Acute myelogenous leukemia (ICD-9 205) also is commonly called "acute myeloid leukemia" or "acute nonlymphocytic leukemia." There are numerous subtypes of the disease. For consistency, this report uses *acute myelogenous leukemia,* or the abbreviation AML, regardless of usage in the source materials. According to ACS estimates, 19,020 men and 14,420 women will be diagnosed with some form of the disease in the United States in 2004, and 12,990 men and 10,310 women will die from it (ACS, 2004a). Collectively, leukemias were expected to account for 2.4% of all new cancer diagnoses and

4.1% of cancer deaths in 2004. 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 6-49.

In adults, acute leukemia is nearly always in the form of AML. Seven distinct morphologic groups were described in the French-American-British (FAB) classification system. FAB M0 and M1 without maturation are characterized by the presence of Auer rods in the leukemic cell. FAB M-2 myeloid leukemia with maturation also has Auer rods and is more likely to have chromosomal abnormalities. FABM-3 progranulocytic leukemia has distinct morphologic, clinical, and cytogenetic features that include tendency toward disseminated intravascular coagulation. FABM-4 myelomonocytic leukemia is characterized by a mixture of large myeloid and monocytic elements. Some patients have prominent eosinophilia. Patients who present with the FAB categories M-2–M-4 generally are between 30 and 40 years of age and experience a favorable outcome with induction

TABLE 6-49 Average Annual Incidence (per 100,000) of Leukemias in United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
All Leukemias									
Males	13.3	13.7	12.2	20.5	21.4	16.2	31.0	33.1	19.4
Females	8.3	8.5	6.7	12.6	12.9	11.3	17.2	18.4	12.5
Acute Lymphocytic Leukemia									
Males	1.0	1.1	0.8	0.7	0.7	0.8	1.2	1.3	[b]
Females	0.7	0.7	0.7	0.9	1.0	0.3	0.7	0.7	[b]
Acute Myeloid Leukemia									
Males	3.4	3.4	3.5	5.3	5.4	3.6	9.0	9.6	5.4
Females	2.5	2.6	1.2	4.4	4.3	4.0	5.6	5.9	4.3
Chronic Lymphocytic Leukemia									
Males	5.0	5.3	4.3	9.2	10.2	5.9	12.5	13.6	8.1
Females	2.2	2.3	2.3	4.2	4.5	3.3	6.1	6.8	3.5
Chronic Myeloid Leukemia									
Males	1.7	1.7	2.7	2.4	2.2	3.2	3.9	3.8	3.8
Females	1.7	1.9	1.2	1.7	1.7	2.0	2.5	2.7	1.7
All Other Leukemia[c]									
Males	0.4	0.4	[b]	0.7	0.6	1.6	1.3	1.3	0.5
Females	0.3	0.2	0.7	0.5	0.5	0.7	1.1	1.1	0.9

[a] SEER (Surveillance, Epidemiology, and End Results Program) nine standard registries, crude age-specific rates, 1997–2001.

[b] Insufficient data to provide a meaningful incidence estimate.

[c] Includes leukemic reticuloendotheliosis (hairy-cell leukemia), plasma-cell leukemia, monocytic leukemia, and acute and chronic erythremia and erythroleukemia.

chemotherapy. Patients with FABM-5, monocytic leukemia; FABM-6, erythroleukemia; or FABM-7, megocariocyticleukemia, often are over the age of 60, and the prognosis generally is poor. None of the subcategories of acute leukemia has a known specific pathogenesis or etiology.

AML is the most common leukemia among adults; its incidence increases steadily with age in people over 40. 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. Risk factors associated with an increased risk of AML include high doses of ionizing radiation, occupational exposure to benzene, and exposure to 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 use is thought to account for about 20% of AML cases.

Acute lymphocytic leukemia (ALL) is a disease of the young and of people over 70. It is relatively uncommon among people in the age groups that characterize most Vietnam veterans. The lifetime incidence of ALL is slightly higher in whites than in blacks and higher in males than in 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.

CLL is the most common of the four primary types of leukemia in men. Because CLL shares many traits with lymphomas (immunohistochemistry; B-cell origin; progression to an acute, aggressive form of NHL), the committee reviews CLL in the next section, separately from the other leukemias.

The incidence of chronic myeloid leukemia (CML) increases steadily with age in people over 30. Its lifetime incidence is roughly equal in whites and blacks and is slightly higher in males than in females. CML accounts for about one-fifth of the cases of leukemia among people in the age groups that characterize most Vietnam veterans. 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; the data that link HTLV-2 to hairy-cell leukemia are less definitive.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and leukemia. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Table 6-50 summarizes the results of the relevant studies.

TABLE 6-50 Selected Epidemiologic Studies—Leukemia

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Miligi et al., 2003	Italian males		
	All types of leukemis, exposure to phenoxy herbicides	6	2.1 (0.7–6.2)
Swaen et al., 2004	Dutch licenced herbicide applicators—mortality	3	1.3 (0.3–3.7)
Studies Reviewed in *Update 2002*			
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)
Amadori et al., 1995	Italian farming and animal-breeding workers		1.8 (1.2–2.6)
Studies Reviewed in *Update 1996*			
Asp et al., 1994	Finnish herbicide 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 exposed to phenoxy herbicides	2	2.2 (0.3–7.9)
Hansen et al., 1992	Danish gardeners		
	All gardeners—CLL	6	2.5 (0.9–5.5)
	All gardeners—all other types of leukemia	3	1.2 (0.3–3.6)
	Male gardeners—CLL	6	2.8 (1.0–6.0)
	Male gardeners—all other types of leukemia	3	1.4 (0.3–4.2)

TABLE 6-50 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
Brown et al., 1990	Residents of Iowa and Minnesota		
	All types of leukemia, ever farmed		1.2 (1.0–1.5)
	CLL, ever farmed		1.4 (1.1–1.9)
	All types of leukemia, any herbicide use		1.2 (0.9–1.6)
	CLL, any herbicide use		1.4 (1.0–2.0)
	Herbicide users, phenoxy acid use		1.2 (0.9–1.6)
	All types of leukemia, 2,4-D use		1.2 (0.9–1.6)
	All types of leukemia, 2,4,5-T use		1.3 (0.7–2.2)
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	1,084	1.3
Burmeister et al., 1982	Residents in Iowa—		
	CCL in white, male farmers	1.9	(1.2–3.1)
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
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)

continues

TABLE 6-50 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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)
Studies Reviewed in *Update 1996*			
Bertazzi et al., 1993	Seveso residents—10-year follow-up—Incidence		
	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			
New Studies			
Akhtar et al., 2004	White Air Force Ranch Hand veterans—lymphopoetic cancers[†]		
	All Ranch Hand veterans		
	Incidence (SIR)	10	0.9 (0.4–1.5)
	Mortality (SMR)	6	1.0 (0.4–2.0)[‡]
	Veterans, tours between 1966–1970—Incidence	7	0.7 (0.3–1.4)
	White Air Force Comparison veterans—lymphopoetic cancers[†]		
	All comparison veterans		
	Incidence (SIR)	9	0.6 (0.3–1.0)
	Mortality (SMR)	5	0.6 (0.2–1.2)[‡]
	Veterans, tours between 1966–1970—Incidence	4	0.3 (0.1–0.8)

TABLE 6-50 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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.

† Lymphopoetic cancers comprise all of the forms of lymphoma (includng Hodgkin's Disease and non-Hodgkin's lymphoma) and leukemia (ALL, AML, CLL, CML).

‡ SMR is for "lymph or hematological system" cancers.

— 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; CI, confience interval; CDVA, Commonwealth Department of Veterans' Affairs; CLL, chronic lymphocytic leukemia; IARC, International Agency for Research on Cancer; USDA, US Department of Agriculture.

Update of the Scientific Literature

Occupational Studies

Swaen et al. (2004) presented results for a 21-year follow-up of mortality in a cohort of 1,341 licensed herbicide applicators working for government agencies in the Netherlands. SMRs were calculated based on age and on calendar-year, cause-specific mortality rates of the general population of the Netherlands. Three deaths from all leukemias combined were recorded for the cohort (2.2 would be expected). The SMR for deaths attributable to all leukemias was 1.3 (95% CI, 0.3–3.7).

Miligi et al. (2003) conducted a population-based case–control study in 11 areas of Italy (5 primarily agricultural, 2 primarily industrial, 4 mixed). All cases of NHL and leukemia diagnosed between 1990 and 1993 in adults 20–74 years of age were included as cases. Control subjects were selected randomly from the general population of the same areas, with frequency matching by age and sex to

the largest diagnostic case group (NHL and CLL combined). Analyses were conducted for two case groups: one for leukemias combined (ICD-9: 204–208) and one for NHL and CLL (ICD-9: 200, 202, 204.1). Questionnaires designed by industrial hygienists and agronomists were used to estimate occupational exposure to chemicals, including specific pesticides. Study subjects from the industrial areas were excluded from the analyses of agricultural exposures.

For medium or high probability of farming-related exposure to phenoxy herbicides, the ORs for NHL and CLL were 1.0 for men (18 exposed cases; 95% CI, 0.5–2.0) and 1.3 for women (11 exposed cases; 95% CI, 0.5–3.7). Similar results were observed for the analysis of likely exposure to 2,4-D. In the analyses of phenoxy herbicides and all leukemias combined, the OR for men was 2.1 (6 exposed cases; 95% CI, 0.7–6.2). The corresponding ratio for all leukemias combined in women was not computed because of the small number (<5) of exposed cases.

Environmental Studies

No environmental studies of leukemia have been published since those reviewed in *Update 2002*.

Vietnam-Veteran Studies

Akhtar et al. (2004) reported on the incidence of cancer in veterans of Operation Ranch Hand and in a comparison cohort of other Air Force veterans who served in Southeast Asia during the same time period but who were not involved in the spraying of herbicides. The occurrence of cancer was ascertained for the period between each veteran's departure from Southeast Asia and December 31, 1999. Information on cancer was derived from study examinations (1982, 1985, 1987, 1992, and 1997), medical records, and death certificates. SIRs were calculated to compare the observed number of cancers with an expected number based on sex, race, age, and calendar-year-specific incidence rates from SEER Program data. The study group consisted of 1,189 Ranch Hand veterans; 1,776 comparison Air Force veterans were included in the analyses using the SEER data.

Because of the small number of site-specific cancers among the veterans, all leukemias were combined with MM and the lymphomas to form the category of lymphopoietic cancers. The analyses were restricted to white veterans (89% of the study population). No excess of lymphopoietic cancers was noted for the Ranch Hand veterans (10 observed, 11.8 expected; SIR, 0.85; 95% CI, 0.43–1.51). The comparison cohort of Air Force veterans actually had a deficit (9 observed, 16.5 expected; SIR, 0.55; 95% CI, 0.27–1.00). This pattern of results did not change when the analyses were restricted to veterans whose tours of duty ended between 1966 and 1970, the years when Agent Orange was the predominant

herbicide in use in Vietnam. The small number of cancers precluded a more detailed analysis by serum dioxin concentrations among Ranch Hand veterans.

Synthesis

Two new studies of leukemia are updates that detail additional follow-up for herbicide applicators in the Netherlands (Swaen et al., 2004) and Ranch Hand veterans and the comparison cohort of Air Force veterans (Akhtar et al., 2004). In each, the small number of cases prevented adequate analysis of leukemia either by types or in an aggregate. The population-based case–control study from Italy (Miligi et al., 2003) presented similar difficulty: Cases were enrolled from the general population, so farming-related exposures (herbicide use) were relatively uncommon. The use of a broad definition of disease is especially problematic for the study of leukemia; *Update 2002* revealed that exposure to herbicides could increase risk for CLL but not for other types of leukemia. Given that the results of the new studies are based on a combination of leukemias or hemato-lymphopoietic cancers, it is not possible to draw separate conclusions about any of the specific cancers.

Conclusions

Strength of Evidence in Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concluded that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and leukemias other than CLL.

Biologic Plausibility

No animal studies have shown an increased incidence of leukemia after exposure to the compounds of interest. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and leukemias other than CLL, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

CHRONIC LYMPHOCYTIC LEUKEMIA

In the proposed WHO classification of non-Hodgkin's lymphoid neoplasms, CLL and its lymphomatous form, small lymphocytic lymphoma, are mature B-cell neoplasms (IARC, 2001). About 8,190 new cases (5,050 in men, 3,140 in women) of CLL will be diagnosed in the United States in 2004; and 4,800 people (2,730 men, 2,070 women) are expected to die from the illness (ACS, 2004a). Nearly all cases occur after the age of 50. For average annual incidence information, see the section on leukemia.

The requirements for diagnosis of CLL include an absolute peripheral-blood lymphocyte count of more than 10×10^9/L, 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 higher 100×10^9/L 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. Hairy-cell leukemia has recently been classified as a rare form of chronic lymphocytic leukemia (AJCC, 2002).

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

CLL was first discussed separately from other leukemias in *Update 2002*. The epidemiologic studies indicated that farming, 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 expo-

sure can contribute to NHL risk. Most cases of CLL and NHL reflect malignant transformation of B-lymphocyte progenitor cells, so those diseases could have a common etiology. Studies reviewed in *Update 2002* and in this report are summarized in Table 6-51.

Update of the Scientific Literature

Akhtar et al. (2004) reported on the incidence of cancer in veterans of Operation Ranch Hand and a comparison cohort of other Air Force veterans who served in Southeast Asia during the same period but who were not involved in spraying herbicides. Because of the small number of site-specific cancers among the veterans, all leukemias were combined with MM and the lymphomas to form the category of lymphopoietic cancers. The analyses of those cancers were restricted to white veterans (89% of the study population). No excess of lymphopoietic cancers was noted for the Ranch Hand veterans (10 observed, 11.8 expected; SIR, 0.85; 95% CI, 0.43–1.51). The comparison cohort of Air Force veterans actually had a deficit of those cancers (9 observed, 16.5 expected; SIR, 0.55; 95% CI, 0.27–1.00). This pattern of results did not change when analyses were restricted to veterans whose tours of duty ended between 1966 and 1970, the years when Agent Orange was the predominant herbicide in use in Vietnam. The small number of cancers precluded a more detailed analysis by serum dioxin concentrations among the Ranch Hand veterans.

No occupational or environmental studies of chronic lymphocytic leukemia have been published since those reviewed in *Update 2002.*

Synthesis

Update 2002 contained a reanalysis of the results from epidemiologic studies specifically concerning CLL. Although considerably more studies support the hypothesis that herbicide exposure can contribute to the development of NHL, exposure to the herbicides 2,4-D and 2,4,5-T also appears to be associated with the occurrence of CLL. Malignant transformation of B-lymphoocyte progenitor cells Is apparent in most cases of CLL and NHL, so it is plausible that these diseases could have a common etiology. The only new study of leukemia in the last two years was an update of cancer incidence among the Ranch Hand veterans compared to a cohort of other Air Force veterans. The small number of cases did not permit analysis by separate types of leukemia, but there was basis to reverse the concluding of the last update.

TABLE 6-51 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—incidence	10	1.8 (0.8–3.2)
Amadori et al., 1995	Workers in northeast Italy		
	Farming or animal-breeding workers	15	2.3 (0.9–5.8)
	Farming workers only	5	1.6 (0.5–5.2)
	Animal-breeding workers only	10	3.1 (1.1–8.3)
Studies Reviewed in *VAO*			
Hansen et al., 1992	Danish gardeners		
	All gardeners	6	2.5 (0.9–5.5)
	Male gardeners	6	2.8 (1.0–6.0)
Brown et al., 1990	Residents of Iowa and Minnesota		
	ever farmed	156	1.4 (1.1–1.9)
	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 only	248	1.7 (CI did not include 1.0)
Burmeister et al., 1982	Residents of Iowa		
	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)
VIETNAM VETERANS			
New Studies			
Akhtar et al., 2004	White Air Force Vietnam Veterans Lymphopoetic leukemia		
	Ranch Hand Veterans—Incidence	10	0.9 (0.4–1.5)
	Comparison Air Force Veterans—Incidence	9	0.6 (0.3–1.0)

[a] Given when available.
— Information denoted by a dash in the original study.
Abbreviation: USDA = US Department of Agriculture.

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 compounds of interest and CLL.

Biologic Plausibility

No animal studies have reported an increased incidence of CLL after exposure to the compounds of interest. A summary of the biologic plausibility of the carcinogenicity of the chemicals of interest in general is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and CLL, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

SUMMARY

On the basis of the occupational, environmental, and veteran studies reviewed, the committee has reached one of four conclusions about the strength of the evidence regarding an association between exposure to 2,4-D, 2,4,5-T, 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 be found in a large, well-designed epidemiologic study in which exposure 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 that were used in *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*.

Health Outcomes with Sufficient Evidence of an Association

For outcomes in this category, a positive association with one of the compounds of interest 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 compounds of interest and four cancers: soft-tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, and chronic lymphocytic leukemia. The scientific literature continues to support the classification of those four cancers in the category of sufficient evidence.

Health Outcomes with Limited or Suggestive Evidence of Association

For outcomes in this category, the evidence must suggest an association with at least one of the compounds of interest that could 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 compounds of interest and laryngeal cancer, lung cancer, bronchial (tracheal) cancer, prostatic cancer, and multiple myeloma. The 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 an Association

The scientific data on many of the kinds of cancer reviewed by the committee were inadequate or insufficient to determine association. For those cancers, the available studies are of insufficient quality, consistency, or statistical power to support a conclusion of the presence or absence of association. Some studies fail to control for confounding or provide inadequate exposure assessment. That category includes studies of hepatobiliary cancers (cancers 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 ovary), 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 known in humans are consistent in *not* showing a positive

association with exposure to one of the compounds of interest. 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 of the available studies. The possibility of a very small increase in risk associated with the exposure studied can never be excluded.

For 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 the compounds of interest: gastrointestinal tumors (of the colon, rectum, stomach, and pancreas) and brain tumors. The most recent scientific evidence continues to support the classification of those diseases 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 compounds of interest 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 from animal studies indicate that TCDD has carcinogenic activity. Several animal species—rats, mice, and hamsters—have been exposed to TCDD and examined for increases in tumor incidence and cancer. TCDD was fed to animals, applied to their skin, injected under their skin, or injected into their abdominal cavities. The research indicates that TCDD can both cause cancers or tumors and act as a promoter. That is, it can 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, oral mucosa and lungs. Studies have demonstrated an increased incidence of liver cancer in animals after TCDD exposure, but only after other adverse changes in the liver were observed. TCDD also is an extremely potent promoter of neoplasia in laboratory rats and mice. Decreased rates of some cancers—including those of the uterus, pancreas, and pituitary and mammary glands—also have been reported; however, this occurred only at doses where there was a decreased body weight gain. The sites at which effects were observed and the exposures required 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 from studies of female rats suggest that complex hormonal

interactions are involved in TCDD-induced carcinogenesis. Recently, the finding that TCDD immortalizes keratinocytes was suggested to be a possible mechanism by which this chemical causes malignancy.

Research results 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. 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 influences normal growth and development, which in turn supports the hypothesis that TCDD could affect cell growth.

The transcriptional alterations induced by TCDD result in changes to some forms of cellular regulation and metabolism at a very basic level. For example, TCDD has been shown to induce cytochrome P450 1A1 (CYP1A1) and other metabolizing enzymes. Those changes result in altered cell metabolism and could be involved in TCDD's carcinogenic activity, especially as involved with 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 the cell pathways that involve growth, maturation, and differentiation, all of which could modulate processes involved in tissue-specific tumor formation. Tissue-specific protective cellular mechanisms also can affect the response to TCDD, further complicating our understanding of the carcinogenic effects of this chemical.

There are differences among various experimental animals in susceptibility to TCDD-induced effects, and the sites at which tumors are induced vary from species to species. Modulated gene expression by TCDD is highly specific for species and cell type. Differences in the induction or repression of responsive genes probably operates in the responses seen in different cell types and species.

Although structural differences in the AhR have been identified among different species, that receptor operates in a similar manner in animals and humans. Therefore, it is likely that there is a common underlying mechanism of the carcinogenic effects of TCDD in humans and animals, and data on animals support a biologic basis for effects in humans. Because of the many species and strain differences in TCDD responses, however, there is disagreement about the magnitude of TCDD exposure that is carcinogenic.

Fewer studies have been conducted on the carcinogenicity of the herbicides. Several studies of 2,4-D, 2,4,5-T, and picloram have been performed in labora-

tory animals. In general, the results were negative, although some of them would not meet current standards for cancer bioassays; others produced equivocal results. Thus, it is impossible to have confidence in the conclusions regarding the carcinogenicity of those compounds. Most of the evidence indicates that 2,4-D is genotoxic only at very high concentrations, and although 2,4,5-T was shown to increase 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 could induce DNA modifications that sensitize it to free-radical injury. Results from some studies indicate that DMA promotes 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 with 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 those compounds. Some animal data might support the conclusion that DMA exposure can result in carcinogenesis, but the data alone are insufficient to support conclusions on the carcinogenicity of that compound in humans.

Considerable uncertainty remains about how to apply the above information to the evaluation of the carcinogenic potential of herbicide or TCDD exposure in Vietnam veterans. A number of health agencies have concluded that TCDD is a human carcinogen. There is, however, considerable uncertainty regarding the doses or body burden at which carcinogenesis may occur.

Increased Risk of Disease Among Vietnam Veterans

In response to 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 for specific cancers, for most outcomes the data are insufficient to quantify an 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 2002* (hereafter, *Update 2002*; IOM, 2003) on the association between exposure to herbicides and adverse reproductive or developmental effects. The categories of association and the approach to categorizing the health outcomes are discussed in Chapters 1 and 2. The literature discussed 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, stillbirth, neonatal and infant mortality, low birthweight and preterm birth, childhood cancer, and alterations in sex ratio. In addition to studies of herbicides and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), studies of populations exposed to polychlorinated biphenyls (PCBs) were reviewed when relevant, because TCDD is sometimes a contaminant of PCBs. For studies new to this update that report only a single reproductive health outcome and that are not revisiting a previously studied population, their design information is summarized here with their results; the design information for all other new studies can be found in Chapter 4.

This chapter's primary emphasis is 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 also were included. Studies investigating the potential reproductive consequences of exposure by either parent were considered; whenever the information was available, an attempt was made to evaluate the effects of maternal and paternal exposure separately.

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, are *congenital anomaly* and *congenital malformation.* Major birth defects, which occur in 2–3% of live births, are abnormalities that are present at birth that are severe enough to interfere with viability or physical well-being. Birth defects are detected in another 5% of babies during follow-up through the first year of life. The causes of most birth defects are unknown. Genetic factors, exposure to some medications, exposure to environmental contaminants, occupational exposures, and lifestyle factors have been implicated in the etiology of birth defects (Kalter and Warkany, 1983). Most etiologic research has focused on the effect of maternal and fetal exposures, but some work has addressed paternal exposures. Paternally mediated exposures might occur by several routes and exert effects in various ways. One way is through direct genetic damage to the male germ cell transmitted to the offspring and dominantly expressed as a birth defect. A hypothesized route is the transfer of toxic compounds through a man's body into his seminal fluid, resulting in fetal exposure during gestation (Chia and Shi, 2002). Another more indirect route of paternally mediated exposure could arise from contact of family members with contamination brought into the home from the workplace.

Summary of *VAO*, *Update 1996*, *Update 1998, Update 2000,* and *Update 2002*

The committee responsible for *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (hereafter, *VAO*; IOM, 1994) determined that there was inadequate or insufficient information to determine an association between exposure to 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (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 *Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996*; IOM, 1996) led it to conclude that there was limited or suggestive evidence of an association between at least one of the compounds of interest and spina bifida in the children of veterans; there was no change in the conclusions regarding other birth defects. Those findings were not modified further in *Veterans and Agent Orange: Update 1998* (hereafter, *Update 1998*; IOM, 1999), *Veterans and Agent Orange: Update 2000* (hereafter, *Update 2000*; IOM, 2001), or *Veterans and Agent Orange: Update 2002* (hereafter, *Update 2002*; IOM, 2003).

Summaries of the studies of birth defects and neural tube defect specifically that were reviewed here and in earlier reports can be found in the Tables 7-1 and 7-2, respectively.

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*			
Kristensen et al., 1997	Norwegian farmers (maternal and paternal exposure)	4,189	1.0 (1.0–1.1)[b]
Dimich-Ward et al., 1996	Sawmill workers (paternal exposure)		
	Cataracts	11[c]	5.7 (1.4–22.6)
	Genital organs	105[c]	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	11	0.9 (0.5–1.7)
	Maternal age > 30	19	2.5 (1.6–2.1)
	Chromosomal	8	1.1 (0.5–2.1)
	Other	48	
	Maternal age < 35	36	1.1 (0.8–1.6)
	Maternal age > 35	12	3.0 (1.6–5.3)
	All births with anomalies	125	1.4 (1.2–1.7)
Studies Reviewed in *VAO*			
Moses et al., 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	11	1.3 (0.5–3.4)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	18	1.1 (0.5–2.2)
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)
Townsend et al., 1982	Follow-up of Dow Chemical plant workers (paternal exposure)	30	0.9 (0.5–1.4)
ENVIRONMENTAL			
New Studies			
Cordier et al. 2004	Residents of the Rhône-Alpes region of France living near municipal solid waste incinerators (maternal and paternal exposure)		
	Minor anomalies	518	0.9 (0.8–1.1)
	Chromosomal anomalies	204	1.0 (0.9–1.2)
	Monogenic anomalies	83	1.1 (0.8-1.4)
	Unknown or multifactoral etiology	964	1.1 (1.0–1.2)

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Schreinemachers, 2003	Rural or farm residents of Minnesota, Montana, and North and South Dakota (maternal and paternal exposure)		
	Any birth anomaly	213	1.1 (0.9–1.3)
	Central nervous system anomalies	12	0.8 (0.5–1.4)
	Circulatory or respiratory anomalies	39	1.7 (1.1–2.6)
	Digestive system anomalies	24	0.9 (0.6–1.5)
	Urogenital anomalies	44	1.0 (0.7–1.5)
	Musculoskeletal or integumental anomalies	70	1.5 (1.1–2.1)
	Chromosomal anomalies	17	0.9 (0.6–1.6)
Studies Reviewed in *Update 2002*			
Loffredo et al., 2001	Mothers in the Baltimore-Washington Infant Study exposed to herbicides during the first trimester (maternal exposures)	66	2.8 (1.3–6.9)
Revich et al., 2001	Residents of Chapaevsk, Russia—congenital malformations	*	(*) NS
ten Tusscher et al., 2000	Infants born in Zeeburg, Amsterdam clinics 1963–1965 with orofacial cleft (maternal exposures)		
	Births in 1963	5	(*) SS
	Births in 1964	7	(*) SS
Studies Reviewed in *Update 2000*			
García et al., 1998	Residents of agricultural areas in Spain—≥median score on chlorophenoxy herbicides exposure duration (months) index (paternal)	14	3.1(0.6–16.9)
Studies Reviewed in *VAO*			
Fitzgerald et al., 1989	Persons exposed to an electrical transformer fire—total birth defects (maternal or paternal exposure)—incidence	1	2.1 (0.05–11.85)
Mastroiacovo et al., 1988	Seveso residents (maternal, paternal, and in utero exposure)		
	Zones A and B total defects	27	1.2 (0.8–1.8)
	Zones A, B, R total defects	137	1.0 (0.8–1.2)
	Zones A and B mild defects	14	1.4 (0.9–2.6)
Stockbauer et al., 1988	Persons in Missouri with documented TCDD soil contamination near residence (maternal; paternal; in utero exposure)		
	Total birth defects	17	0.8 (0.4–1.5)
	Major defects	15	0.8 (0.4–1.7)
	Midline defects	4	0.7 (0.2–2.3)

continues

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Hanify et al., 1981	Residents of areas of Northland New Zealand subject to aerial 2,4,5-T spraying[d]		
	All birth malformations	164	1.7 (1.4–2.1)[e]
	All heart malformations	20	3.9 (2.1–7.4)[e]
	Hypospadias, epispadias	18	5.6 (2.7–11.7)[e]
	Talipes	52	1.7 (1.2–2.3)[e]
	Cleft lip	6	0.6 (0.3–1.3)[e]
	Isolated cleft palate	7	1.4 (0.6–3.2) [e]
VIETNAM VETERANS			
Studies Reviewed in *Update 2002*			
Kang et al., 2000	Female Vietnam veterans	4,140	
	"Likely" 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)
	Tracheo-oesophageal fistula	10	23 expected (14–32)
	Anencephaly	13	16 expected (8–24)
	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., 1998a	Air Force Ranch Hand veterans (paternal exposures)		
	Before service in Southeast Asia	*	0.7 (*)
	After service in 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 and 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)

TABLE 7-1 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
	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 *Update VAO*			
AFHS, 1992	Air Force Operation Ranch Hand veterans—birth defects in conceptions following service in Southeast Asia		
	Congenital anomalies	229	1.3 (1.1–1.6)
	Nervous system	5	1.9 (0.5–7.2)
	Respiratory system	5	2.6 (0.6–10.7)
	Circulatory system or heart	19	1.4 (0.7–2.6)
	Urinary system	21	2.5 (1.3–5.0)
	Chromosomal	6	1.8 (0.6–6.1)
	Other	5	2.6 (0.6–10.7)
Aschengrau and Monson, 1990	Vietnam veterans whose children were born at Boston Hospital for Women (paternal exposure)		
	All congenital anomalies (crude OR)		
	Vietnam veterans compared to men without known military service	55	1.3 (0.9–1.9)
	Vietnam veterans compared to non-Vietnam veterans	55	1.2 (0.8–1.9)
	One or more major malformations (crude OR)		
	Vietnam veterans compared to men without known military service	18	1.8 (1.0–3.1)
	Vietnam veterans compared to non-Vietnam veterans	18	1.3 (0.7–2.4)
CDC, 1989	Vietnam Experience Study—interview data (paternal exposure)		
	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 defects	426	1.2 (1.1–1.5)
	Hydrocephalus	11	5.1 (1.1–23.1)
	Spina bifida	9	1.7 (0.6–5.0)
	Hypospadias	10	3.1 (0.9–11.3)
	Multiple defects	71	1.6 (1.1–2.5)
	Birth defects in childrens of veterans reporting 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—hospital records (paternal exposure)		
	Birth defects	130	1.0 (0.8–1.3)
	Birth defects—black Vietnam veterans only	21	3.4 (1.5–7.6)
	Major birth defects	51	1.2 (0.8–1.9)
	Digestive system defects	18	2.0 (0.9–4.6)
Donovan et al., 1984	Australian Vietnam veterans (paternal exposure)		
	Vietnam veterans vs all other men	127	1.02 (0.8–1.3)
	National Service veterans—Vietnam service vs no Vietnam service	69	1.3 (0.9–2.0)
Erikson et al., 1984a	Vietnam veterans identified through the CDC Metropolitan Atlanta Congenital Defects Program (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)

[a] Given when available.
[b] 95% confidence intervals contained one for all outcomes. Anencephaly and spina bifida included in this calculation.
[c] Number of workers with maximal index of exposure (upper three quartiles) for any job held up to three months prior to conception.
[d] Excludes stillbirths, neonatal death, or dislocated or dislocatable hip.
[e] 90% confidence interval
* 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; OR, oddds ratio; 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	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	Norwegian farmers—spina bifida (parental exposure)		
	Tractor spraying equipment	28	1.6 (0.9–2.7)
	Tractor spraying equipment and 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 only	18[b]	1.8 (0.8–4.1)
Garry et al., 1996	Private pesticide appliers—central nervous system defects (paternal exposure)	6	1.1 (0.5–2.4)
ENVIRONMENTAL[c]			
New Studies			
Cordier et al. 2004	Residents of the Rhône-Alpes region of France living near municipal solid-waste incinerators (maternal and paternal exposure)	49	0.9 (0.6–1.2)
Studies Reviewed in *VAO*			
Stockbauer et al., 1988	Persons in Missouri with documented TCDD soil contamination near residence—central nervous system defects (maternal; paternal; in utero exposure)	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 (0.7–2.9)[d]
	Spina bifida	13	1.1 (0.6–2.1)[d]
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
AIHW, 1999	Australian Vietnam veterans—Validation Study (paternal exposure)		
	Spina bifida—maxima	50	33 expected (22–44)
	Anencephaly	13	16 expected (8–24)
Studies Reviewed in *Update 1996*			
Wolfe et al., 1995	Air Force Operation Ranch Hand personnel—neural tube defects[e] (paternal exposure)	4	(*)

continues

TABLE 7-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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	(*)
	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[f]	2.7 (1.2–6.2)
	EOI-5: anencephaly	7[f]	0.7 (0.2–2.8)
Australia Department of Veteran 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 prior to conception.
[c] Either or both parents potentially exposed.
[d] 90% confidence interval.
[e] Of the four neural tube defects reported among Ranch Hand offspring there were two spina bifida (high dioxin level), one spina bifida (low dioxin), and one anencephaly (low dioxin). No neural tube defects were reported in the comparison cohort. 454 post-service births were studied in Ranch Hand veterans; 570 in comparison cohort.
[f] 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; AIHW, Australian Institute of Health and Welfare; CDC, Centers for Disease Control and Prevention; CI, confidence interval; EOI, exposure opportunity index; NR, not reported.

Update of the Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2002* (IOM, 2003).

Enviromental Studies

Cordier et al. (2004) studied the impact of exposure to emissions from municipal solid-waste incinerators on birth defects in a region of southeast France for a 10-year period (1988–1997), under the assumption that such emissions increase the environmental load of dioxin and other hazardous compounds. Data on congenital malformations obtained from a regional registry were categorized into four groups: minor, chromosomal, monogenic, and other major anomalies. Communities with more than 50,000 residents were categorized by a detailed scoring system into 194 exposed and 2,678 unexposed. The rates of congenital malformations were compared in analyses adjusted for year of birth, maternal age, department of birth, population density, average family income, and local road traffic (when available). Congenital anomalies were not significantly associated with exposure overall (relative ratio [RR], 1.04; 95% confidence interval [CI], 0.97–1.11), but some specific anomalies (facial clefts, renal dysplasia, obstructive uropathies, cardiac anomalies) showed significant dose–response relationships. The defined exposure indicator could not, however, differentiate exposure to dioxins from exposure to metals in this ecologic study.

Schreinemachers (2003) conducted an ecologic study that compared rates of adverse birth outcomes in US agricultural states: Minnesota, Montana, North Dakota, and South Dakota. Counties, the unit of analysis, were included in the study if at least half of the population in the county was rural and if more than 20% of the land was dedicated to raising crops. The 147 counties were then classified according to their acreage of wheat fields, as a surrogate for exposure to chlorophenoxy herbicides (including 2,4-D) as high-wheat ($N = 74$) and low-wheat ($N = 73$). National birth and infant death data for 1995–1997 were used to derive gender-specific rates of malformations at birth for white singleton births, adjusting for several covariates, including maternal age, parity, maternal education, prenatal care, previous preterm or small-for-gestational-age (SGA) birth, tobacco use during pregnancy, alcohol use during pregnancy, sex of child, and season of conception. A strong association was observed for circulatory and respiratory anomalies (odds ratio [OR], 1.7; 95% CI, 1.1–2.6), which became even stronger after excluding heart malformations (OR, 2.0; 95% CI, 1.1–3.6). Conception during the season of heavy herbicide application (April–June) was the only significant adjustment covariate (OR, 1.7; 95% CI, 1.1–2.8). After covariate adjustment, increased risk for musculoskeletal and integumental anomalies was observed (OR, 1.5; 95% CI, 1.1–2.1). Boys appeared to be more susceptible to congenital anomalies than girls (male-to-female ratios of births with any congenital anomaly were 1.67 and 1.60 in the low- and high-wheat counties, respectively). The authors noted that the use of herbicides other than the chlorophenoxy herbicides should also be considered as a possible cause. Moreover, since acreage was used as an exposure surrogate, lack of directly measured herbicide exposure is a major limitation.

Vietnam-Veteran Studies

Correa-Villasenor et al. (2003) documented the methodology, use, and related results of the Metropolitan Atlanta Congenital Defects Program, in which 35 years of birth defects surveillance was done at the Centers for Disease Control and Prevention. Data from the registry were used by Erickson et al. (1984b), who showed that there was no greater risk among Vietnam veterans for fathering babies with serious birth defects. No health effects analysis was conducted by Correa-Villasenor et al. (2003).

Synthesis

Cordier et al. (2004) found significant associations with exposure to emissions from municipal solid-waste incinerators only for some facial cleft, renal dysplasia, and "other renal anomalies." The validity of this ecologic study is limited considerably by the possibility of exposure misclassification and residual confounding. Furthermore, the researchers did not have actual dioxin measurements and the subjects were probably exposed to other toxic substances, particularly metals, in the incinerator emissions.

Schreinemachers (2003) reported increased incidences of circulatory or respiratory anomalies (possibly more pronounced among male children) in association with agricultural activity. Because of the study's ecological design, its results are valid only for regional differences and might not translate to individual comparisons. The use of a county's wheat-producing acreage as a surrogate for parental exposure to agricultural chemicals and, even more indirectly, for dioxin exposure severely limits the value of these findings in evaluating the exposure experience of Vietnam veterans.

Conclusions

Strength of Evidence from Epidemiologic Studies

There were no new relevant studies on the association between parental exposure to 2,4-D, 2,4,5-T, TCDD, cacodylic acid, or picloram and spina bifida in offspring. The committee concludes that the evidence is still limited or suggestive of an association between exposure to the compounds of interest and spina bifida.

Its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports leads the committee to conclude that there is still inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and all other birth defects.

Although there were reports of increased risks of transposition of the great arteries, non-syndromal orofacial clefts, and congenital morphogenetic condi-

tions in the various studies reviewed for *Update 2004*, those studies suffer from various pitfalls in design, sample size, and nonspecific exposure ascertainment. The two epidemiologic studies discussed in this report also suggest associations with specific groups of anomalies, as discussed above. But the studies have several limitations, and the associations might have been caused by other types of environmental contaminants that are not necessarily relevant to the charge of this committee.

Biologic Plausibility

Laboratory studies of potential male-mediated developmental toxicity attributable to exposure to TCDD and herbicides, specifically with regard to birth defects, are too limited to permit conclusions. Notably, one investigation that was reviewed for *Update 2002* did not show evidence that paternal exposure to a herbicide formulation containing 2,4-D and picloram caused birth defects or any other adverse reproductive outcomes in experimental animals. Studies of chemical production workers exposed to TCDD suggest there are associated hormonal changes, but it is unclear whether those changes could be responsible for an increase in spina bifida or other birth defects.

A discussion of the biologic plausibility of reproductive effects in general arising from exposure to the chemicals of interest is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and birth defects among offspring, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in the risk of this outcome in their children.

Although there are data to suggest an association between exposure to the chemicals of interest and spina bifida, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in the risk of this outcome (or other birth defects) in their children.

FERTILITY

Male reproductive function is under the control of several components whose proper coordination is important for normal fertility. Several of those components and some endpoints related to male fertility, including reproductive hormones and sperm characteristics, can be studied as indicators of fertility. 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 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 Seretoli 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, affect the neuroendocrine system and spermatogenesis (for reviews see Bonde and Giwercman, 1995; Tas et al., 1996).

Summary of *VAO*, *Update 1996*, *Update 1998 Update 2000,* and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and altered sperm characteristics or infertility. Additional information available to the committees responsible for *Update 1996*, *Update 1998, Update 2000,* and *Update 2002* did not change that finding. Reviews of the relevant studies are presented in the earlier reports. Table 7-3 summarizes those studies.

Update of the Scientific Literature

Occupational Studies

No relevant occupational studies have been published since *Update 2002* (IOM, 2003).

Environmental Studies

Greenlee et al. (2003) studied factors possibly associated with infertility in a case–control study of women living in an agricultural region of Wisconsin. A woman was considered infertile if she had 12 months of unprotected intercourse without conceiving a pregnancy that ended in live birth. Case subjects included women who were 18–35 years old and who sought treatment for infertility. They were identified through a review of medical records, and the group included women with endometriosis; infertility attributable to anovulation or pituitary–hypothalamic dysfunction; infertility of tubal, uterine, cervical, or vaginal origin; or infertility of other-specified or other-unspecified origin. Control subjects were identified through review of medical records of women with new visits to an obstetrics and gynecology department. Cases and controls were matched for age and clinic service date. Participants were asked about their activities during the 2 years before their pregnancy attempt. In 322 case subjects and 322 control

TABLE 7-3 Selected Epidemiologic Studies—Fertility (Altered Hormone Concentrations, Decreased Sperm Counts or Quality, Subfertility, or Infertility)

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in Update *2000*			
Abell et al., 2000	Female greenhouse workers in Denmark— fecundibility ratio (maternal exposure)		
	>20 hours 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	Danish farmers who used any potentially spermatotoxic pesticides, including 2,4-D —fecundibility ratio (paternal exposure)		
	Farmers using pesticides vs organic farmers	523	1.0 (0.8–1.4)[b]
	Used three or more pesticides		0.9 (0.7–1.2)[b]
	Used manual sprayer for pesticides		0.8 (0.6–1.1)[b]
Studies Reviewed in *Update 1998*			
Heacock et al., 1998	Workers at sawmills using chlorophenates (paternal exposure)		
	Standardized fertility ratio	18,016 (births)	0.9 (0.8–0.9)[c]
	Mantel-Haenszel rate ratio estimator	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]
Lerda and Rizzi, 1991	Argentinean farmers exposed to 2,4-D	32	
	Sperm count (millions/ml)		Exposed: 49.0 vs control: 101.6
	Motility (%)		Exposed: 24.8 vs control: 70.4
	Sperm death (%)		Exposed: 82.9 vs control: 37.1[d]
	Anomalies (%)		Exposed: 72.9 vs control: 33.4 ($p<0.01$ overall)

continues

TABLE 7-3 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Greenlee et al., 2003	Women from Wisconsin, US ± infertility (maternal exposure)		
	Mixed or applied herbicides	21	2.3 (0.9–6.1)
	Used 2,4,5-T	9	9 cases (2.7%) 11 controls (3.4%)
	Used 2,4-D	4	4 cases (1.2%) 4 controls (1.2%)
Swan et al., 2003	Men from Missouri, US ± low sperm quality		
	Elevated urinary metabolite marker for 2,4-D	5	0.8 (0.2–3.0)
Studies Reviewed in Update *2002*			
Staessen et al., 2001	Adolescents in communities close to industrial sources of heavy metals, PCBs, VOCs, and PAHs— delays in sexual maturity		
	In Antwerp, Belgium	15	4.0 (*)
	In Wilrik, Belgium	8	1.7 (*)
VIETNAM VETERANS			
Studies Reviewed in *Update 1996*			
Henriksen et al., 1996	Effects on specific hormone levels or sperm count 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)
	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)

TABLE 7-3 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
	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 having children	49	1.3 ($p < .01$)

[a] Given when available.
[b] For this study, relative risk has been replaced with the fecundability ratio, for which a value less than 1.0 indicates an adverse effect.
[c] For this study, relative risk has been replaced with the standardized fertility ratio, for which a value less than 1.0 indicates an adverse effect.
[d] Table 1 in the reference reverses these figures—control: 82.9%; exposed: 37.1%—but the text ("The percentages of asthenospermia, mobility, necrosperma and teratospermia were greater in the exposed group than in controls…") suggests that this is a typographic error.
* Information not provided by study authors.

subjects, when the broad category of "mix/apply herbicides" in the 2 years before trying to conceive, the crude OR was 2.3 (95% CI, 0.9–6.1) based on 21 exposed cases; adjustment for maternal education, maternal and paternal hours of passive smoke exposure, maternal and paternal time spent reviewing occupational and pesticide exposure lists, and per capita income was said to increase the OR to 26.9 (95% CI, 1.9–384.8). No equivalent data were presented for men. Numbers were too small to analyze individual pesticide products, but 9 case subjects (2.7%) and 11 control subjects (3.4%) reported being exposed to 2,4,5-T. Four case subjects (1.2%) and 4 controls (1.2%) reported exposure to 2,4-D.

Swan et al. (2003) performed a nested case–control study to examine whether previously observed poor semen quality in men from rural relative to urban areas was attributable to use of pesticides including herbicides, fungicides, and other pest control substances. Urine samples were analyzed for 15 non-persistent pesticide metabolites. None of the 36 subjects from Minnesota (9 cases and 27

controls) had detectable 2,4-D metabolites. Other pesticide metabolites were detected in the study—none of them were compounds of interest for this study. In the set of 25 cases and 25 controls from Missouri, the difference in the levels of the 2,4-D metabolite (means of 0.56 versus 0.10 micrograms/gram [μg/g] creatinine, respectively) was only of borderline statistical significance ($p = 0.10$). With exposure classification dichotomized at 0.10 μg/g, there was no association with semen quality (5 cases and 6 controls exposed; OR, 0.8; 95% CI, 0.2–3.0). Treating metabolite level and sperm quality parameters as continuous variables, 2,4-D was not association with sperm motility or concentration, but showed a weak association with sperm morphology ($\beta = -2.36$, $p = 0.11$). It should be noted that, because of the short half-life of 2,4-D, urine samples can be used to indicate exposures only for a few days.

Eskenazi et al. (2002a) studied the association between TCDD exposures, as measured first from serum samples collected immediately after an industrial explosion in 1976 at Seveso, Italy, and then in menstrual-cycle characteristics 20 years after the explosion. Several menstrual outcomes were analyzed, including length of menstrual cycle (in days), number of days of menstrual flow, regularity of menstrual cycle (binary: regular, irregular), and heaviness of menstrual flow (categorical: scanty, heavy, moderate). Multiple linear or logistic regression models were fitted (for continuous and categorical data, respectively), with heaviness of menstrual flow that was dichotomized in various ways; women with irregular cycles were excluded from analysis of length of menstrual flow.

A 10-fold increase in TCDD concentrations was marginally associated with about 0.40 day's increase in estimated menstrual cycle length (95% CI, –0.14–0.94). In a model that included an interaction between TCDD concentration and menarchial status (interaction $p = 0.08$); that marginal association was present only in premenarchial women (adjusted β, 0.93; 95% CI, –0.01–1.86). For days of menstrual flow, no association was observed with TCDD concentration, even after stratification by menarchial status. In models for irregular flow and heaviness of flow, there was no significant association between TCDD concentration and heavy compared with moderate flow (adjusted OR, 0.95; 95% CI, 0.61–1.50). In models that compared the scanty flow category with others (moderate and heavy categories combined), there was a modest, but insignificant, association with TCDD concentration (adjusted OR, 0.84; 95% CI, 0.44–1.61). The association was significantly stronger for women who were premenarchial at the time of the explosion (interaction $p = 0.03$) with an adjusted OR of 0.33 (95% CI, 0.10–1.06), and completely absent for postmenarchial women (adjusted OR, 1.36; 95% CI, 0.70–2.64). After adjustment for age at interview and age at menarche, a 10-fold increase in serum TCDD concentration was significantly associated with reduced odds of having an irregular cycle (adjusted OR, 0.46; 95% CI, 0.23–0.95), with no apparent effect modification by menarchial status at the time of the explosion. It is possible that the finding was influenced by the exclusion of women who reported using hormones to regulate menstrual cycles. When those

29 women were included in the analysis, there was no longer a significant association with TCDD concentration (adjusted OR, 0.67; 95% CI, 0.40–1.10). The sensitivity analysis that refitted models after restricting to women aged 40 or younger did not result in any appreciable differences in conclusions. The main conclusion from the study was that serum TCDD concentration was associated with some menstrual cycle characteristics, with possible effect modification by menarchial status.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2002* (IOM, 2003).

Synthesis

Greenlee et al. (2003) presented intriguing findings with respect to female infertility, although the inability to examine the effects of specific herbicides because of the small sample sizes was a limitation. Moreover, information on risk factors was obtained from self-reports, which can be subject to recall bias.

Swan et al. (2003) conducted various statistical analyses in an effort to detect relationships between semen quality and the urinary metabolite levels of various herbicides. In that the results were at most of borderline statistical significance and rather inconsistent, they did not demonstrate a clear association between fairly recent exposure to 2,4-D and low semen quality.

The study by Eskenazi et al. (2002a) had several strengths, including its population-based (albeit historical) cohort design, the sophisticated and thorough analytic approaches employed, and the attempts to conduct a thorough sensitivity analysis. By using TCDD levels from previously gathered serum samples, they demonstrated some long-term effects of TCDD on current menstrual cycle. The menstrual-cycle characteristics, which were assessed retrospectively, are not directly predictive of fertility status. Eskenazi et al. (2002b) also investigated endometriosis, another outcome that does not actually measure fertility, but could be relevant to it.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and altered hormone concentrations; decreased sperm counts; or sperm quality, subfertility, or infertility.

Biologic Plausibility

Evidence from tests with laboratory animals suggests that TCDD can alter testosterone synthesis, follicle development, and ova production. Those effects occur at relatively high doses. The reproductive significance of those changes to Vietnam veterans exposed to much lower concentrations of TCDD is not clear.

A discussion of the biologic plausibility of reproductive effects in general arising from exposure to the chemicals of interest is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and altered sperm characteristics or infertility, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

SPONTANEOUS ABORTION

Spontaneous abortion is the expulsion of a nonviable fetus, generally before 20 weeks of gestation, that is not induced through physical or pharmacologic means. The background risk of recognized spontaneous abortion is generally 7–15% (Hertz-Picciotto and Samuels, 1988), but it is established that many more pregnancies terminate before women become aware of pregnancy (Wilcox et al., 1988); those are known as subclinical pregnancy losses and generally are not included in studies of spontaneous abortion. Estimates of the risk of recognized spontaneous abortion vary with the design and method of analysis. Study designs include cohorts of women asked retrospectively about 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, particularly of spontaneous abortions that took place a long time before. Studies that enroll 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 can attract nonrepresentative study groups because protocols are demanding.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000* and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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 that finding. Information available to the committee responsible for *Update 2002*, however, led to the conclusion that there was suggestive evidence that paternal exposure to TCDD is not associated with the risk of spontaneous abortion, but that there was insufficient information to determine whether an association exists between maternal exposure to TCDD and the risk of spontaneous abortion, or between maternal or paternal exposure to 2,4-D, 2,4,5-T, picloram, or cacodylic acid and the risk of spontaneous abortion. The relevant studies are reviewed in the earlier reports. Table 7-4 summarizes the studies.

Update of the Scientific Literature

Eskenazi et al. (2003) evaluated data from the Seveso Women's Health Study (SWHS) for an association between individual serum TCDD concentrations and birth outcomes in women who resided in Seveso at the time of the 1976 accident. For the analysis of spontaneous abortions, all pregnancies that ended in the period immediately after the explosion to the time of enrollment into the SWHS were considered, excluding those that ended by voluntary abortion ($N = 108$) or by ectopic or molar pregnancy ($N = 11$). The group of pregnancies that ended between 1976 and 1984—the first half-life of TCDD after the explosion—was analyzed separately. Exposure was based on serum TCDD concentrations, which were entered as a continuous variable in the statistical analyses (as a logarithmic function; an increase of 1 unit change corresponded to a 10-fold increase in TCDD concentration). The median maternal serum TCDD concentration at the time of the explosion was 46.6 parts per trillion (ppt) (range, 2.5–9,140 ppt). TCDD concentrations were highest for the youngest women, who were nulliparous at the time of the explosion. The analysis considered potential confounders and effect modifiers a priori from the literature, including maternal age at pregnancy, tobacco use, alcohol use, parity, and many other factors. Statistical adjustments were made for women who had multiple pregnancies during the study period. All information on pregnancy, pregnancy outcomes, and covariates was obtained in interviews conducted about 20 years after the explosion. The average length of recall between interview and pregnancy was 10.7 years. TCDD concentrations were highest for women with the shortest time of recall to pregnancy.

During the study period, 769 spontaneous abortions were reported (from 476 women; some had multiple spontaneous abortions). Those women had slightly lower TCDD concentrations than were found in women who had live births. No increase in rate was found in association with serum TCDD concentrations either in the group as a whole (adjusted OR, 0.8; 95% CI 0.6–1.2) or when the analysis was restricted to pregnancies that occurred between 1976 and 1984. However, almost one-third of the pregnancies reported within the first year of the explosion ended in voluntary abortions (a rate that decreased to 11% thereafter). Although it could be hypothesized that this could bias the results towards the null (assum-

TABLE 7-4 Selected Epidemiologic Studies—Spontaneous Abortion

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2002*			
Arbuckle et al., 2001	Ontario farm families (paternal and/or maternal exposure)		
	Phenoxyacetic acid herbicide exposure in the pre-conception period and the risk of first trimester spontaneous abortions	48	1.5 (1.1–2.1)
Schnorr et al., 2001	Wives and partners of men in the NIOSH cohort (paternal exposure)		
	Estimated paternal TCDD serum level at the time of conception		
	<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)
Studies Reviewed in *Update 2000*			
Driscoll, 1998	Women employed by the US Forest Service—pregnancies ending in miscarriage	141	2.0 (1.1–3.5)
Studies Reviewed in *VAO*			
Moses et al., 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	14	0.9 (0.4–1.8)
Suskind and Hertzberg, 1984	Follow-up of 2,4,5-T production workers (paternal exposure)	69	0.9 (0.6–1.2)
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)
Townsend et al., 1982	Wives of men employed involved in chlorophenol processing at Dow Chemical Co. (paternal exposure)	85	1.0 (0.8–1.4)
Carmelli et al., 1981	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 aged 18–25 years		
	Forest and commercial exposure	8	3.1 (0.9–9.6)
	Exposure during conception period		
	Father aged 31–35 years, farm exposure	10	2.9 (0.8–10.9)

TABLE 7-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
New Studies			
Eskenazi et al., 2003	Seveso (Italy) Women's Health Study participants living in exposure Zones A and B in 1976		
	Spontaneous abortions in pregancies 1976–1998	97	0.8 (0.6–1.2)
	Spontaneous abortions in pregancies 1976–1984	44	1.0 (0.6–1.6)
Studies Reviewed in *Update 2002*			
Revich et al., 2001	Residents of Chapaevsk, Russia (maternal and paternal exposure)	*	24.4% (20.0–29.5%)[b]
	Residents of surrounding towns in the Samara Region (maternal and paternal exposure)		
	Samara	*	15.2% (14.3–16.1%)[b]
	Toliatti	*	10.6% (9.8–11.5%) [b]
	Syzran	*	15.6% (13.4–18.1%)[b]
	Novokuibyshevsk	*	16.9% (14.0–20.3%)[b]
	Other small towns	*	11.3% (9.4–13.8%)[b]
Tuyet and Johansson, 2001	Vietnamese women who were or whose husbands were exposed to herbicides sprayed during the Vietnam war	*	(*) [anecdotal reports of miscarriage in pilot study]
Studies Reviewed in *Update 2000*			
Axmon et al., 2000	Wives of Swedish fishermen		
	Miscarriages and stillborn infants before week 12		0.5 (0.3–1.0)
	East coast residents	12	(*)
	West coast residents	54	(*)
Petrelli et al., 2000	Wives of pesticide appliers	26	3.8 (1.2–12.0)
VIETNAM VETERANS			
Studies Reviewed in *Update 2002*			
Kang et al., 2000	Female Vietnam-era veterans—spontaneous abortions or stillbirths		
	Vietnam veterans (1,665 pregnancies)	278	(*)
	Vietnam-era veterans who did not serve in Vietnam (1,912 pregnancies)	317	(*)

continues

TABLE 7-4 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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 (paternal exposure)	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*			
Aschengrau and Monson, 1989	Wives of Vietnam veterans presenting at Boston Hospital for Women		
	Spontaneous abortions through 27 weeks gestation	10	0.9 (0.4–1.9)
	First-trimester (through 13 weeks gestation) spontaneous abortions only	*	1.2 (0.6–2.8)
CDC, 1989	Vietnam Experience Study (paternal exposure)		
	Overall	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)
Field and Kerr, 1988	Follow-up of Australian Vietnam veterans (paternal exposure)	195	1.6 (1.3–2.0)
Stellman et al., 1988	American Legionnaires who served in Southeast Asia 1961–1975 (paternal exposure)		
	Vietnam veterans compared to 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 to 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)

[a] Given when available.

[b] Spontaneous abortion rate per 100 full-term pregnancies for the years 1991–1997.

* 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.

ing that perhaps if the pregnancies had not been ended voluntarily, they might have ended spontaneously). However, the rates of spontaneous abortion did not vary by exposure concentration of serum TCDD. If TCCD exposure were to increase preclinical early losses, the study could have missed the effect completely.

No relevant occupational or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

The only study reviewed for spontaneous abortion in relation to TCDD exposure did not reveal an association. The study of Eskenazi et al. (2003) reported on serum TCDD concentrations from the time of the Seveso accident for all study participants, carefully considered many potential confounders, and had a high participation rate. The outcomes and covariates related to pregnancy were all based on self-report, however, and on average they occurred 10 years after the pregnancy ended. In addition, although the range of exposures as measured by serum TCDD varied substantially within the study group, all women in the study had resided in the two most heavily contaminated areas in Seveso. It could be argued that more definitive results would have been produced if a truly unexposed control population had been included. Finally, because all spontaneous abortions were self-reported and referred to clinical loses, it could be hypothesized that the study does not rule out the possibility of a TCDD effect during the earliest period of gestation.

Conclusions

Strength of Evidence from Epidemiologic Studies

Additional information available to committee responsible for *Update 2002* led that committee to note that there was suggestive evidence that paternal exposure to TCDD is not associated with the risk of spontaneous abortion, but that the information remained insufficient to determine whether an association exists between the risk of spontaneous abortion and maternal exposure to TCDD or either maternal or paternal exposure to 2,4-D, 2,4,5-T, picloram, or cacodylic acid. On the basis of its evaluation of the epidemiologic literature examining spontaneous abortion reviewed here and in previous *VAO* reports, the current committee concurs with the overall conclusion of the previous committees that the data are inadequate or insufficient to determine whether an association exists between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and the risk of spontaneous abortion.

Biologic Plausibility

Evidence from studies with laboratory animals suggests that TCDD can alter hormones after low-dose exposure and 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. Exposure to 2,4-D and 2,4,5-T causes fetal toxicity and lethality after maternal exposure in experimental animals. However, that effect occurs only at high doses and in the presence of maternal toxicity. No fetal toxicity or lethality has been reported to attend paternal exposure to 2,4-D.

A discussion of the biologic plausibility of reproductive effects in general arising from exposure to the chemicals of interest is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and spontaneous abortion, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

STILLBIRTH, NEONATAL DEATH, AND INFANT DEATH

Stillbirth or *late fetal death* typically refers to the delivery at or after 20 weeks of gestation of a fetus that shows no signs of life; 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 28 days of birth.

Because the causes of stillbirth and early neonatal death overlap considerably, they are commonly analyzed together in a category, referred to as *perinatal mortality* (Kallen, 1988). Stillbirths occur in less than 1% of all births (CDC, 2000). The most common causes of perinatal mortality (Kallen, 1988) among low-birthweight (500–2,500 g) liveborn and stillborn infants are placental and delivery complications—abruptio placenta, placenta previa, malpresentation, and umbilical-cord complications. 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, Update 2000, and Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and stillbirth, neonatal death, or

infant death. Additional information available to the committees responsible for *Update 1996, Update 1998, Update 2000,* and *Update 2002* did not change that finding. Reviews of the relevant studies are presented in the earlier reports.

Update of the Scientific Literature

No relevant occupational, environmental, or Vietnam-veteran studies have been published since *Update 2002*.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and stillbirth, neonatal death, or infant death.

Biologic Plausibility

Laboratory studies of the potential male-mediated developmental toxicity attributable to exposure to TCDD and herbicides in adult male animals are too limited to support conclusions.

A discussion of the biologic plausibility of reproductive effects in general arising from exposure to the chemicals of interest is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and stillbirth, neonatal death, or infant death, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

BIRTHWEIGHT AND PRETERM DELIVERY

The World Health Organization recommends 2,500 g as the threshold determination for low birthweight (Alberman, 1984). Low infant weight at birth is among the important predictors of neonatal mortality and morbidity in the United States, and preterm delivery is a significant cause. The concept of low birthweight actually encompasses two 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 can have other long-term conse-

quences. 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 are low birthweight. The incidence of IUGR is much more difficult to quantify because there are no standards for distributing birthweight by gestational age. When no distinction is made between the causes of low birthweight (IUGR or PTD), the factors most strongly associated with reduced birthweight are maternal tobacco use during pregnancy, multiple births, and race or ethnicity. Other potential risk factors are 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; previous low birthweight or PTD; multiple gestations; tobacco use; and cervical, uterine, or placental abnormalities (Berkowitz and Papiernik, 1993).

Summary of *VAO*, *Update 1996*, *Update 1998, Update 2000,* and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to the compounds of interest and low birthweight. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000,* and *Update 2002* did not change that finding. Reviews of the relevant studies are presented in the earlier reports.

Update of Scientific Literature

Occupational Studies

Dabrowski et al. (2003) conducted a case–control study to examine birthweight in the offspring of women who were involved in farming for 7 or more days during pregnancy. The subjects were found originally at 25 maternity hospitals in the area of Lodz in central Poland. Sampling for the study was first cross-sectional and additional cases were added from 2 hospitals in other counties because the original group was small. In total, the study included 117 women who delivered low-birthweight infants (cases) and 377 women who delivered infants weighing at least 2,500 g (controls). Exposures were grouped by trimester, and because most women (83.6%) for whom pesticide exposure was reported for the first trimester also had exposure during the second trimester (95 women), those groups were combined. No significant differences were exhibited in the birthweights in the exposed and the non-exposed groups. The offspring of women who were exposed to phenoxyacetic acid derivatives during first or second trimester had a mean birthweight of 2,711 g (standard deviation [SD], 752); the

expected weight was 2,746 g (a difference of –35, $p = 0.822$). Pregnancy duration also was the same, with a mean of 38 weeks (SD, 2.5) in exposed women and in controls ($p = 0.846$). The study was limited by its retrospective nature; interviews were conducted after the birth by the physician. Although recall bias in favor of higher exposures among mothers of low birth weight infants is a potential problem in such studies, the bias usually tends to be away from the null, and hence does not explain the null finding.

Environmental Studies

Eskenazi et al. (2003) examined the association of TCDD exposure with reproductive outcomes among 510 SWHS participants. The women all had complete pregnancies within the 20 years after the accident. The analysis of birthweight and SGA was restricted to the 608 singleton live births (414 women). Exposure was based on individual TCDD serum measurements obtained from most women shortly after the explosion. Information on pregnancy outcomes and covariates was obtained by self-report in interviews conducted 20 years after the accident. The average length of recall between interview and pregnancy was 10.7 years.

Mean birthweight was 3,281 g, and there was a low-birth-weight (<2,500 g) rate of 5.1%. The mean gestational age was 39.4 weeks and a PTD rate of 4.9%; there were 59 (9.7%) SGA infants. There was no increase in association with serum TCDD concentrations after controlling for confounding factors. Overall, there was no decrease in birthweight with increasing serum TCDD (OR, -4 g; 95% CI, -68–60, for each 10-fold increase in maternal TCDD concentration). When the analysis was restricted to pregnancies that occurred during the first 8 years after the accident (corresponding to the first half-life of TCDD), there was a trend of decreased birthweight that was not statistically significant (adjusted OR, -92 g; 95% CI, -204–19). When data were analyzed for SGA, the results were consistent, with small, non-significant increases (OR, 1.2; 95% CI, 0.8–1.8 for the group; OR, 1.4; 95% CI, 0.6–2.9 for pregnancies in the earlier period). There was a similar trend for preterm births (<37 weeks of gestation) (OR, 1.3; 95% CI, 0.7–2.3 for the group as a whole, and OR = 1.5, CI, 0.7–3.2) for the earlier pregnancies). The results were slightly stronger for SGA when the analysis was confined further to first the post-explosion pregnancy (among women who had more than one pregnancy) during the earlier period, but they still did not reach statistical significance (OR, 1.8; 95% CI, 0.7–4.3).

The study presents individual serum TCDD concentrations, and outcome information based on personal interviews, on average 10 years after pregnancy. Recall biases therefore could affect the results, particularly if women suspected that their proximity to the plant could have adversely affected their pregnancies. Although they did not know their TCDD measurements at the time of the interview, zone of residence was positively associated with serum TCDD. The most

heavily exposed women were the youngest ones at the time of the explosion, and many of those younger women might not have had pregnancies by the time of enrollment in SWSH. Finally, the study included only those women who had resided closest to the Seveso plant (in zones A or B), and a totally unexposed control group was not available for comparison.

Vietnam-Veteran Studies

No relevant Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

The study of reproductive outcomes among participants of the SWHS shows a small, non-significant association between maternal dioxin concentrations and decreased birthweight and prematurity among infants born during the 20 years after the explosion. Although the results could suggest an association, they should be interpreted with caution because of the possibility of recall and other biases. The study has several flaws: All information on pregnancy outcomes and covariates were obtained by self-report; the average recall time was more than 10 years after the pregnancy had ended; women living closest to the explosion might believe they were at greater risk of adverse pregnancy outcomes; about one-third of the pregnancies that began soon after the explosion were ended by elective abortion; no unexposed control group or measurement of background dioxin was included. Added to that list of uncertainties is a lack of statistical significance among the various outcomes investigated, despite the accurate measurements of exposure. Thus, no conclusion is possible with respect to a connection between dioxin exposures in the SWHS cohort and decreased birthweight or increased prematurity.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest 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. TCDD and herbicides are known to cross the placenta and lead to direct exposure of the fetus. Data from studies in experi-

mental animals also suggest that the preimplantation embryo and developing fetus are sensitive to the toxic effects of 2,4-D and TCDD after maternal exposure. However, the significance of those animal effects to humans is not clear.

A discussion of the biologic plausibility of reproductive effects in general arising from exposure to the chemicals of interest is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and low birthweight, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

CHILDHOOD CANCER

The American Cancer Society estimated that 9,200 children under the age of 15 would be diagnosed with cancer in the United States in 2004 (ACS, 2004). Treatment and supportive care of children with cancer have greatly improved, and mortality rates have declined by 49% over the past 30 years. Despite those advances, cancer remains the leading cause of death from disease in children under the age of 15, and 1,510 deaths were projected for 2004 (ACS, 2004).

Leukemia is the most common cancer in children. It accounts for about one-third of all childhood cancer cases; nearly 2,760 children are expected to be diagnosed in 2004 (ACS, 2004). Of those, nearly 2,000 will be diagnosed with acute lymphocytic leukemia (ALL); most of the rest will have acute myelogenous leukemia (AML). AML (*International Classification of Diseases*, Ninth Edition [ICD-9] 205) also is commonly referred to as "acute myeloid leukemia" and "acute non-lymphocytic leukemia." There are numerous subtypes of the disease. For consistency, this report uses *acute myelogenous leukemia,* or the abbreviation AML, regardless of usage in the source materials. ALL is most common in early childhood, peaking between the ages of 2 and 3, and AML is most common during the first 2 years of life. ALL incidence is consistently higher in boys than in girls; AML has a similar incidence rate in boys and girls (NCI, 2001). Through early adulthood, ALL rates are about twice as high in whites as in blacks; AML exhibits 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, Update 2000* and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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 *VAO* 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 compounds of interest and AML. After the release of *Update 2000*, investigators from one study 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 summarizes the results of the relevant studies. The committees responsible for *Update 2000* and *Update 2002* reviewed the material in earlier *VAO* reports and in newly available published literature and agreed that there remained inadequate or insufficient evidence to determine an association between exposure and childhood cancers.

Update of Scientific Literature

As part of the Agricultural Health Study, Flower et al. (2004) examined childhood cancer in the offspring of male pesticide applicators in Iowa. Childhood cancer was defined as cancer diagnosed from birth through 19 years of age. The potential associations between pesticide exposure and individual types of cancer were not examined. Incidence was compared with the expected number of age-, sex-, race-, and time-period-specific cancer rates from Iowa Surveillance, Epidemiology, and End Result data. For maternal use of chlorophenoxy herbicides, there was a childhood cancer OR of 0.7 (95% CI, 0.3–1.5) based on 7 exposed cases. There was a higher rate of childhood cancers for paternal exposure to chlorophenoxy herbicides (OR, 1.3; 95% CI, 0.62–2.58) based on 28 exposed cases. Specifically, for 2,4-D, the OR was 0.7 (95% CI, 0.3–1.6) for maternal exposure based on 7 exposed cases. For paternal exposure, based on 6 exposed cases, the OR was 1.29 (95% CI, 0.71–2.35).

No relevant environmental or Vietnam-veteran studies have been published since *Update 2002* (IOM, 2003).

Synthesis

The only new study reviewed for this update (Flower et al., 2004) does not show any significant association between the relevant exposures and childhood

TABLE 7-5 Selected Epidemiologic Studies—Childhood Cancers

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Flower et al., 2004	Offspring of Male Pesticide Applicators in Iowa		
	Maternal exposure to chlorophenoxy herbicides	7	0.7 (0.3–1.5)
	Paternal exposure to chlorophenoxy herbicides	28	1.3 (0.6–2.6)
	Maternal exposure to 2,4-D	7	0.7 (0.3–1.6)
	Paternal exposure to 2,4-D	6	1.3 (0.7–2.4)
Studies Reviewed in *Update 2000*			
Heacock et al., 2000	Offspring of sawmill workers exposured to fungicides contaminated with PCDDs and PCDFs (paternal exposure)		
	Leukemia		
	All workers offspring—incidence	11	1.0 (0.5–1.8)
	Offspring of workers with high chlorophenate exposure	5	0.8 (0.2–3.6)[b]
	Brain cancer		
	All workers offspring—incidence	9	1.3 (0.6–2.5)
	Offspring of workers with high chlorophenate exposure	5	1.5 (0.4–6.9)[b]
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	2.3 (p = .05)
	AML in children with paternal exposure >1,000 days	17	2.7 (1.0–7.0)
	AML in children with maternal exposure >1,000 days	7	undefined (no cases in controls)

continues

TABLE 7-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
Daniels et al., 2001	Neuroblastoma risk in children (case–control study)		
	Parents reported using pesticides in the home	*	1.6 (1.0–2.3)
	Parents reported using herbicides in the home	*	1.9 (1.1–3.2)
	Mother reported applying pesticides in the garden	*	2.2 (1.3–3.8)
Buckley et al., 2000	NHL diagnosed at the age of ≤ 20 years in children with potential prenatal exposure to herbicides	*	(*)[c]
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 *Herbicide/Dioxin Exposure and AML in the Children of Veterans*			
Kristensen et al., 1996	Children of agricultural workers in Norway		
	Children with AML whose parents purchased pesticides	12	1.4 (0.6–2.9)
Studies Reviewed in *Update 2000*			
Meinert et al., 2000	Childhood cancer—population-based case-control study		
	Leukemias		
	Paternal exposure; year before pregnancy	62	1.5 (1.1–2.2)
	Paternal exposure; during pregnancy	57	1.6 (1.1–2.3)
	Maternal exposure; year before pregnancy	19	2.1 (1.1–4.2)
	Maternal exposure, during pregnancy	15	3.6 (1.5–8.8)
	Lymphomas		
	Paternal exposure, year before pregnancy	11	1.5 (0.7–3.1)
	Paternal exposure, during pregnancy	10	1.6 (0.7–3.6)
	Maternal exposure, year before pregnancy	3	2.9 (0.7–13)
	Maternal exposure, during pregnancy	4	11.8 (2.2–64)
Pearce and Parker, 2000	Kidney cancer in subjects (1–15 yrs) whose father listed an agricultural occupation on the child's death certificate	21	0.9 (0.2–3.8)

TABLE 7-5 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Infante-Rivard et al., 1999	Childhood ALL in households using herbicides during the pregnancy (in utero exposure, others not excluded)—population-based case-control study	118	1.8 (1.3–2.6)
Studies Reviewed in *Update 1996*			
Pesatori et al., 1993	Seveso residents aged 0–19 years—10-year follow-up, morbidity, all exposure zones		
	All cancers	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 aged 0–19 years—10-year follow-up, mortality, all exposure zones		
	All cancers	10	7.9 (3.8–13.6)
	Leukemias	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 *Herbicide/Dioxin Exposure and AML in the Children of Veterans*			
AIHW, 2001	Australian Vietnam veterans' children—Revised Validation Study		
	AML	12[d]	1.3 (0.8–4.0)
Studies Reviewed in *Update 2000*			
AIHW, 2000	*Australian Vietnam veterans' children—Validation Study*		
	AML	*This study, which incorrectly calculated the expected number of AML cases, is superceded by AIHW, 2001 above.*	

continues

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—outcomes in the offspring of veterans (paternal exposure)		
	Cancer	25	1.5 (0.7–2.8)
	Leukemia	12	1.6 (0.6–4.0)
Field and Kerr, 1988	Cancer in children of Australian Vietnam veterans (paternal exposure)	4	(*)
Erickson et al, 1984b	CDC Birth Defects Study—children of Vietnam veterans (paternal exposure)		
	"Other" neoplasms	87	1.8 (1.0–3.3)

[a] Given when available.
[b] OR estimated using low exposure subjects as the comparison cohort.
[c] No information on herbicides as a class, distinct from insecticides or other pesticides, was available; exposures before conception were not singled out, and no distinction between maternal and paternal exposure was made.
[d] Of the 12, 9 were observed and 3 additional cases were estimated to have occurred in the portion of the cohort whose data were not validated.
* 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; SS, statistically significant.

cancer. The lack of significance could be attributable to the very small number of cases and the attendant lack of statistical power.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or

insufficient evidence of an association between exposure to the compounds of interest and childhood cancers.

Biologic Plausibility

Susceptibility to cancers in childhood after environmental exposures could be influenced by several factors, one of which is that a child could 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 during infancy or early childhood either directly or through breast milk. TCDD and dioxin-like compounds cross the placenta and are present in breast milk, so a pathway of exposure is demonstrated. Some data suggest that the effects of TCDD and dioxin-like compounds with estrogen like activity persist beyond childhood, even into adult life. For example, recent work indicates that animals exposed in utero to TCDD become more susceptible to mammary cancers induced by some polycyclic aromatic hydrocarbons but that the timing of exposure to TCDD is likely important for the development of malignancies.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and childhood cancers, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

SEX RATIO

Sex ratio (males to females at birth)—about 106 males per 100 females (Pyeritz, 1998), or about 51% males among all births—has been used as a potential marker of genetic damage. It has been hypothesized that the induction of lethal mutations before birth could alter sex ratio. For instance, a lethal mutation on the paternal X chromosome would differentially affect female conceptuses. For some years, investigators have evaluated the sex ratio among various species in relation to such exposures as radiation. More recently, it has been suggested that the ratio is controlled by parental hormones at conception and that changes in gonadotropin and steroid concentrations could exert an effect (James, 1996). The specific mechanisms, such as zygote formation, implantation, regulation of sex-determining factors, and selective fetal loss, are not clear, and direct experimental evidence to support or refute the hypothesis is lacking. James (1997) suggested that a reduction in testosterone and high gonadotropin after TCDD exposure would result in an excess of female offspring. Potential confounding factors for

an altered sex ratio are uncertain, but parental age, social class, illness, race, tobacco use, and stress have been considered.

Summary of *VAO*, *Update 1996*, *Update 1998, Update 2000*, and *Update 2002*

The potential association between exposure to 2,4-D, 2,4,5-T, 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*, *Update 2000*, and *Update 2002* reviewed papers addressing altered sex ratio as part of their examination of literature on fertility. There was inadequate or insufficient information to determine an association between exposure to the compounds of interest and sex ratio. The relevant studies are summarized in Table 7-6.

Update of Scientific Literature

Ryan et al. (2002) studied the sex ratio of children of workers at agrochemical plants in Ufa, Bashkortostan, Russia. More than 650 workers produced the 2,4,5-trichlorophenol (TrCP) from 1961 to 1988, and more than 250 others produced 2,4,5-T from 1964 to 1967. Blood samples from 29 and 55 individuals from these two groups, respectively, were analyzed for TCDD and other dioxins. The median of toxic equivalent values over both cohorts was 243 ppt, with a range of 17–8,520 ppt. The concentrations were generally higher among the TrCP workers (median, 672 ppt) than in the 2,4,5-T workers (median, 177 ppt).

Information about the workers and their children was obtained from company archival records and later verified in personal or telephone interviews with the workers or with close relatives. Birth data were available for 110 and 88 workers, respectively, from the TrCP and 2,4,5-T groups. Only children born at least 9 months after the start of relevant employment (but including any who were deceased) were used in analysis. The sex ratio for the city of Ufa, estimated at 0.512 from data supplied by the State Regional Statistical Department of the Republic of Bashkortostan, was used as a basis for comparison. Statistical analysis was conducted via z-test for ratios and χ-square analysis of contingency. The two cohorts (2,4,5-T and TrCP) were analyzed separately and in combination. Comparisons were made for fathers and mothers separately (15 and 30% of the workers were females in the 2,4,5-T and TrCP groups, respectively) and for all parents combined. The sex ratio for the combined cohorts was 0.40 (z, 3.21, $p < 0.001$), indicating a significant excess of female births. Despite the comparable degree of exposure for fathers and mothers, that significant excess in female births was higher when only fathers were exposed (sex ratio, 0.38; z, 3.60, $p < 0.001$), and virtually nonexistent when the mothers alone were exposed (sex ratio, 0.51, not significant [NS]).

No relevant environmental or Vietnam-veteran studies have been published since *Update 2002* (IOM, 2003).

TABLE 7-6 Selected Epidemiologic Studies—Sex Ratio

Reference	Study Population	Sex Ratio of Offspring (boys/total)[a]	Comments
OCCUPATIONAL			
New Studies[b]			
Ryan et al., 2002	Workers manufacturing 2,4,5,-trichlorophenol (1961–1988) or 2,4,5-T production (1964–1967)	0.40 (91 boys: 136 girls) 0.38 (71 boys: 117 girls) 0.51 (20 boys: 19 girls)	$p < 0.001$, either parent exposed; $p < 0.001$, only father exposed; NS, only mother exposed
Studies Reviewed in *Update 2002*			
Schnorr et al., 2001	Workers producing trichlorophenol and derivatives, including 2,4,5-T: exposed fathers vs unexposed	0.53 vs 0.54	NS overall; no difference on basis of age at first exposure
Okubo et al., 2000	Japanese workers exposed to dicyclopentadiene, cyclopentadiene, epoxy resin, bisphenol A epichlorohydrin vs Japanese population	0.25 (6 boys: 18 girls)	$p < 0.01$
Moshammer and Neuberger, 2000	Austrian chloracne cohort (children born after start TCDD exposure in 1971 vs children born before 1971)	0.46 (26 boys: 30 girls) vs 0.70 (19 boys: 12 girls)	Fewer sons, especially if father was <20 yr when exposed (0.20 for 1 boy: 4 girls)
Savitz et al., 1997	Ontario Farm Family Health Study: fathers with "chemical activity" vs "no chemical activity" vs "no farm activity" during 3 mo before conception (some phenoxy herbicides)		NS overall; lower sex ratio if use of protective equipment not reported
Studies Reviewed in *Update 1998*			
Heacock et al., 1998	Sawmill workers in British Columbia	0.515 0.519 0.512	Chlorophenate-exposed workers; Unexposed workers; Province overall

continues

TABLE 7-6 *Continued*

Reference	Study Population	Sex Ratio of Offspring (boys/total)[a]	Comments
ENVIRONMENTAL			
Studies Reviewed in *Update 2002*			
Karmaus et al., 2002	Michigan fish-eaters (serum PCBs and DDE levels)	>0.5	Significantly more sons, if paternal blood level PCBs >8.1 μg/L;
		<0.5	NS more daughters, if maternal blood level PCBs >8.1 μg/L
Yoshimura et al., 2001	Births 1967–1977 among individuals exposed to PCBs and PCDFs in Yusho, Japan, in 1968		NS
Revich et al., 2001	Residents near chemical plant in Chapaevsk, Russia	0.5 0.4 0.6	1983-1997 min, 1989 max, 1987 and 1995
Studies Reviewed in *Update 2000*			
Mocarelli et al., 2000	Individuals aged 3 to 45 and in Zones A, B, or R at time of Seveso accident	0.4	Father exposed (especially if had been <19 yr)
		0.6	Only mother exposed
		0.6	Neither parent exposed
Studies Reviewed in *Update 1998*			
Mocarelli et al., 1996	Seveso—sex ratio of births in Zone A (1977–1984)	SR = 0.35 (26 boys: 48 girls)	$p < 0.001$
VIETNAM VETERANS			
Studies Reviewed in *Update 2000*			
Michalek et al., 1998b	Sex ratio of births to Ranch Hand personnel (high, low, or background dioxin level) vs other servicemen		SR higher for higher dioxin levels

[a] Given when available.

[b] *VAO*-series reports prior to *Veterans and Agent Orange: Update 1998* did not address the association between to the chemicals of interest (2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid) and perturbations in the sex ratio of offspring.

* Information not provided by study authors.

— Information denoted by a dash in the original study.

ABBREVIATIONS: NS, not significant; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofurans; TCDD, tetrachlorodibenzo-*p*-dioxin.

Synthesis

Despite some strengths, including the use of blood samples, and its obvious relevance to the charge of this committee, the study by Ryan et al. (2002) had several limitations. Samples were obtained many years after initial exposure, and no attempt was made to extrapolate concentrations at the time of employment. TCDD might be a strong candidate for explaining the altered sex ratios, but other compounds (perhaps with a shorter half-life) cannot be ruled out. The authors' assertion that the hypothesis that youth at the time of exposure is an important factor could not be tested because "the mean age of the parents at the birth of their children was about 29 (range 20–43) years," but the parents' ages at the time of first exposure would be critical information for assessing the hypothesis. The nature of the analysis in the study did not allow for covariate adjustments for confounders or for effect modification. The results are similar to those observed for the Seveso population (Mocarelli et al., 1996, 2000, as reviewed in *Update 1998* and *Update 2000*), but different from those reported for the US chlorophenol cohort (Schnorr et al., 2001, as reviewed in *Update 2002).*

Biologic Plausibility

There has been no work with experimental animals that specifically examined the effects of TCDD on sex ratios of offspring, nor have any alterations in sex ratio been reported for animal studies that have examined developmental effects of TCDD on offspring. However, several publications have suggested mechanisms by which an altered sex ratio might occur. James (2002), argued that paternal exposure to organochlorines could have different effects on sex ratios than does maternal exposure; because paternal and maternal exposures can lead to opposite effects on sex ratios, there could be confounding; and the effects of some organochlorines should be examined more closely because some could exhibit estrogenic behavior, whereas others could show antiestrogenic or antiandrogenic behavior. He also suggests that mammalian sex ratios depend partly on hormone concentrations in both parents around the time of conception: Low parental testosterone and high gonadotropin is associated with a higher prevalence of daughters. Numerous animal studies have shown that dioxin disrupts the production of several hormones and that it modulates hormone-dependent pathways, including those involved in reproduction (see Chapter 3). It is plausible that similar effects could disrupt the hormones that affect sex ratio.

Jongbloet et al. (2002) pointed out that experimental data are consistent with the possibility that the antiandrogenic effects of dioxin on male sperm (after paternal exposure) alter sperm transit time and mating behavior, causing fertilization of an "over-ripe" oocyte and leading to a reduced number of male progeny. Furthermore, the antiestrogenic properties of dioxin at the midcycle (after mater-

nal exposure) could result in preferential fertilization of non-optimally matured oocytes by Y-bearing sperm, thus resulting in more male offspring.

To have a better understanding of the issues involved, James (2002) suggested several lines of research focusing on closer examination of specific contaminants or congeners that could be associated with different exposure; endocrinologic assays of exposed women with different exposures, to study the hormonal profiles; and animal studies to obtain more decisive data on the effects of dioxin exposure on sex ratios under defined experimental conditions.

The above mechanisms are based on sex as determined by the chromosomal constitution of the fetus. The hormonal environment of the mother during gestation also might modify expression of developing genitalia, which are the likely basis of assignment of sex to children at birth. That would not, however, correspond to the tendency for any suggestive observed effects to be associated with paternal exposure.

SUMMARY

Strength of the Evidence in Epidemiologic Studies

There is inadequate or insufficient evidence to determine an association between exposure to 2,4-D, 2,4,5-T, 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; altered sex ratio; and childhood cancers. There is limited or suggestive evidence for an association between spina bifida and exposure to the compounds of interest.

Overall Biologic Plausibility for Reproductive and Hormonal Effects

This section summarizes the general biologic plausibility of a connection between exposure to the compounds of interest 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 reproductive and developmental effects in laboratory animals. Effects on male and female reproductive organs are not always accompanied by adverse reproductive outcomes. The administration of TCDD to male animals elicits reproductive toxicity by affecting testicular and seminal vesicle weight and function and by decreasing the rate of sperm production. The mechanisms of those effects are not known, but a primary hypothesis is that they are mediated through effects on hormones. Exposure to TCDD has been accompanied by decreased concentrations of hormones such as gonadotropin and testosterone, which regulate sperm production. However, high doses of TCDD are required to elicit many of those effects. Furthermore, TCDD-exposed male rats

were able to sire viable fetuses. Many studies have examined the effects of TCDD on the female reproductive system. Abnormal follicle development and decreased numbers of ova have been observed. Although oocytes appear to be directly responsive to TCDD, effects on hormones, their metabolism, and the ability of hormones to act within the ovaries also are likely contributors to those effects. A recent study indicates that exposure of mice to TCDD during pregnancy disrupts mammary gland differentiation and lactation. 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 suggest that exposure to TCDD in utero could affect the body weight of newborn humans.

TCDD is teratogenic in mice, inducing cleft palate and hydronephrosis. Research indicates that coexposure with either of two other compounds, hydrocortisone or retinoic acid, synergistically enhances expression of cleft palate. The synergy suggests that the pathways controlled by those 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. Some evidence suggests that the endothelial lining of blood vessels is a primary target site of TCDD-induced cardiovascular toxicity. Cytochrome P450 1A1 induction or alterations in pathways controlled by vascular endothelial growth factor might mediate the early lesions that result in TCDD-related vascular derangements. That antioxidant treatment provides protection against TCDD-induced embryotoxicity in some systems suggests that reactive oxygen species might be involved in the teratogenic effects of exposure to TCDD. Several reports of studies in exposed animals and humans suggest that low perinatal exposure to TCDD and 2,4-D could impair brain development. Outcomes can be subtle, ranging from altered learning and memory to modified sex-related behavior. The mechanisms of those effects are unclear.

Studies in several rodent species show that administration of a single maternal dose of TCDD produces malformations of the external genitalia and functional reproductive alterations in female progeny, including decreased fertility rate, reduced fecundity, cystic endometrial hyperplasia, and disrupted estrus cycles. 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 produced 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, decreasing prostatic dihydrotestosterone concentrations, or interfering with androgen-signaling pathways. In utero exposure to TCDD also caused decreased seminal vesicle weight and branching, and it decreased sperm production and increased sperm transit time in male offspring.

Studies in female offspring of TCDD-exposed dams are few but demonstrate that in utero and lactational exposure can reduce fertility, decrease the ability to carry pregnancy to term, decrease litter size, increase fetal death, impair ovarian function, and decrease concentrations of estradiol and progesterone. Most of those effects could occur as a result of TCDD's general toxicity to the pregnant dam, however, and not as the result of any TCDD-specific mechanism. TCDD also induces changes in serum concentrations of reproductive hormones in immature female rats given TCDD by gastric intubation, partly because of the action of TCDD on the pituitary gland. As indicated above, some effects observed in the fetuses or offspring from TCDD-treated dams may be due to toxicity to the dams. However, it is clear that many effects of TCDD on development also occur at doses where there is no overt maternal toxicity.

The mechanism by which TCDD could exert reproductive and developmental effects is not established. Although the types of developmental effects reported in numerous toxicology experiments have been observed in highly exposed human populations, extrapolating results from animals to humans is difficult, because the factors that determine susceptibility to reproductive and developmental effects vary among species. TCDD has a variety of effects on growth regulation, hormone systems, and other factors associated with the regulation of activities in normal cells; those effects in turn could lead to reproductive or developmental toxicity.

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 to lead to changes in transcription; that is, to genes that are differentially regulated. Modulation of those genes could alter cell function.

Although structural differences in the AhR have been identified among species, it operates similarly 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 for 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. However, biologic plausibility for effects of TCDD on development in humans is also supported by several studies reporting effects on children exposed in utero to PCBs containing dioxin-like compounds. Furthermore, some of these effects were reported to occur at near background levels of exposure.

Little information is available on the reproductive and developmental effects of exposure to 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 was reduced, and their mortality increased (Charles et al., 1999); very high doses of 2,4-D and 2,4-DB were required to elicit those effects. Exposure to 2,4-D also alters 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. Picloram alone could 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; by the lack of strong evidence of organ-specific effects among species; and by differences in route, dose, duration, and timing of exposure. Experiments with 2,4-D and 2,4,5-T indicate that they have subcellular effects 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 those 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 can be used to predict human health outcomes and about the extent to which the health effects resulting from high-dose exposure can be extrapolated to low-dose exposure. The investigation of the biologic mechanisms that underlie TCDD's toxic effects continues to be an active field of research, and updates of this report could have more and better information on which to base conclusions, at least for TCDD.

Increased Risk of Disease Among Vietnam Veterans

The lack of data supporting an association of reproductive and developmental effects with exposure to the chemicals of interest, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

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8

Neurologic Disorders

Neurologic disorders include a wide variety of medical conditions. The nervous system can be divided anatomically and functionally into the central nervous system (CNS) and the peripheral nervous system (PNS). Distinguishing between CNS and PNS dysfunction is a useful starting point for understanding and evaluating neurologic disorders.

The CNS includes the brain and spinal cord. CNS disorders can be broadly divided into neurobehavioral disorders and movement disorders. Neurobehavioral disorders can involve cognitive syndromes, including memory problems, dementia, and Alzheimer's disease; and neuropsychiatric problems, including neurasthenia (a collection of such symptoms as difficulty in concentrating, headache, insomnia, and fatigue), post-traumatic stress disorder, anxiety disorder, depression, and suicide. Those disorders result from problems in the cerebral cortex or limbic system. Movement disorders, such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), involve weakness, tremors, involuntary movements, incoordination, or gait abnormalities. Those disorders result from problems in the basal ganglia, cerebellum, or spinal cord.

The PNS includes the spinal nerve roots that exit the spinal cord through the vertebral column, traverse the brachial and lumbar plexuses, and end in the peripheral nerves that connect with muscles, skin, and internal organs. PNS disorders are classified as various types of peripheral neuropathy, which can involve sensory changes, motor weakness, or autonomic instability. Those disorders result from problems in somatic or autonomic nerves or both.

Neurologic disorders also can be classified on the basis of anatomic distribution as either global or focal; on the basis of timing relative to exposure as early

or delayed onset; or on the basis of duration as transient or persistent. For example, global CNS dysfunction can lead to a general abnormality, such as an altered level of consciousness, whereas focal CNS dysfunction might lead to an isolated abnormality, such as difficulty with language function (aphasia). Early onset disorders are seen within days or weeks or exposure; delayed onset may occur after months or years. Transient disorders are short-lived; persistent disorders produce lasting deficits. Timing is important in assessing the effects of chemical exposure on neurological function and must be considered in the design and critique of epidemiologic studies. In the original Veterans and Agent Orange (*VAO*) report (IOM, 1994), attention was deliberately focused on persistent neurobehavioral disorders. Later reports—*Veterans and Agent Orange: Update 1996* (hereafter, *Update 1996* [IOM, 1996]), *Veterans and Agent Orange: Update 1998* (hereafter, *Update 1998* [IOM, 1999]), *Veterans and Agent Orange: Update 2000* (hereafter, *Update 2000* [IOM, 2001]) and *Veterans and Agent Orange: Update 2002* (hereafter, *Update 2002* [IOM, 2003])—and this report review data pertinent to all neurologic disorders.

Case identification in neurologic disorders is often difficult, because there are few disorders for which there are specific diagnostic tests. Many disorders involve cellular or molecular biochemical effects, so even the most advanced imaging techniques can miss an abnormality. Because the nervous system is not readily accessible for biopsy, pathologic confirmation usually is not feasible. Neurologic disorders are by their nature largely subjective; so there often is no objective evidence with which to confirm diagnosis.

Many studies have addressed the possible contribution of herbicides and pesticides to neurologic disorders. Studies relevant to this report investigated exposures from one of three general settings: in the workplace, from the environment, or during military service in Vietnam.

This chapter reviews the association between exposure to 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); 4-amino-3,5,6-trichloropicolinic acid (picloram); and cacodylic acid (dimethylarsenic acid or DMA) and neurobehavioral disorders, movement disorders, and peripheral neuropathy. The scientific evidence for biologic plausibility also is reviewed briefly. More complete discussions of the categories of association and this committee's approach to categorizing health outcomes are presented in Chapters 1 and 2. A more thorough discussion of biologic plausibility is found in Chapter 3. For studies new to this update that report only a single neurological health outcome and that are not revisiting a previously studied population, their design information is summarized with their results; the design information for all other new studies can be found in Chapter 4.

NEUROBEHAVIORAL DISORDERS (COGNITIVE OR NEUROPSYCHIATRIC)

Summary of *VAO*, *Update 1996*, *Update 1998, Update 2000,* and *Update 2002*

On the basis of the data available at the time, it was concluded in *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* that there was inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and neurobehavioral disorders. Much of the data that informed that conclusion came from the Air Force Health Study (AFHS, 1984, 1987, 1990, 1991, 1995, 2000), an ongoing, longitudinal study of a cohort of Air Force veterans (Ranch Hands) whose duties involved spraying pesticides during their service in Vietnam. *VAO* and the *Updates* offer more complete discussions of the AFHS protocols and results; a brief summary is included here. The AFHS study design and methods of exposure assessment, respectively, are discussed in Chapters 4 and 5 of this report.

The studies reviewed in *VAO* (IOM, 1994) revealed no association between serum TCDD concentration and reported sleep disturbance or variables from the Symptom Checklist-90-Revised (SCL-90-R). In contrast, serum TCDD was significantly associated with responses on some scales of the Millon Clinical Multiaxial Inventory (MCMI).

In *Update 2000* (IOM, 2001), results were reviewed from AFHS (2000). Some self-reported symptoms on a checklist (anxiety, hostility, obsessive–compulsive behavior, paranoid ideation, somatization, global severity index, other neuroses) were significantly more frequent in Ranch Hands, but associations for some of those variables were not significant after adjustment for covariates. In addition, a repeat psychological assessment was performed with SCL-90-R, and reported psychological disorders were verified through medical record review and combined with those obtained on previous examinations. Of the five categories of psychological diagnosis—psychosis, alcohol dependence, drug dependence, anxiety, and other neurosis—a dose–response pattern was found for serum TCDD concentration and "other neuroses" in the enlisted groundcrew. However, when the relationship between the serum TCDD and the psychological diagnoses was examined for all Ranch Hands, there were no significant results.

Three new studies were reviewed in *Update 2002* (IOM, 2003). Neuropsychological tests of cognitive functioning indicated significant group differences on some scales. However, the findings did not support a dose–response relationship with serum TCDD; poorer performance was seen in groups with background or low exposure, and the lower performance on only one memory test for one subgroup of subjects suggested a chance finding.

Uncertainty in interpreting results from the AFHS relates to variations in diagnostic approach, variable findings within subgroups on similar tests, and the lack of dose–response relationships with objective measures of exposure. The

committee has summarized data from many other studies; similar limitations have affected interpretation of those results, as described in *Update 1998*. Therefore, that committee maintained the conclusion that there has been inadequate or insufficient evidence of an association between exposure to the compounds of interest and neurobehavioral disorders (cognitive or neuropsychiatric).

Update of the Scientific Literature

Since *Update 2002* (IOM, 2003), five reports have investigated associations between neurobehavioral disorders (cognitive or neuropsychiatric) and possible exposure to the compounds of interest: an update of the AFHS (Barrett et al., 2003), a cross-sectional study of a cohort of Korean veterans who served in Vietnam (Kim et al., 2003), an update of an occupational cohort from the Czech Republic (Pelclova et al., 2002), a cohort study from the Bordeaux region of France (Baldi et al., 2003a), and a semi-ecological study from a community adjacent to a wood treatment plant (Dahlgren et al., 2003).

Psychological functioning was compared in Ranch Hand veterans and other Vietnam veterans (Barrett et al., 2003). The number of study participants changed depending on the year of examination, with a range of 921–953 Ranch Hands (roughly 75% participation rate) and 1,037–1,202 comparison subjects. Exposure was determined by serum dioxin measured in 1987 or 1992 and back-extrapolated to the final year of each subject's tour of duty, assuming a constant half-life for dioxin of 8.7 years. Ranch Hands were placed in background-, low-, and high-exposure groups (with the mean serum concentration marking the cutoff between low and high). The characteristics of the study groups indicated that those with high exposure were more likely to be younger enlisted personnel; those with background or low exposure were older officers. Two standard psychological test instruments were administered: the Minnesota Multiphasic Personality Inventory (MMPI) in 1982 and 1985, and MCMI in 1987 and 1992. MMPI results were inconsistent over time and showed no clearly significant associations with exposure. There was no association between dioxin concentration and PTSD symptoms as measured on the MMPI. MCMI results were essentially identical for all groups for both years, with a single exception—1992 results showed significantly elevated personality scores in the background-exposure group. Although this was a well-designed study, capitalizing on the opportunity to evaluate potential associations between objective measures of exposure and psychological functioning using validated test instruments, the conclusions are limited by the possibility of misclassification of exposure, selection bias, and uncontrolled confounding (by subjects' educational achievement). The authors quite reasonably conclude that there were "few consistent differences in psychological functioning" between groups based on serum dioxin concentrations.

Pelclova et al. (2002) published an updated description of neuropsychological test results from 12 members of a group of workers at a 2,4,5-T production

facility in the Czech Republic. Although a prior report (Pazderova-Vejlupka et al., 1981) indicated significant correlations between TCDD concentrations and cognitive-test results (see *Update 2002*), test scores were higher at the follow-up examination and the correlations were no longer significant when the cognitive tests were repeated in 2001 (Peclova et al., 2002). Previous publications from that group were reviewed in *VAO* and *Update 2002*, which identified significant methodologic problems in selection bias and lack of control for confounding by educational achievement, tobacco use, or alcohol use. An essential limitation is the lack of a comparison group, which precludes any causal inference.

A cross-sectional study of Korean veterans who served in Vietnam described a variety of health outcomes (Kim et al., 2003). The subjects were recruited from a roster of the Korean Ministry of Patriots and Veterans Affairs, with participation rates for Vietnam veterans of 27.6% and for a comparison group of non-Vietnam-veteran pensioners of 5.7%. The demographic characteristics of the participating Vietnam veterans were significantly different both from the source population and from the comparison group. Participants were older and had served in Vietnam longer than had non-participants. Among the participants, Vietnam veterans were younger, less likely to be married, less likely to smoke tobacco or drink alcohol, and less likely to have advanced education than were non-Vietnam-veteran comparison subjects. Participants were assigned to one of four Agent Orange exposure categories, based on a combination of self-report of personal exposure and duty within specific geographic–military regions. Further details on the exposure assessment can be found in Chapter 5 of this report.

Health outcomes were assessed by a group of four family practitioners, blinded to subjects' exposure status, using a "standardized comprehensive clinical investigation." Associations between exposure and health outcome were estimated using χ-square tests or multiple linear regression to control for potential confounders. There was a significantly higher prevalence of PTSD and mood disorder in Vietnam veterans than in the non-Vietnam-veteran comparison group; although the association was not significant after controlling for multiple potential confounders, and it did not differ by exposure in Vietnam veterans. The study is limited because of the possibility of selection bias. There is also a chance of residual confounding because of the demographic differences between groups, although the authors appropriately included potential confounders in their statistical models. The study does not provide evidence for a significant association between exposure to the compounds of interest and neurobehavioral disorders.

The Bordeaux study (Baldi et al., 2003a) focused on a cohort of 2,792 persons over age 65, enrolled in 1987 for the purposes of studying normal and pathological cerebral aging and loss of independence in the elderly. By the time of the 5-, 8-, and 10-year follow-ups, the cohort had decreased to 1,507, 1,118, and 1,026 persons, respectively. Exposures were categorized into quartiles by the likelihood of occupational use of chemical pesticides on the basis of self-reports,

which introduce the possibility of recall bias. The high drop-out rate raises concerns of selection bias. The authors also could not identify exposure to specific compounds, although fungicides were implicated as the most heavily used compounds in grape cultivation in Bordeaux. It is unlikely that those exposures were comparable to herbicide exposures in Vietnam, so the study offers no evidence that would implicate the compounds of interest.

The final study used a semi-ecological design to assess the possibility that self-reported symptoms suggesting neurobehavioral disorders in a group of people from eastern Mississippi were related to residence near a creosote treatment plant (Dahlgren et al., 2003). The study suffers from design weaknesses, including selection and ascertainment bias, lack of objective exposure data, and lack of physician-confirmed diagnoses; its design is not suited to address the presence of an association.

Synthesis

There is no consistent evidence for any association between neurobehavioral disorders (cognitive or neuropsychiatric) and Agent Orange exposure. Difficulties in case identification and diagnosis, misclassification of exposures because of a lack of contemporaneous measures, subject ascertainment and selection bias, and uncontrolled confounding from many comorbid conditions are common weaknesses in the studies reviewed. The variability of the test results over time, the weak and inconsistent associations, and a lack of consistent dose–response relationships, also prevent those studies from supporting an association between the exposures of interest and neurobehavioral disorders.

Conclusion

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiological evidence reviewed here and in previous *VAO* reports, the committee concludes that there is still inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and neurobehavioral disorders (cognitive or neuropsychiatric).

Biologic Plausibility

No new animal studies are relevant to the compounds of interest and neurobehavioral disorders (cognitive or neuropsychiatric). A summary of biologic plausibility is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and neurobehavioral disorders, including cognitive and neuropsychiatric, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

MOVEMENT DISORDERS

This section summarizes the data from previous *VAO* reports and updates the scientific literature on movement disorders, including PD and ALS.

Parkinson's Disease and Parkinsonism

PD is a progressive neurodegenerative disorder that affects millions of people worldwide. Its primary clinical manifestations are bradykinesia, resting tremor, cogwheel rigidity, and gait instability. These signs were first described in 1817 as a single entity by James Parkinson, who believed that severe fright from a traumatic experience was a probable cause. Despite nearly two centuries of investigation, the true causes of the disease remain enigmatic, and the diagnosis still relies on a characteristic constellation of signs from clinical neurologic examination. Unfortunately, the signs are not pathognomonic; they are seen in other disorders, including parkinsonism resulting from syndromes that are virtually indistinguishable from PD. Ultimately, a diagnosis of PD can be confirmed with postmortem pathologic examination of brain tissue for the characteristic loss of neurons from the substantia nigra and telltale Lewy body intracellular inclusions. Pathology findings in other causes of parkinsonism show different patterns of brain injury.

Estimates of population-based incidence for PD range from 2 to 22 per 100,000 person-years, and estimates of prevalence range from 18 to 182 per 100,000 person-years (both age adjusted to the 1970 US census). That makes PD the second most common neurodegenerative disease (after Alzheimer's disease). Age is the only definite risk factor for PD; peak incidence and prevalence are consistently found in the seventh or eighth decades of life.

Heredity has long been suspected as a primary risk factor for PD, and identification of the evidence for genetic transmission has accumulated over the past decade, marked by the determination of specific mutations in two genes, Parkin and α-synuclein. However, it has become clear that simple Mendelian transmission can account only for some rare forms of familial and young-onset PD.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

Based on growing concerns about a possible link between PD and pesticide exposures, the original committee suggested that attention be paid to the pattern

of new cases in exposed and non-exposed Vietnam veterans, especially as they entered the decades during which PD becomes more prevalent. That recommendation was echoed in each subsequent *Update.* It was noted that many published studies have used similar methodology, with diagnostic criteria based either on clinical signs of PD or on *International Classification of Diseases*, Ninth Edition (ICD-9) diagnostic coding from death certificates or hospital admission records. Usually, pesticide exposure was considered relevant only when it occurred before disease onset, although the specific timing relative to onset usually was not clear.

The *Update 1996* and *Update 1998* committees considered the detection of early-onset cases to be vital to test the hypothesis that cases are related to a toxic exposure.

The *Update 2000* committee noted that most studies have grouped cases of all ages; those that have separated early-onset cases have yielded inconsistent results (Butterfield et al., 1993; Stern et al., 1991). Estimates of relative risk have been quite inconsistent: Five studies demonstrate positive associations (Butterfield et al., 1993; Gorell et al., 1998; Liou et al., 1997; Seidler et al., 1996; Semchuk et al., 1992), two demonstrate negative associations (Kuopio, 1999; Stern et al., 1991), and one shows no association (Taylor et al., 1999). A meta-analysis indicated significant heterogeneity among the published work (Priyadarshi et al., 2000). Evidence for a dose–response relationship was limited: only one study (Gorell et al., 1998) demonstrated an increased incidence of PD with increasing dose as measured by duration of exposure.

Update 2002 reviewed reports of two cohort studies (Engel et al., 2001; Petrovich et al., 2002), the results of which were similar to those of the many other studies reviewed for earlier volumes. Lengthy agricultural occupation was associated with parkinsonism in many reports; however, the results did not show consistent dose–response trends, and no association was identified for any individual compound or class of pesticides.

None of the studies has described specific exposures to the compounds of interest. Table 8-1 summarizes the relevant studies.

Update of the Scientific Literature

Since *Update 2002* (IOM, 2002), three reports have examined the possible association between PD and pesticide exposures: one (Baldi et al., 2003a) was described above (see the section on neurobehavioral disorders); the second is a nested case–control study related to the Bordeaux cohort (Baldi et al., 2003b); the third comes from a Belgian case–control study that examined associations with a variety of environmental factors, including exposure to pesticides (Pals et al., 2003).

In the Bordeaux cohort study (Baldi et al., 2003a), incident (new) cases at the 8- and 10-year follow-up were identified by self-report in response to the question, “Do you have Parkinson’s disease?” Prevalent (existing) cases of PD were excluded at the time of enrollment and after 5 years of follow-up; for those cases,

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
Baldi et al., 2003a; France	585 men (age > 70 years)		Questionnaire—detailed occupational histories	+	Occupational pesticides (mostly fungicides) 5.6 (1.5–21.6)	Self-report at 8 and 10 year follow-ups
Baldi et al., 2003b; France	84 (age > 70 years)	252 (age > 70 years)	Interview –Occupational history coded by experts –Residential history	+	Occupational pesticides (mostly fungicides) 2.2 (1.1–3.4)	UK PD Society Brain Bank clinical criteria
Pals et al., 2003	423	205	Questionnaire–occupational history not interpreted with respect to pesticide use			Neurologic exam
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 exam
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 exam by trained nurse

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
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
Fall et al., 1999; Sweden[b]	113	263	Questionnaire—any job handling pesticides		Pesticides 2.8 (0.9–8.7)	Neurologic exam
Kuopio et al., 1999; Finland	123 (onset of PD before 1984)	279	Interview—pesticides or herbicides regularly or occasionally used		Regular use of herbicides 0.7 (0.3–1.3)	Neurologic exam
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 exam
Chan et al., 1998; Hong Kong[b]	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 exam

continues

TABLE 8-1 *Continued*

Reference and Country	Study Group	Comparison Group	Exposure Assessment	Significant Association with Pesticides	OR (95 % CI)	Neurologic Dysfunction
Gorrell et al., 1998; US[b]	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
Hubble et al., 1998; US	3 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 exam
McCann et al., 1998; Australia[b]	224	310	Questionnaire—daily or weekly exposure to industrial herbicides and pesticides >6 months		Herbicides or pesticides 1.2 (0.8–1.5)	Neurologic exam
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
Smargiassi et al., 1998; Italy[b]	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

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 exam
Schulte et al., 1996; US[c]	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 exam
Chaturvedi et al., 1995; Canada[b]	87 (age > 64 years)	2,070	Survey—exposure positive if frequently used		Pesticides 1.8 (0.9–3.4)	History of PD
Hertzman et al., 1994; Canada[b]	127	245	Interview—occupation with probable pesticide exposure	+	Pesticides in men 2.3 (1.1–4.9)	Neurologic exam
Morano et al., 1994; Spain[b]	74	148	Interview—direct and indirect exposure to pesticides		Pesticide 1.73 (1.0–3.0)	Neurologic exam

continues

TABLE 8-1 *Continued*

Reference and Country	Study Group	Comparison Group	Exposure Assessment	Significant Association with Pesticides	OR (95 % CI)	Neurologic Dysfunction
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
Hubble et al., 1993; US[b]	63	76	Questionnaire—pesticide or herbicide use 20 days per year for >5 years	+	Pesticide or herbicide 3.4 (1.3–7.3)	Neurologic exam
Jimenez-Jimenez et al., 1992; Spain[b]	128	256	Interview—exposure: applied pesticides, or lived and ate vegetables where pesticides use		Pesticide 1.3 (0.9–2.1)	Standard criteria of PD by history
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 exam
Stern et al., 1991; US[b]	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)	Standard criteria of PD by history

					Insecticide—young onset 0.6 (0.2–1.7) Insecticide—old onset 0.8 (0.3–2.1)	
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[b]	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 exam
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 exam
Hertzman et al., 1990; Canada	57	122	Questionnaire—ever worked in an orchard	+	Working in orchards 3.7 (1.3–10.3)	Neurologic exam
Koller et al., 1990; US[b]	150	150	Interview—acre-years = acres multiplied by years of herbicide or pesticide used		Herbicide or pesticide use 1.1 (0.9–1.3)	Neurologic exam

continues

TABLE 8-1 *Continued*

Reference and Country	Study Group	Comparison Group	Exposure Assessment	Significant Association with Pesticides	OR (95 % CI)	Neurologic Dysfunction
Ho et al., 1989; Hong Kong[b]	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 exam
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 exam

[a] Modified from Le Couteur et al. (1999).
[b] Studies used in meta-analysis (Priyadarshi et al., 2000).
[c] Previously quoted in *Update 1996* or *Update 1998*.
ABBREVIATIONS: PMR, proportionate mortality ratio.

diagnosis was based on an initial screening questionnaire and subsequently verified by a neurologist. The incidence for exposed and unexposed subjects, respectively, was estimated at 8.9 and 4.1 cases per 1,000 person-years. The results do suggest increased risk to men with occupational exposure to pesticides, but the use of fungicides in vineyards predominated, rather than any of the compounds of interest with respect to Vietnam veterans. Other design flaws limit inferences regarding associations.

The case–control study from Bordeaux (Baldi et al., 2003b) compared 84 subjects over age 70 with PD who had been recruited from hospital-based specialty clinic practices with a control group of 252 subjects without PD, identified from the previously described cohort. There is no evidence from that study to implicate the compounds of interest to Vietnam veterans.

In a cross-sectional case–control study from Belgium (Pals et al., 2003), numerous historical exposures were compared for case subjects with PD and spouse controls. Relevant to the committee's charge was the assessment of pesticide exposure; however, no data are presented on herbicides in general or specifically for any of the compounds of interest.

There is no evidence in any of the studies for an association between PD and exposure to the compounds of interest.

Synthesis

Epidemiologic studies have pursued a variety of environmental exposures as potential risk factors for PD, and pesticide use among those receiving the most attention. It has rarely been possible to isolate the effects of selected chemical herbicides, because exposures often are mixed and assessments usually are retrospective, relying on such broad categories as "ever…exposed to any pesticide." In addition, reported associations have been inconsistent and only rarely has there been evidence for dose–response relationships. Thus, the data are weakened for the committee's purposes by persistent limitations in methodology and by the lack of specificity for the compounds of interest.

Conclusions

Strength of Evidence from Epidemiologic Studies On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there remains inadequate or insufficient evidence of an association between exposure to the compounds of interest and PD.

Biologic Plausibility Interest in possible environmental causes of PD was increased by the observation that 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (MPTP) poisoning induces a movement disorder that recapitulates the classic features of PD. The effects of MPTP and its bioactive metabolite MPP+ have

become well-known, and MPTP toxicity has become the preeminent animal model for PD research. Although it is notable that the chemical structure of MPP+ is quite similar to that of paraquat (a commonly used herbicide), it is quite different from the compounds of interest in this report.

Increased Risk of Disease Among Vietnam Veterans The lack of data on the association between exposure to the chemicals of interest and PD, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

Amyotrophic Lateral Sclerosis

ALS is a progressive, adult-onset, motor neuron disease that presents with muscle atrophy, weakness, and fasciculations. Most cases of ALS are sporadic; only 5–10% of cases are familial. The annual incidence of sporadic ALS is 1–2 per 100,000 person-years, and the incidence of ALS peaks between 55 and 75 years of age (Brooks, 1996). One-fifth of familial-ALS patients have mutations in the gene-encoding superoxide dismutase-1 (Rosen et al., 1993). A specific diagnostic test does not exist, but clinical diagnosis has a high degree of accuracy (Rowland, 1998; Rowland and Shneider, 2001).

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

ALS was first considered by the committee for *Update 2002*. Table 8-2 summarizes research on the possible association between ALS and pesticide exposures. The research has covered heavy metal exposure (McGuire et al., 1997; Roelofs-Iverson et al., 1984); occupational exposure in chemical plants (Deapen and Henderson, 1986; McGuire et al., 1997); exposure to animal carcasses (Hanisch et al., 1976); the effects of heavy manual labor (Breland and Currier, 1967); working with electricity (Deapen and Henderson, 1986; Savettieri et al., 1991); working with pneumatic tools (Gallagher and Sanders, 1987; Savettieri et al., 1991); working in the plastic industry (Deapen and Henderson, 1986); and working as a truck driver (Kurtzke and Beebe, 1980).

Pesticide exposure has been associated with increased risk of ALS, including a twofold increased risk from long-term occupational exposure to pesticides (Deapen and Henderson, 1986), a threefold increased risk from exposure to agricultural chemical products (Savettieri et al., 1991), and a threefold increased risk from exposure to herbicides (McGuire et al., 1997), although none of those risk estimates was statistically significant.

A population-based case–control study demonstrated associations between exposure to agricultural chemical products and ALS in men, with a statistically significant 2.4-fold increased risk, a statistically significant trend by duration of exposure, and a 4.4-fold increased risk from heavy exposure in accidents or spills

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

(McGuire et al., 1997). A mortality study of Dow Chemical Company employees exposed to 2,4-D included 3 deaths from ALS, with a significantly increased mortality rate of 3.45 (Burns et al., 2001).

Although the findings were intriguing, the committee was concerned about the small number of subjects involved.

Update of the Scientific Literature

No relevant epidemiologic studies have been published since *Update 2002*.

Synthesis

As with PD, epidemiologic studies of ALS have pursued a variety of environmental exposures as potential risk factors. Among those receiving the most attention have been pesticides. It has rarely been possible to isolate the effects of selected herbicides, as exposures often have been mixed and assessment usually has been retrospective, relying on such broad categories as "ever…exposed to any pesticide." Reported associations have been inconsistent and only rarely has there been evidence for dose–response relationships. Thus, although there is some evidence that exposure to pesticides might be associated with increased risk for ALS, those data are weakened for the committee's purposes by persistent methodologic limitations and by the lack of specificity for the compounds of interest.

Conclusions

Strength of Evidence from Epidemiologic Studies On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there remains inadequate or insufficient evidence of an association between exposure to the compounds of interest and amyotrophic lateral sclerosis.

Biologic Plausibility There were no new animal studies that were relevant to the compounds of interest and ALS. A summary of biologic plausibility is presented at the end of this chapter.

Increased Risk of Disease Among Vietnam Veterans The lack of data on the association between exposure to the chemicals of interest and ALS, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

PERIPHERAL NEUROPATHY

Peripheral neuropathy consists of disorders of the PNS. Manifestations of this syndrome can include a combination of sensory changes, motor weakness, or autonomic instability. Clinically, various forms of peripheral neuropathy can be characterized by the distribution of nerve abnormalities and their patterns of progression. For example, a peripheral neuropathy resulting from toxic exposure usually affects the limbs in a symmetric pattern, beginning distally (in the toes) and moving proximally (toward the spine), providing the basis for the term "dying-back neuropathy," now more rigorously referred to as "distal axonopathy." Thus, sensory deficits at the ankle generally occur after deficits in the toes. Physiologically, various forms of peripheral neuropathy can be characterized by results from electrodiagnostic testing to indicate which neural structures are affected. Most toxin-induced neuropathies involve injury to the nerve cell bodies (neurons) or nerve fibers (axons), which produce changes in the amplitude of a nerve's response to an electrical stimulus. In severe cases, there also can be slowing in the speed of nerve impulses. This contrasts with the prominent slowing of nerve conduction velocity (NCV) resulting from injury to myelin, as seen with inflammatory conditions such as Guillain-Barré syndrome.

The clinical appearances of several peripheral neuropathies can be virtually identical, so it is often difficult to determine whether a peripheral neuropathy is caused by a toxic exposure. Sometimes there are clues from particular features of the clinical history and presentation that suggest toxic exposure, but complaints of peripheral nerve disorders often occur in isolation and are monotonously similar, making etiologic determination difficult. As many as 30% of cases are "idiopathic," having no etiology determined despite exhaustive clinical evaluation.

The most common toxin-induced neuropathy occurs as a result of chronic alcohol exposure. Peripheral neuropathy also occurs commonly as a complication of diabetes, with a prevalence of up to 50% reported in people with chronic diabetes. It is important to include assessment of alcohol use and diabetes as covariates in epidemiologic studies, because the neuropathies that are related to those conditions are clinically and physiologically indistinguishable from other toxin-induced neuropathies.

Clinically, in cases of toxin-induced peripheral neuropathy, stabilization or improvement is the rule after exposure ends. Recovery might not be complete, however, and the degree of recovery can depend on the severity of the initial deficits. Furthermore, there is the possibility of "subclinical" effects, and a person might be unaware of symptoms, although evidence for nerve dysfunction can be found in a detailed neurologic examination or through electrodiagnostic testing.

In *VAO*, peripheral neuropathy was considered a single category of disease. Before revising the conclusion regarding neuropathies, the committee for *Update 1996* subdivided them into "acute and subacute" or "chronic" classifications (on the basis of when an outcome occurs relative to exposure). In this section of this

report, however, the terms "acute" (brief) and "chronic" (prolonged or protracted) describe the time course of toxin exposure. "Early" and "delayed onset" are used to describe the time course of the neuropathy. The distinction between "transient" and "persistent" is not always clear, because recovery may be incomplete. The committee considers a neuropathy to be early onset and transient if abnormalities appear and resolve within two years after cessation of external exposure.

Summary of *VAO*, *Update 1996*, *Update 1998, Update 2000*, and *Update 2002*

VAO and subsequent *Updates* noted that the literature on peripheral neuropathy has been difficult to integrate because it is characterized by variable methodologies that lack uniform operational definitions. The techniques used to identify affected persons, to define comparison populations, and to assess exposures differ considerably among studies. Also, many of the studies are limited by non-random selection, which raises concern for bias, and by the relatively small number of participants, which decreases confidence of risk estimates and limits the power to detect a true association. There have been variable results, with some studies demonstrating abnormalities of peripheral nerve function and others not.

In *VAO*, the committee reviewed results from four occupational-cohort studies of workers previously exposed to the compounds of interest (Moses et al., 1984; Singer et al., 1982; Suskind and Hertzberg; 1984; Sweeney et al., 1993). Singer et al. (1982) reported slowed NCVs in 2,4-D and 2,4,5-T production workers who were examined 2 months after exposures were reduced. In former 2,4,5-T production workers with a history of chloracne (10 years after last exposure), Moses et al. (1984) found diminished pin-prick sensation, but Suskind and Hertzbert (1984) did not find differences in NCVs. Similarly, Sweeney et al. (1993) reported decreased pin-prick sensation, but no differences in NCVs in former herbicide production workers (evaluated 15 years or more after their last exposure).

VAO also reviewed epidemiologic studies of populations potentially exposed to TCDD in the environment. A series of studies from Italy evaluated peripheral neuropathy in the population from Seveso after the industrial accident on July 10, 1976 (Assennato et al., 1989; Barbieri et al., 1988; Boeri et al., 1978; Filippini et al., 1981; Gilioli et al., 1979). Boeri et al. (1978) reported more frequent symptoms and signs of neuropathy in a cohort of residents living in the contaminated area than in a comparison group who were last examined 7–10 months after the explosion. There was no statistical difference in conduction velocity between groups. Gilioli et al. (1979) noted electrodiagnostic abnormalities in laboratory technicians potentially exposed to TCDD from analytical samples; however, the technicians also were exposed to solvents used in the analytical process. Fillipini et al. (1981) reported an increased prevalence of peripheral neuropathy in Seveso residents with evidence of high exposure to TCDD (chloracne or liver enzyme abnormalities) who were last examined 21 months after the accident. Barbieri et

al. (1988) reported a higher rate of abnormalities on neurologic examination and electrodiagnostic testing in subjects with a history of chloracne who were examined 6 years after the accident, but there was no significant increase in peripheral neuropathy as defined by World Health Organization (WHO) criteria. Assennato et al. (1989) described electrodiagnostic evaluation of that group 9 years after the accident; no differences were observed in NCV or neuropathy as defined by WHO criteria. Other environmental studies reviewed in *VAO* were of Missouri residents potentially exposed to TCDD in the early 1970s when waste oil was sprayed to control dust (Hoffman et al., 1986; Stehr et al., 1986; Webb et al., 1987). Although more frequent sensory abnormalities were reported in potentially exposed subjects, the differences were not statistically significant, and the semi-ecological study design was not suited to causal inference. Some of the data from epidemiologic studies of environmental exposures have suggested increased risk of peripheral nerve abnormalities, but evidence of an association between exposure to the compounds of interest and peripheral neuropathy is inconsistent.

Studies of Vietnam veterans were also reviewed in *VAO* (AFHS, 1984, 1987, 1991; CDC, 1988). A Centers for Disease Control and Prevention study (CDC, 1988) focused on service in Vietnam, not on exposure to the compounds of interest, and it therefore provided no evidence for the possible effects of exposures. There was no indication of increased risk of peripheral neuropathy from the first Ranch Hand reports (AFHS, 1984, 1987, 1991). No evidence of an association between exposure and peripheral neuropathy in Vietnam veterans was derived from the studies reviewed in *VAO*.

Update 1996 reviewed two new epidemiologic studies. Using an administrative database, Zober et al. (1994) found no evidence of increased medical service utilization for diagnosis of peripheral neuropathy in workers previously exposed to TCDD at a BASF plant. Decoufle et al. (1992) reported no association between self-reported exposure to herbicides in Vietnam and peripheral neuropathy. The limitations of those studies were such that they did not confirm or refute a possible relationship between exposure and neuropathy.

In addition, the committee responsible for *Update 1996* reviewed case reports that described peripheral neuropathy after exposures to the compounds of interest (Berkley and Magee, 1963; Goldstein et al., 1959; Todd, 1962). In each instance, the peripheral neuropathy improved gradually, but had not resolved completely even after several months or years. The possibility cannot be entirely excluded that the five cases reported in these publications were unrelated to herbicide exposure and represented examples of other disorders, such as idiopathic Guillain-Barré syndrome. The committee also considered several supportive animal models (Grahmann et al., 1993; Grehl et al., 1993; see section on biological plausibility). The committee concluded that there was limited or suggestive evidence of an association between exposure to the compounds of interest and early-onset transient peripheral neuropathy.

Update 1998 reviewed no new studies. The context for the issue of peripheral

neuropathy, its relationship with toxic exposures, and the occurence of diabetes mellitus was discussed. In particular, it was noted that neuropathy is a common consequence of diabetes. That was particularly relevant, and the committee issued a special report a year later concluding that there was limited or suggestive evidence of an association between diabetes and exposure to Agent Orange.

Update 2000 reviewed the most recent Ranch Hand report available at that time (AFHS, 2000), which combined signs of peripheral neuropathy to produce increasingly specific, graded indexes of neuropathy—a common approach in epidemiologic studies. Ranch Hand subjects were significantly more likely than were comparison subjects to have abnormalities in the indexes, and the prevalence of abnormalities increased with dioxin concentration. Although the clinical relevance of epidemiologic indexes of neuropathy is never certain, the strong associations described between the indexes and the conditions known to produce peripheral neuropathy, such as diabetes and alcohol use, supported their validity in this study. The AFHS investigators included those conditions as potential confounders in the statistical analysis. However, the effect of diabetes could not be eliminated in the most specific neuropathy index, because there were not enough non-diabetic subjects. It therefore was impossible to estimate the association between dioxin exposure and neuropathy, absent any effect of diabetes.

The committee responsible for *Update 2002* considered one peer-reviewed article that described the peripheral neuropathy data from the Ranch Hand cohort (Michalek et al., 2001). In primary analysis, the investigators had included diabetes as a potential confounder in the statistical model. In a secondary analysis, subjects with conditions that were known to be associated with neuropathy were excluded, and subjects with diabetes were enumerated. In both sets of analyses, there were strong and significant associations between possible and probable neuropathy and dioxin concentration, and significant trends were found with increasing concentrations of dioxin. However, there were too few non-diabetic subjects to produce meaningful estimates of risk, absent the contribution of diabetes. Thus, questions remained about the specific association between exposure to the compounds of interest and peripheral neuropathy absent any effect of diabetes.

Update of the Scientific Literature

Peripheral neuropathy was one outcome considered in the study of Korean Vietnam veterans (Kim et al., 2003; see the section on neurobehavioral disorders and Chapter 4 for a description of the study). It was significantly more common in Vietnam veterans than in non-Vietnam veterans, with a 2.4-fold risk (95% CI, 1.04–5.48) even after controlling for alcohol use and age, although there were no differences based on estimated TCDD exposure within subgroups of Vietnam veterans. Diabetes was also more common in Vietnam veterans. The authors concluded that there was an excess frequency of peripheral neuropathy in Vietnam veterans.

Although the study groups differed in terms of known risk factors for neuropathy, including age, alcohol use, and diabetes, the authors controlled for possible confounding by including age and alcohol use in the statistical analysis. It is not clear how diabetes was handled, but the report distinguishes between "peripheral neuropathy," for which there was a significant difference between groups, and "neuropathy with diabetes," which was not significantly different between the groups. The possibility of selection bias is a concern—only 28% of eligible Vietnam veterans participated in the study and participation may have been related to health status. Therefore, the study provides some evidence of an association between service in Vietnam and peripheral neuropathy, although the weight of the results is limited because of the study's limitations. The study does not provide evidence for an association between specific exposure to the compounds of interest and peripheral neuropathy.

Synthesis

Over the past 50 years, a body of literature has accumulated suggesting an association between the compounds of interest and peripheral neuropathy. Past committees have concluded that there is evidence for an association between "acute and subacute transient" peripheral neuropathy and exposure to at least one compound of interest (*Update 1996*). However, there remained questions about whether there was evidence for an association with persistent neuropathy.

Human case reports have documented peripheral neuropathy after acute exposure to large amounts of 2,4-D as shown by neurologic examination and electrodiagnostic testing. Reports have indicated eventual symptom stabilization and improvement, but sensory and motor deficits have persisted in some people for months or years after the exposure ended.

Several epidemiologic studies have reported increased risk of peripheral neuropathy in populations exposed to the compounds of interest in a variety of occupational and environmental settings. However, the literature is inconsistent and has suffered from methodological limitations. The most dramatic exposures have involved industrial accidents that caused environmental contamination, such as the one in Seveso, Italy, in 1976. Studies of residents in that region have shown early onset neuropathy and subclinical abnormalities in some subjects have been demonstrated using electrodiagnostic testing.

Epidemiologic studies, using appropriate comparison groups and standard techniques for diagnosis and assessment of exposure, have not demonstrated consistent associations between exposure to the compounds of interest and the development of peripheral neuropathy. Several reports have shown no significant association, and for the reports that did indicate an association, chance, bias, or confounding could not be ruled out with confidence. In particular, it is possible that diabetes confounds the results, because many of the subjects with neuropathy also had diabetes, and diabetes is a known cause of neuropathy. Controlling for

the effects of diabetes is a technical challenge because there is evidence for an association between diabetes and exposure to at least one of the compounds of interest (IOM, 2003); in many cases, diabetes could be in the causal pathway that links exposure and peripheral neuropathy.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to the compounds of interest and early onset transient peripheral neuropathy.

However, on the basis of its evaluation of the epidemiologic evidence reviewed here and previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and delayed or persistent peripheral neuropathy.

Biologic Plausibility

Toxicology experiments have demonstrated adverse effects of the compounds of interest on nerve cells at a molecular and cellular level; many common mechanisms of neurotoxicity are involved. Neuronal cell cultures treated with 2,4-D showed decreased neurite extension associated with intracellular changes, including a decrease in microtubules, inhibition of the polymerization of tubulin, disorganization of the Golgi apparatus, and inhibition of ganglioside synthesis. Those mechanisms are important for maintaining synaptic connections between nerve cells and supporting the mechanisms involved in axon regeneration during recovery from peripheral neuropathy. Whole-animal experiments have demonstrated that TCDD treatments affect the fundamental molecular events that underlie neurotransmission. Grahmann et al. (1993) and Grehl et al. (1993) reported on abnormalities in electrophysiology and pathology, respectively, observed in the peripheral nerves of a set of rats treated with TCDD. Those treatments did not produce a wasting syndrome, nor was there evidence of general organ system failure. When the animals were sacrificed 8 months after exposure, there was pathologic evidence for persistent axonal nerve damage and histologic findings typical of toxin-induced injury. Those results provide evidence for the biologic plausibility of an association between peripheral neuropathy and exposure to the compounds of interest. Replicating and extending these findings would strengthen the observation and could provide additional insight regarding pathophysiologic mechanisms.

Increased Risk of Disease Among Vietnam Veterans

The lack of data supporting an association between exposure to the chemicals of interest and peripheral neuropathy, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

SUMMARY

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest (2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid) and neurobehavioral disorders (cognitive or neuropsychiatric) or movement disorders (PD or ALS). The evidence regarding association is drawn from studies of Vietnam veterans and from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components.

In *Update 1996*, the committee stated that there was limited or suggestive evidence of an association between exposure to at least one of the compounds of interest and "acute and subacute transient" peripheral neuropathy. The evidence 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 *Update 2002* supported that conclusion. In this report, the committee concludes that there is limited or suggestive evidence of an association between exposure and "early onset, transient" peripheral neuropathy.

The committee also concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and "delayed or persistent" peripheral neuropathy. That conclusion is based on evaluation of the accumulated epidemiologic evidence described here, some of which was detailed in earlier *VAO* reports. The evidence is drawn from epidemiologic studies of Vietnam veterans, from occupational studies, and from other studies in which subjects were exposed to at least one compound of interest.

Biologic Plausibility

This section summarizes the biologic plausibility of a connection between exposure to the compounds of interest and various neurologic disorders, on the basis of data from experimental studies (see Chapter 3 for a more detailed discussion).

The effects of TCDD are mediated by interaction with the AhR, a protein

found in animal and human cells. The AhR complex is known to bind DNA and produce changes in transcription, thereby influencing genetic function. The AhR complex can produce an array of molecular effects that influence cell growth, hormone regulation, and normal cellular metabolism. Although some structural differences have been identified in the AhRs of different species, the AhR is functionally similar across species. Therefore, data from animal studies can be used to support the biologic plausibility of human neurotoxicity.

Basic scientific studies have emphasized the importance of alterations in neurotransmitter systems as potential mechanisms that underlie TCDD-induced neurobehavioral disorders. Neuronal cultures treated with 2,4-D exhibited decreased neurite extension associated with intracellular changes, including a decrease in microtubules, inhibition of the polymerization of tubulin, disorganization of the Golgi apparatus, and inhibition of ganglioside synthesis. Those mechanisms are important for maintaining the connections between nerve cells that are necessary for neuronal function and that are involved in axon regeneration and recovery from peripheral neuropathy. Animal experiments have demonstrated that TCDD treatments affect the fundamental molecular events that underlie neurotransmission initiated by calcium uptake. Mechanistic studies have demonstrated that 2,4,5-T can alter cellular metabolism and cholinergic transmission necessary for neuromuscular transmission.

TCDD treatment produces peripheral neuropathy in rats. Treatment at doses that do not cause general systemic illness or wasting disease produces electrodiagnostic changes in peripheral nerve function and pathologic findings that are characteristic of toxin-induced axonal peripheral neuropathy.

The foregoing evidence demonstrates biologic plausibility for a connection between Agent Orange exposure and neurotoxic effects in humans. Experiments with 2,4-D, 2,4,5-T, and TCDD indicate toxic effects on nerve cells that are molecular and cellular, demonstrating evidence for common mechanisms of neurotoxicity. Experiments with TCDD in whole animals demonstrate specific effects on the PNS at doses that do not cause general systemic illness. As discussed in Chapter 3, extrapolation of those observations to humans is complicated by differences in sensitivity and susceptibility among animals, strains, and species; by the lack of strong evidence of organ-specific effects across species; and by differences in route, dose, duration, and timing of chemical exposures. Thus, although the observations in themselves cannot support a conclusion that Agent Orange produces neurotoxic effects in humans, the studies provide evidence for the biologic plausibility of an association.

Increased Risk of Disease Among Vietnam Veterans

The inadequacy of data supporting an association of neurological effects (other than early onset, transient peripheral neuropathy) with exposure to the

chemicals of interest, coupled with the lack of exposure information on Vietnam-eterans, precludes quantification of any possible increase in their risk.

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9

Other Health Effects

This chapter discusses data on the possible association between exposure to 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 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD], a contaminant of 2,4,5-T) and the following non-cancer health outcomes: chloracne, porphyria cutanea tarda (PCT), 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. For studies new to this update that report only a single health outcome and that are not revisiting a previously studied population, their design information is summarized with their results; the design information for all other news studies can be found in Chapter 4. Within health outcome, the studies are grouped by exposure type (occupational, environmental, or Vietnam veteran). Each section ends with the *Veterans and Agent Orange: Update 2004* committee's conclusions 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

Chloracne is a skin disease that is characteristic of exposure to TCDD and other cyclic organochlorine compounds. It shares some pathologic processes (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 and sebaceous gland epithelium. Although chloracne is typically distributed over the eyes, ears, and neck, patterns of chloracne among chemical industry workers exposed to TCDD also include the trunk, genitalia, and buttocks (Neuberger et al., 1998).

Chloracne has been studied extensively 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. It also should be noted that lack of chloracne does not necessarily indicate the absence of exposure to substantial levels of TCDD**,** as is apparent from studies of individuals exposed in the Seveso accident. There is not necessarily a correlation between serum concentrations of TCDD and the occurrence or severity of chloracne.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (hereafter referred to as *VAO*; IOM, 1994) determined there was sufficient evidence of an association between exposure to at least one compound of interest and chloracne. Additional information available to the committees responsible for *Veterans and Agent Orange: Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), *Update 2000* (IOM, 2001), and *Update 2002* (IOM, 2003) did not change that finding. Reviews of the studies that underlie the finding can be found in the earlier reports.

Update of the Scientific Literature

No relevant occupational or environmental studies have been published since *Update 2002*.

Vietnam-Veteran Studies

Kim J-S et al. (2003) conducted a cross-sectional study of Korean veterans who served in Vietnam to determine what adverse health effects might be associated with service in Vietnam and, by inference, attributable to exposure to Agent Orange. The Korean-veteran cohort consisted of 1,224 male veterans (27.6% participation rate); a non-exposed group consisted of 154 male Korean veterans (5.7% participation rate) who did not serve in Vietnam. Chloracne occurred in nine veterans who served in Vietnam; it did not occur in the veterans who served elsewhere. The prevalence of chloracne did not increase for higher exposures. Selection and recall bias could limit the usefulness of the results.

Synthesis

Chloracne is clearly associated with high exposure to cyclic organochlorine compounds, but because it appears shortly after exposure, a long latency would be inconsistent with information in previous studies. The study conducted by Kim J-S et al. (2003) had significant limitations and did not contribute to better understanding chloracne as it relates to organochlorines.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is sufficient evidence of an association between exposure to at least one compound of interest and chloracne.

Biologic Plausibility

As noted in previous reports, chloracne-like skin lesions have been reported to occur in several animal species in response to exposure to TCDD but not to purified phenoxyacetic herbicides. TCDD induces differentiation in human keratinocytes, and it is reported to decrease an acidic type I keratin involved in epidermal development and thus lead to keratinocyte hyperproliferation and skin irritations, such as chloracne. The data provide a biologically plausible mechanism for the induction of chloracne by TCDD.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and chloracne, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

PORPHYRIA CUTANEA TARDA

Porphyrias are uncommon disorders caused by deficiencies of enzymes involved in the heme (iron-containing, non-protein portion of the hemoglobin molecule) biosynthesis pathway. PCT is a heterogeneous group of disorders caused by a deficiency of a specific enzyme (uroporphyrinogen decarboxylase). PCT, the most common of the porphyries, can be inherited, but more often it is acquired. Type I PCT, which accounts for 80–90% of all cases, is an acquired disease that typically becomes evident in adulthood. Type I PCT can occur spontaneously, but more commonly it occurs in conjunction with environmental factors, such as alcohol consumption, exposure to estrogens, or use of some medications.

The most significant clinical finding is cutaneous photosensitivity. Sensitivity to sunlight is thought to result from the excitation of excess porphyrins in the skin by long-wave ultraviolet light, which leads to cell damage. Fluid-filled vesicles and bullae develop on sun-exposed areas of the face and on the dorsa of the hands, feet, forearms, and legs. Other features include hypertrichosis (excess hair) and hyperpigmentation (increased pigment), especially on the face. In individuals with PCT, porphyrins are increased in the liver, plasma, urine, and stools. Iron, estrogens, alcohol, viral hepatitis, and chlorinated hydrocarbons can aggravate the disorder. Iron overload is almost always present in individuals with PCT.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* determined that there was sufficient evidence of an association between exposure to at least one compound of interest (2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid) and PCT in genetically susceptible people. The data, however, indicated that PCT manifests shortly after exposure to TCDD. Therefore, new cases of PCT attributable 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*, *Update 2000*, and *Update 2002* did not change that conclusion. Reviews of the relevant studies are found in the earlier reports.

Update of the Scientific Literature

No relevant occupational or environmental studies have been published since *Update 2002*.

Vietnam-Veteran Studies

Kim J-S et al. (2003) conducted a cross-sectional study of Korean veterans who served in Vietnam to determine what adverse health effects might be associ-

ated with service in Vietnam and, by inference, attributable to exposure to Agent Orange. The Korean-veteran cohort consisted of 1,224 male veterans (27.6% participation rate); a non-exposed group consisted of 154 male Korean veterans (5.7% participation rate) who served elsewhere. Uroporphyrin (a common porphyrin that is increased in persons with PCT) occurred with greater frequency in the urine of veterans who served in Vietnam than it did in non-Vietnam veterans ($p < 0.0001$). However, the authors did not include uroporphyrin concentrations or specify urine collection procedures (24-h urine or spot urine sample) in their report.

Synthesis

PCT would be an early response to TCDD; recovery would occur after exposure ceased. Although PCT has been seen after exposure to TCDD in industrial settings, Vietnam veterans enrolled in the Ranch Hand study have not exhibited symptoms suggestive of the disorder.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one compound of interest and PCT.

Biologic Plausibility

PCT has not been replicated in animal studies with TCDD, although other porphyrin abnormalities have been reported. Porphyrin accumulation was found in fish and chick embryo hepatocyte cultures treated with TCDD.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and PCT in genetically susceptible people, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

RESPIRATORY DISORDERS

Non-malignant respiratory disorders comprise acute and chronic lung diseases other than cancer. Acute respiratory disorders include pneumonia and other

respiratory infections. Those disorders could be increased in frequency and severity when the normal defense mechanisms of the lower respiratory tract are compromised. Chronic non-malignant respiratory disorders generally take one of two forms: *Airways disease* is a general term for disorders characterized by obstruction of the flow of air out of the lungs, among them asthma and chronic obstructive pulmonary disease (COPD), which includes emphysema and chronic bronchitis. *Parenchymal disease*, or *interstitial disease*, generally includes numerous disorders that cause inflammation and scarring of the deep lung tissue, including the air sacs and supporting structures. Parenchymal disease is less common than is airways disease, and its disorders are characterized by reductions in lung capacity, although they can include a component of airway obstruction. Some severe chronic lung disorders, such as cystic fibrosis, are hereditary. Because Vietnam veterans received health screenings before entering military service, few severe hereditary chronic lung disorders are expected in that population.

The major risk factor for many non-malignant respiratory disorders is cigarette-smoking. Although cigarette-smoking is not associated with every disease of the lungs, it is the major cause of many airways disorders, it contributes to some interstitial disease, and it 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 habitual cigarette-smoking 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. Vietnam veterans are reported to smoke more heavily than are non-Vietnam veterans (McKinney et al., 1997).

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to the compounds of interest (2,4-D, 2,4,5-T, 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 non-malignant respiratory disorders among study subjects, particularly males, who were 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 non-malignant respiratory disorders, particularly COPD, among people exposed to TCDD, the observation is tentative and the information insufficient to determine an association between the exposures of interest and respiratory dis-

orders. Additional information available to the committee responsible for *Update 2002* did not change that finding.

Update of the Scientific Literature

Dahlgren et al. (2003) described a cross-sectional environmental study that used questionnaires to gather information on potential adverse health effects among residents near a wood treatment plant. The plant always used creosote, and pentachlorophenol was used from 1951 to 1971. Soil and sediment samples from a ditch in the neighborhood contained dioxins and furans. The exposed population consisted of 1,269 residents of a neighborhood near the plant; they also were plaintiffs or potential plaintiffs in a lawsuit against the plant. A representative sample group of 214 residents was chosen to participate in the study. The unexposed population consisted of 139 age- and sex-matched residents of a distant neighborhood. Exposed and unexposed subjects completed questionnaires; were examined by a physician; and underwent blood, urine, and respiratory function tests.

Exposed residents reported greater frequency of chronic bronchitis by history (17.8% versus 5.7%, $p < 0.0001$) and asthma by history (40.5% versus 11.0%, $p < 0.0001$). Selection bias and recall bias limit the utility of the results. Also, it is unclear whether the authors adequately controlled for history of tobacco use. In addition, multiple environmental exposures occurred in the neighborhood near the plant, and the authors could not determine which exposures were responsible for the reported adverse health effects.

No relevant occupational or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

No new studies provide evidence of a direct risk of non-malignant respiratory disorders in adults since those reviewed in *Update 2002*.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and non-malignant acute or chronic respiratory disorders.

Biologic Plausibility

Lung tissue contains high concentrations of the aryl hydrocarbon receptor (AhR), which mediates the effects of TCDD. Recent data also show that human lung cells are responsive to TCDD in terms of altered gene expression. Therefore, it is biologically plausible that exposure to TCDD may result in acute and chronic lung disorders, but cigarette-smoking also is a major risk factor for those disorders. 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 compounds to which human lungs are exposed. Bronchiolar metaplasia of the alveolar epithelium has been observed in the lungs of rats exposed to TCDD for 2 years.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and the respiratory disorders specified above, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

IMMUNE SYSTEM DISORDERS

The immune system defends the body against infection by viruses, bacteria, and other disease-producing microorganisms (pathogens). The immune system also operates in cancer surveillance, destroying cells that have become transformed and might otherwise develop into tumors. As it recognizes the wide array of pathogens in the environment, the immune system relies on many different cell types that operate together to generate immune responses. The immune system's cells arise from stem cells in the bone marrow; they are found throughout the body's lymphoid tissues, and they circulate in the blood as white blood cells (WBCs).

Granulocytes are WBCs that respond quickly to infection. They display a variety of protein receptors on their surfaces that can bind to molecules on different pathogens. When the receptors are bound, they signal the granulocytes to release toxins contained in their granules that kill the pathogens. Macrophages ("large eaters") are WBCs that ingest bacteria and other microbes; once inside the macrophage, the microbes are killed by oxidative and enzymatic processes. Natural killer (NK) cells are WBCs that recognize and kill virus-infected cells and cancer cells. Granulocytes, macrophages, and NK cells are the first line of defense against infectious disease and cancer.

A second line of defense specifically targets the infecting microorganism. The response is mediated by the T and B lymphocytes. B lymphocytes produce

antibodies, which bind to infectious microbes or their toxins, causing them to be destroyed by other immune system cells. There are several antibodies (immunoglobulins [Ig]): IgM, IgG, IgA, IgE, and IgD. All have different functions, but each is quite specific in action. For example, antibodies produced in response to a tuberculosis infection will bind only to tuberculosis bacteria. T lymphocytes carry out many functions: They help B lymphocytes make antibodies, activate macrophages to kill bacteria more effectively, and directly kill tumor cells and virus-infected cells. T lymphocytes regulate the magnitude of the immune response and turn it off when the infection is cleared. T cells function primarily by secreting soluble chemical messengers called interleukins and they acquire their different functions by passing through the thymus gland before entering the blood and lymph nodes. An intact T and B lymphocyte response is highly effective at preventing disease. Once a person is exposed to a particular disease organism or is vaccinated against that disease, memory T and B cells are present in larger numbers. The cells respond more quickly upon reinfection to prevent disease.

When the immune system responds to a foreign substance that is not actually pathogenic, such as animal dander, pollen, or poison ivy, the immune system can generate an allergic response. Many allergic responses are associated with the production of IgE or sometimes IgG antibodies. Once produced, those antibodies bind to the surface of other cells—mast cells—that occur in tissues throughout the body, including lung airways, the gut wall, and blood vessel walls. When a person is exposed again to the allergen, the allergen binds to the antibodies on the mast cells, causing them to release histamine and leukotrienes, which cause the allergic symptoms. Allergic responses also are mediated by memory T cells that release inflammatory substances when the allergen binds to receptors. Rashes caused by contact with poison ivy or nickel are T-cell-mediated allergies.

Autoimmune disease is another example of the immune system's causing rather than preventing disease. In this case, the immune system mistakenly attacks the body's own cells and tissues as if they were foreign. The autoimmune reaction in multiple sclerosis is directed against the myelin sheath of the nervous system; in Crohn's disease, the gut is the target of attack; in type 1 diabetes mellitus, the insulin-producing cells of the pancreas are destroyed by the immune response. In some autoimmune diseases, B cells mistakenly make antibodies against specific cellular components. Those autoantibodies may interfere with the normal function of the tissues or cause the tissue to break down. Sometimes altered T-cell function is the underlying cause of autoimmunity. Genetic predisposition and environmental factors such as infectious diseases and stress are thought to facilitate the development of autoimmune diseases.

Suppression of the immune response can result in reduced resistance to infectious disease and to increased risk of cancer. In persons suffering from allergies or autoimmune disease, immune suppression can relieve symptoms. Immune deficiencies can be congenital or acquired in a variety of ways. Infection with HIV is a well-recognized example of an acquired immune deficiency in

which a specific type of T cell is the target of the virus. The decline in the number of CD4+ T cells after HIV infection correlates with an increased incidence of infectious diseases, including fatal opportunistic infections, and with an increased incidence of several types of cancer. On a more modest scale, nutritional deficiencies, chronic stress, and exposure to toxic chemicals also have been shown to suppress some functions of the immune system. However, because the immune system has considerable reserve capacity and redundancy, that modest suppression does not usually translate directly into increased disease. The ability of an individual to combat infection depends on many factors that are difficult to control in a scientific study, including the virulence of the microbe; the presence of other diseases (diabetes); age; vaccination status; stress; and exposure to toxic compounds, both intentionally (alcohol, tobacco or drug use, medications) and unintentionally (through workplace or environmental exposures). Because of those many confounding factors, few studies have been able to identify significant changes in immune response after environmental exposure in humans, and the evidence of direct links between chemical-compound-induced changes in immune function and altered disease is not strong.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committees responsible for *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*, concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and immune system disorders. Reviews of the studies that underlie those conclusions are presented in the previous reports (IOM, 1994, 1996, 1999, 2001, 2003).

Update of the Scientific Literature

Occupational Studies

No relevant occupational studies of immune disorders have been published since those reviewed in *Update 2002*.

Environmental Studies

In a new study about Seveso, Italy, Baccarelli et al. (2002) measured plasma immunoglobulin and complement concentrations in a random sample of the population in the most highly exposed zone ($N = 62$) and in the surrounding uncontaminated area ($N = 58$). Plasma IgG significantly decreased with increasing TCDD concentration. That association was present after adjusting for age, sex, tobacco use, and consumption of domestic livestock and poultry. There was no effect on concentrations of IgM, IgA, or complement; IgE was not measured.

Two studies have evaluated the influence of exposure to TCDD-like compounds on immune response in children. Van Den Heuvel et al. (2002) characterized the immune status of adolescent boys and girls in Flanders, Belgium, in relation to their blood concentrations of PCBs and dioxin-like compounds. The latter data were obtained using the chemical-activated luciferase expression (CALUX) bioassay. The percentages of eosinophils and NK cells in the blood and IgG concentrations in the serum were negatively correlated with increasing CALUX toxicity equivalents (TEQ). The concentrations of IgE to specific allergens were negatively correlated with CALUX TEQs, in parallel with a significant decrease in self-reported allergies. The decrease in IgE found in the adolescents might suggest a dioxin-induced suppression of the immune response, consistent with the findings in laboratory animals exposed to TCDD. In a follow-up study of 8-year-old Dutch children ($N = 27$) perinatally exposed to dioxin, ten Tusscher et al. (2003) found a decrease in allergy in relation to increasing dioxin exposure. They also found increased numbers of CD4+ T cells and CD45RA+ cells, a phenotype associated with naïve T cells. An increased percentage of naïve versus activated T cells is consistent with a generalized decrease in immune responsiveness associated with dioxin exposure.

Vietnam-Veteran Studies

Kim H-A et al. (2003) studied Korean Vietnam War veterans for evidence of immune system changes in relation to their operation in various areas of Vietnam. Study subjects were classified into three groups, based on self-reported Agent Orange exposure history and health status. A veteran patient group ($N = 24$) consisted of veterans exposed to Agent Orange and suffering from chronic illness; the veteran control group ($N = 27$) had similar exposure but no chronic illness. Control subjects ($N = 36$) were healthy age-matched subjects with no Vietnam War military service. A significant increase in plasma IgE was found in both groups of veterans compared with control subjects. The patient group also had significantly decreased plasma IgG1. Those changes correlated with decreased production of interferon gamma in the patient group and with increased production of interleukin 4 in both veterans' groups when the T cells from the subjects were cultured in vitro. No changes in the plasma concentrations of antibodies against double-stranded DNA or extractable nuclear antigens, both markers of autoimmune disease, were found in the veterans, nor were changes found in frequency distribution of peripheral blood leukocyte subpopulations.

Synthesis

TCDD is a well-known immunosuppressive agent in laboratory animals; it is among the most potent immunotoxicants in the environment. Therefore, one would expect that exposure of humans to sufficiently high doses of TCDD would

result in immune suppression. However, several studies of various parameters of human immune function have failed to reveal consistent correlations with TCDD exposure, and no detectable pattern of increased infectious disease has developed in veterans exposed to high concentrations of TCDD or other herbicides used in Vietnam. Although suppression of the immune response by TCDD could increase the risk of some cancers in Vietnam veterans, there is no evidence to support that connection.

Recent studies that have examined the influence of TCDD on IgE production have generated additional conflicting data. Two studies revealed a significant reduction in IgE production and associated allergic responses correlated with increasing exposure to TCDD and related compounds among children in Belgium and the Netherlands (ten Tusscher et al., 2003; Van Den Heuvel et al., 2002). In contrast, Korean Vietnam veterans had increased rather than decreased IgE concentrations in plasma—independent of health status (Kim H-A et al., 2003). The basis for those different outcomes is not known; however, it is possible that the immunosuppressive effects of TCDD observed in the children resulted from exposure during development, which is a more sensitive period for the induction of immunotoxicity than is adulthood. The increase in IgE observed in the Korean veterans had not been reported in previous studies.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and immune suppression or autoimmunity.

Biologic Plausibility

TCDD is a known immunosuppressive chemical in laboratory animals, and exposure to TCDD has been shown to influence the incidence and severity of a variety of infectious diseases. The effects of TCDD on the immune system are species- and strain-specific. Relative to recent studies demonstrating an effect of TCDD on allergic disease in humans, Luebke et al. (2001) reported that TCDD exposure suppressed the allergic immune response of rats to house-dust-mite antigens and decreased allergen-associated lung pathology. Those effects occurred in the absence of any change in serum IgE. A study by Kimata (2003) isolated B cells from allergic (atopic) and non-allergic persons and cultured the cells with TCDD in vitro. Exposure to TCDD significantly enhanced IgE production by B cells isolated from atopic but not from non-atopic persons. The author suggested that TCDD exposure may aggravate preexisting allergic disease by

enhancing IgE production. In previous studies, TCDD treatment was shown to decrease the secretion of IgG1 and IgE in murine B-cell cultures stimulated with bacterial lipopolysaccharide and interleukin 4 (Karras et al., 1995). Hinsdill et al. (1980) reported that feeding juvenile and adult mice various quantities of TCDD for 5 weeks or more produced no evidence of enhanced IgE synthesis but reduced contact sensitization to dinitrofluorobenzene. Chapter 3 discusses recent toxicologic studies that demonstrate the immunotoxic effects of the compounds of interest.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and immune system disorders, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

DIABETES

Diabetes mellitus is a group of heterogeneous metabolic disorders characterized by hyperglycemia and quantitative or qualitative deficiency of insulin action (Orchard et al., 1992). Although all forms share hyperglycemia, the pathogenic processes involved in its development differ. Most cases of diabetes fall into two categories: type 1 diabetes is characterized by an absolute deficiency of insulin caused by the destruction of insulin-producing cells in the pancreas (β-cells); type 2 diabetes is characterized by a combination of resistance to the actions of insulin and inadequate secretion of insulin (called relative insulin deficiency). In old classification systems type 1 diabetes was called insulin-dependent diabetes mellitus or juvenile-onset diabetes mellitus; type 2 diabetes was called non-insulin-dependent diabetes mellitus or adult-onset diabetes mellitus. The modern classification system recognizes that type 2 diabetes can occur in children and also can require insulin. For both types, long-term complications can include cardiovascular disease, nephropathy, retinopathy, neuropathy, and increased vulnerability to infections. Maintaining blood sugar concentrations within the normal range is crucial for preventing complications.

About 90% of all cases of diabetes mellitus are type 2 cases. Onset can occur before 30 years of age, and incidence increases steadily with age thereafter. The main risk factors are age, obesity, central fat deposition, a history of gestational diabetes (if female), physical inactivity, ethnicity (prevalence is greater in blacks and Hispanics than in whites), and—perhaps most important—family history. The relative contributions of those features, however, are not known.

The etiology of type 2 diabetes is unknown, but three major components have been identified: peripheral insulin resistance (thought by many to be primary) in target tissues (muscle, adipose tissue, liver); a defect in β-cell insulin

secretion; and hepatic glucose overproduction. In states of insulin resistance, insulin secretion is initially higher for each concentration of glucose, compared with that for people without diabetes. That hyperinsulinemic state is a compensation for peripheral resistance and often can maintain normal glucose levels for years. Eventually, β-cell compensation becomes inadequate, and there is progression to overt diabetes with concomitant hyperglycemia. The reason the β-cells cease to produce sufficient insulin is not known.

Type 1 diabetes occurs as a result of immunologically mediated destruction of β-cells in the pancreas and causes an absolute deficiency of insulin. It often develops during childhood, but it can develop at any age. As with many autoimmune diseases, genetic and environmental factors influence pathogenesis. Some viral infections are believed to be important environmental factors that could trigger the autoimmunity associated with type 1 diabetes.

Pathogenetic diversity and diagnostic uncertainty are among the important problems associated with epidemiologic study of diabetes. Given the multiple likely pathogenetic mechanisms that lead to diabetes mellitus—which include diverse genetic susceptibilities (ranging from autoimmunity to obesity) and the variety of potential environmental and behavioral factors (viruses, nutrition, activity)— many agents or behaviors can contribute to risk, especially in genetically susceptible people. The multiple mechanisms also can lead to heterogeneous responses to various exposures. Because up to half the affected diabetic population is undiagnosed, the potential for ascertainment bias in population-based surveys is high (more intensively followed groups or those with more frequent health care contact are more likely to be diagnosed), thereby emphasizing the need for formal standardized testing (to detect undiagnosed cases) in epidemiologic studies.

Summary of Previous IOM Reports

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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), the Institute of Medicine (IOM) called together a committee to conduct an interim review of the scientific evidence regarding type 2 diabetes. That review focused on information published after the deliberations of the *Update 1998* committee, and it resulted in the report *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (IOM, 2000). The committee responsible for that report determined that there was limited or suggestive evidence of an association between type 2 diabetes and exposure to at least one compound of interest. The committees responsible for *Update 2000* and *Update 2002* upheld

that finding. Reviews of the pertinent studies are found in the earlier reports. Table 9-1 presents a summary.

Update of the Scientific Literature

Occupational Studies

No occupational studies have been published since those reviewed in *Update 2002*.

Environmental Studies

Fierens et al. (2003) completed a population-based cross-sectional study in several Belgian towns to assess the association between serum dioxin concentration and the prevalence of diabetes. Diabetes was reported by 3.6% of adults from the exposed communities (7/194) and in 3.2% of adults from a comparison community (2/63). No data were presented on the average dioxin concentrations in adults from either area. Although the incidence of diabetes was similar in both groups, analyses that compared the 9 diabetic adults with the 248 non-diabetic adults showed significantly higher concentrations of dioxins (diabetics, 46.6 picograms [pg] TEQ/g fat; non-diabetic, 25.2 pg TEQ/g fat, $p < 0.05$). In a covariate-adjusted analysis contrasting adults in the top decile of dioxin concentration with adults below the 90th percentile, the odds ratio (OR) for diabetes associated with the top decile was 5.1 (95% CI [confidence interval], 1.2–21.7). The dioxin concentrations at the 90th percentile and actual number of adults with diabetes in each category were not given.

Vietnam-Veteran Studies

Michalek et al. (2003) analyzed data from the Ranch Hand study to address a question of methodology: Is the association between serum TCDD concentration and diabetes incidence attributable to some characteristic that affects both diabetes and TCDD elimination rate? The researchers chose 343 participants from the Ranch Hand study who had multiple measurements of TCDD during their periodic examinations. An elimination rate was estimated for each veteran from those repeated measures. Proportional hazards and logistic regression models were computed to assess the association between TCDD elimination rate and the incidence of diabetes, both before and after adjustment for initial TCDD concentration and other risk factors for diabetes (age, family history, body mass index, tobacco use). The initial inverse association between TCDD elimination rate and diabetes was largely eliminated with the addition to the models of the other predictors. The baseline measurement of TCDD was a statistically significant ($p = 0.03$) predictor of time to onset of diabetes and a positive but non-

TABLE 9-1 Selected Epidemiologic Studies—Diabetes and Health Outcomes Related to Diabetes

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
Studies Reviewed in *Update 2002*			
Kitamura et al., 2000	Workers exposed to PCDD at a municipal waste incinerator	8	NS
Steenland et al., 2001	Ranch Hand veterans, workers exposed to TCDD-contaminated products, and comparison cohorts		
	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[b]	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)
	20 <TCDD <75 pg/g (ng/kg) of lipid	6	1.5 (0.5–4.3)
	75 < TCDD <238 pg/g (ng/kg) of lipid	3	0.7 (0.2–2.6)
	238 <TCDD <3,400 pg/g (ng/kg) of lipid	10	2.0 (0.8–4.9)
Steenland et al., 1999[b]	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[b]	Exposed production workers and sprayers in 12 countries[a]	33	2.3 (0.5–9.5)
Steenland et al., 1992[b,c]	Dioxin-exposed workers—Mortality		
	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—Mortality	4	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—Mortality	4	SMR = 0.7 (0.2–1.9)

continues

TABLE 9-1 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Moses et al., 1984	2,4,5-T and TCP production workers with chloracne	22	2.3 (1.1–4.8)
May, 1982	TCP production workers	2	*
Pazderova-Vejlupkova et al., 1981	2,4,5-T and TCP production workers	11	*
ENVIRONMENTAL			
New Study			
Fierens et al., 2003	Belgium residents (142 women; 115 men) environmentally exposed to dioxins and PCBs		
	Subjects in the top decile for adjusted concentrations of dioxins		5.1 (1.2–21.7)
Studies Reviewed in *Update 2002*			
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[b]	Non-diabetic residents near the Vertac/Hercules Superfund site—OR 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[b]	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[b]	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)

TABLE 9-1 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
VIETNAM VETERANS			
New Studies			
JS Kim et al., 2003	Korean veterans of Vietnam association between diabetes and exposure status	144	2.7 (1.1–6.7)
Michalek et al., 2003	Ranch Hand participating in a pharmacokinetic study (N = 343)		
	Rate of TCDD elimination and occurrence of diabetes (proportional hazards model)	92	NS
	Rate of TCDD elimination and time to onset of diabetes (proportional hazards model)	92	NS
Studies Reviewed in *Update 2000*			
AFHS, 2000[b]	Air Force Ranch Hand veterans and comparisons		(Numerous analyses discussed in the text of *Herbicide/Dioxin Exposure and Type 2 Diabetes*)
Longnecker and Michalek, 2000[b]	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]
CDVA, 1998a[b]	Australian Vietnam veterans—male	2,391 reported[e] (6% of respondents)	1,780 expected (1,558–2,003)
CDVA, 1998b[b]	Australian Vietnam veterans—female	5 reported[e] (2% of respondents)	10 expected (9–11)
Henriksen et al., 1997[b]	Ranch Hand—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 Hand		
	High-exposure category	57	1.5 (1.2–2.0)
	All Ranch Hand	146	1.1 (0.9–1.4)
O'Toole et al., 1996	Australian Vietnam veterans	12	1.6 (0.4–2.7)[e]

continues

TABLE 9-1 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
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] Given when available.
[b] Study is summarized in *Update 2002* and discussed in greater detail in *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (IOM, 2000).
[c] May include some of the same subjects covered in the NIOSH cohorts addressed in the other references cited in the Occupational cohorts category.
[d] Adjusted for age, race, body mass index, waist size, family history of diabetes, body mass index at the time dioxin was measured, serum triglycerides, and military occupation.
[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 diabetes?"
* = Information not provided by study authors.
ABBREVIATIONS: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 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; HDL, high-density lipoprotein; N/A, not applicable; NS, not significant; SS, statistically significant; TCP, trichlorophenol.

statistically significant ($p = 0.16$) predictor of incidence of diabetes in the subset of Ranch Hand veterans. The data suggest that the association between serum TCDD and diabetes is not simply attributable to a factor that causes diabetes and slows elimination of TCDD.

Kim J-S et al. (2003) reported the results of a cross-sectional study of exposure to Agent Orange and the prevalence of a large number of health outcomes in veterans from Korea. The researchers recruited male Vietnam veterans in 1995 from a list of veterans who had contacted the Korean government to be examined for possible medical care and compensation for service-related conditions. A total of 4,432 veterans were on the list; 1,514 (34.2%) agreed to participate in the study; 1,224 (27.6%) were age-eligible for the study (45–64 years) and completed the study interview and examination. Of the 2,682 male non-Vietnam veterans identified who received military pensions and resided near Seoul, only 154 (5.7%) of those persons agreed to participate as the comparison group for the study.

These researchers created an index to estimate exposure to Agent Orange in the Vietnam veterans. The index included information on time spent in different regions of Vietnam and on reports of exposure to Agent Orange on the skin, in the air, or through drinking or swimming in contaminated water. Quartiles were formed from scores on the index, and the categories of exposure were compared

with lipid-adjusted TCDD concentrations from pooled blood samples drawn during a 1995 examination for the study. The mean values of TCDD by quartile of the overall exposure index in the Vietnam veterans were 0.6, 0.62, 0.78, and 0.87 pg/g, respectively; non-Vietnam veterans had an average serum TCDD of 0.3 pg/g. As discussed in greater detail in Chapter 5, the decision to pool blood samples (from 745 sampled individuals to produce 13 analytic readings) greatly compromised validation of the exposure categories derived from interview responses. The resulting range of serum TCDD levels was very narrow, putting into question the biologic relevance of any differences. The assessment of health outcomes was done without knowledge of exposure status, but the researchers did not provide specific information on the definition of diabetes for the analysis.

Diabetes was present in 5.8% (9/154) of the non-Vietnam veterans and in 12.6% (154/1,224) of the Vietnam veterans (prevalence ratio, 2.17; $p = 0.02$ with adjustment for age). Within the group of Vietnam veterans, the prevalence of diabetes varied only slightly by quartile of Agent Orange exposure index (range, 11.5–14.1%, $p = 0.78$ for differences across categories). In a logistic regression model adjusting for alcohol consumption, tobacco use, body mass index, education, age, and marital status, the odds ratio for diabetes in Vietnam versus non-Vietnam veterans was 2.69 (95% CI, 1.09–6.67, $p = 0.03$).

Synthesis

The study by Fierens et al. (2003) has several important weaknesses that limit its contribution to the literature on exposure to herbicides and the risk of developing diabetes. Diabetes is based on self-report, and there is no description of the communities or the study population to reveal differences in ascertainment of diabetes (attributable to differences in access to medical care). The small number of adults with diabetes is reflected in the imprecise results, and the confidence interval ranges from barely above one to a 22-fold increased risk. Dioxins are reported in aggregate, so the relationship between TCDD concentrations and diabetes cannot be ascertained.

Michalek et al. (2003) provide additional support for a causal relationship between TCDD exposure and the development of diabetes by demonstrating in data from the Ranch Hand study that the association is not an artifact of differences in the elimination rate of TCDD. The study of Korean veterans (Kim J-S et al., 2003) shows an elevated prevalence of diabetes in Vietnam veterans compared with those who served elsewhere. The difference is not attributable to confounding by several important risk factors for diabetes, and the Vietnam veterans did have higher serum TCDD than did the comparison group. However, the report does not significantly strengthen the evidence for an association between herbicide exposure and diabetes. Limitations in measurement could be responsible for the absence of any trend in diabetes among the Vietnam veterans by quartile of Agent Orange exposure index. A more serious concern is the

possibility of selection bias in the formation of the study population. As the authors concede, it is likely that veterans with chronic health conditions were overrepresented in the sample of Vietnam veterans because of their interest in potential compensation or medical care for the health effects of exposure to herbicides. The adjustment for confounders will not automatically correct for that form of selective participation. The relatively low participation in both groups (28% and 6% in Vietnam and non-Vietnam veterans, respectively) underscores the need for additional data on the influence of selection bias in this cross-sectional study.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one compounds of interest and diabetes.

Biologic Plausibility

The evaluation of the potential of TCDD to induce clinical diabetes in animals has been impaired by the lack of an appropriate animal model for type 2 diabetes. TCDD's effects on triglycerides and high-density lipoproteins, glucose transport, protein kinase C, and other lipoproteins in animals suggest that it 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. Chronic active inflammation, acinar cytoplasmic vacuolization, acinar atrophy, and arterial chronic active inflammation have been observed in the pancreata of rats exposed to TCDD for 2 years. The lesions indicate that the pancreatic acinar cells are susceptible to the toxic effects of TCDD; the islet cells appear to be more resistant. Nevertheless, until appropriate animal models are developed to show the etiology and pathogenesis of diabetes, confirmation of TCDD's ability to induce diabetes in animals will remain elusive.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and type 2 diabetes, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

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). Cholesterol and triglycerides, the two major types of lipids, are carried in the blood attached to proteins to form lipoproteins, which are classified by 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. 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 liver; it also results from the catabolism of VLDL. LDL is thought to be involved in the delivery of cholesterol to the tissues; 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 can be familial (because of an LDL receptor genetic defect) or polygenic (attributable to multiple minor genetic susceptibilities); familial hypertriglyceridemia (sometimes linked to susceptibility to diabetes); and mixed hyperlipidemias in which cholesterol and triglycerides are elevated. The mixed hyperlipidemias group includes familial combined hyperlipidemia, which could result from hepatic overproduction of VLDL and apoprotein B, and type III dyslipidemia, 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, use of drugs, age, gender, hormones) have major effects. In particular, the saturated-fat content of the diet might raise LDL concentrations via decreased LDL-receptor activity; obesity and a high-carbohydrate diet can increase VLDL triglycerides and possibly are linked to insulin resistance and reduced lipoprotein lipase activity. Intercurrent illness can increase triglyceride concentration and decrease cholesterol concentration. Diabetes also is associated with increased triglycerides and decreased HDL cholesterol, whereas other diseases (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 considered before the effect of a new factor can be assessed (LaRosa, 1990). In the current context, any analysis must control for obesity as a primary determinant of triglyceride and TCDD concentrations. The ability of acute or chronic illness to raise triglyceride and glucose and lower HDL and LDL cholesterol concentrations must be recognized.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and lipid and lipoprotein disorders. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Reviews of the relevant studies are found in the earlier reports. Table 9-2 provides a summary.

Update of the Scientific Literature

Environmental and Vietnam-Veteran Studies

No relevant environmental or Vietnam-veteran studies of lipid and lipoprotein disorders have been published since those reviewed in *Update 2002*.

Occupational Studies

Pelclová et al. (2002) measured cholesterol and triglyceride concentrations in 12 men who were exposed to extremely high concentrations of TCDD in the 1960s while they were employed in herbicide production at a chemical factory in the former Czechoslovokia. The exposure occurred because of excessive temperature and pressure in the production process over an extended period of time (between 1965 and 1968) rather than as a consequence of a major release at a single point in time. More than 80 workers were affected, but the researchers provided little information about those who were not included in the study. The average plasma TCDD concentrations for the 12 study participants in 1996 (approximately 30 years after the exposure) was 271 pg/g, with a range from 14 to 760 pg/g. Elevated triglycerides (>1.69 millimolar [mmol]/L) and cholesterol (>5.2 mmol/L) were observed in 6 and 7 of the 12 men, respectively. The correlation between TCDD in 1996 and highest recorded measurement of triglyceride or cholesterol at any point between 1968 and 2001 was 0.66 for triglyceride and 0.78 for cholesterol. No information was given about follow-up measures of lipids collected in standard or periodic fashion for participants and there is no discussion of how individual differences in treatment of elevated cholesterol could influence the highest recorded value for total cholesterol.

Hu et al. (2003) conducted a cross-sectional study of dioxin–furan exposures and lipids in workers at municipal-waste incinerator plants in Taipei City, Taiwan. A total of 133 workers were randomly sampled from 3 plants; workers had to have been employed for at least 6 months in the operation and control or maintenance departments. Nearly all (98.5%) who were randomly selected agreed to participate in the study. In all, 17 congeners were measured, including TCDD

(which had a range from undetectable to 14 pg/g, with a median of 1.6 pg/g). Workers with TCDD above the median had higher average cholesterol and were more likely to have cholesterol above 220 mg/dL ($p < 0.05$ for both measures of cholesterol, actual means and proportions not shown). The relationship between TCDD and cholesterol was not statistically significant when TCDD was measured by tertiles, quartiles, or as a continuous variable. TCDD was not associated with triglyceride as a continuous or categorical measure.

Synthesis

The report by Pelclová et al. (2002) has some important shortcomings—the small sample, uncertainty about whether the 12 exposed workers are representative of the exposed group, and the lack of an explicit comparison group. The correlation between TCDD and lipids is impressive but difficult to interpret because the lipids were measured as early as 1970 and as late as 2001. The relatively extreme concentrations of TCDD also affect the generalizability of the findings.

The study by Hu et al. (2003) successfully recruited a cross-section of workers and did show significant variation in cholesterol by a dichotomous measure of TCDD. The loss of statistical significance with more detailed categories or along the full continuum of TCDD values suggests that the findings from the initial analysis are not robust or consistent. The researchers adjusted for age and body mass index but not for diet (intake of fiber and specific fats) that are potential confounders. Several individual congeners other than TCDD were identified as statistically significant correlates of elevated cholesterol. The study design does not allow for isolation of the effect of any single exposure. The relationship between herbicide exposure and lipid remains uncertain.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and lipid and lipoprotein disorders.

Biologic Plausibility

The induction of lipid mobilization and alterations in lipid metabolism are well-known effects of high-dose exposure to TCDD in laboratory animals resulting in hyperpipidemia and loss of body fat. Increased serum triglycerides were

TABLE 9-2 Selected Epidemiologic Studies—Lipid and Lipoprotein Disorders

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
OCCUPATIONAL			
New Studies			
Hu et al., 2003	Workers exposed to PCDDs/PCDFs at 3 municipal waste incinerators in Taipei City		
	Comparison between high and low exposure groups using the low exposures group as controls	67 high; 66 low	
	Total cholesterol		2.8 (1.0–7.9)
	Triglycerides		1.5 (0.5–4.3)
Pelclová et al., 2002	Workers exposed to 2,3,7,8-TCDD during herbicide production at a factory in Spolana, Czech Republic	12	
	Correlation between the year (1968 to 2001) in which the highest level of the parameter was measured and serum 2,3,7,8-TCDD level in 1966		
	Cholesterol		r = 0.78; p = 0.01
	Triglycerides		r = 0.66; p = 0.02
Studies Reviewed in *Update 2002*			
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)

TABLE 9-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Ott and Zober, 1996	Production workers exposed to TCDD	42	
	Cholesterol		NSE[b]
	Triglycerides		NSE[b]
	HDL cholesterol		Increased; $p = 0.05$[b]
Studies Reviewed in *VAO*			
Martin, 1984	Production workers exposed to TCDD		
	Workers without chloracne	53	
	Cholesterol		Increased; $p < 0.005$[b]
	Triglycerides		Increased; $p < 0.005$[b]
	HDL cholesterol		NSE[b]
	Workers with chloracne	39	
	Cholesterol		Increased; $p < 0.05$[b]
	Triglycerides		Increased; $p < 0.01$[b]
	HDL cholesterol		NSE[b]
Moses et al., 1984	TCP and 2,4,5-T production workers	118	
	Cholesterol		NSE[c]
	Triglycerides		NSE[c]
Suskind and Hertzberg, 1984	TCP production workers	204	
	Cholesterol		NSE[b]
	Triglycerides		NSE[b]
	HDL cholesterol		NSE[b]
May, 1982	TCP production workers	94	
	Cholesterol		NSE[b]
	Triglycerides		NSE[b]
Pazderova-Vejlupkova et al., 1981	TCP and 2,4,5-T production workers	55	
	Cholesterol		NSE[b]
	Triglycerides		Increased VLDL; $p = 0.01$[b]
ENVIRONMENTAL			
Studies Reviewed in *VAO*			
Assennato et al., 1989	Seveso Zone A subjects who developed chloracne	193	
	Cholesterol		NSE[b]
	Triglycerides		NSE[b]

continues

TABLE 9-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]
Mocarelli et al., 1986	Children exposed near Seveso	63	
	Cholesterol		NSE[b]
	Triglycerides		NSE[b]
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	Air Force Ranch Hand veterans (1992 exam data)	884	
	Cholesterol		NSE (cholesterol: HDL ratio)[b]
	Triglycerides		NSE[d]
	HDL cholesterol		NSE[d] (cholesterol: HDL ratio)
O'Toole et al., 1996	Australian Vietnam veterans, compared with the Australian population	20	
	Cholesterol		3.0 (1.3–4.7)
Studies reviewed in *VAO*			
AFHS, 1991	Air Force Ranch Hand veterans Serum dioxin analysis (1987 exam data)	283–304[f]	
	Cholesterol		$p = 0.175$[e]
	Triglycerides		$p < 0.001$[e,g]
	HDL cholesterol		$p < 0.001$[e]
AFHS, 1990[h]	Air Force Ranch Hand veterans Original exposure group analysis (1987 exam 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; Wolfe et al., 1990	Air Force Ranch Hand veterans exposed to herbicide spraying (1982 data)	1,027	
	Cholesterol		NSE[h]
	Triglycerides		NSE[h]
	HDL cholesterol		NSE[h]

TABLE 9-2 *Continued*

Reference	Study Population	Exposed Cases[a]	Estimated Relative Risk (95% CI)[a]

[a] Given when available.
[b] *p*-values comparing means of subjects and controls. Univariate analysis.
[c] *p*-values comparing means in production workers with subsequent chloracne to those without.
[d] Comparing change over time between exposed and comparison groups.
[e] Comparing mean dioxin across lipid groups.
[f] Number of exposed Ranch Hand with "high" lipid values.
[g] Continuous analysis.
[h] Comparing means.
NOTE: Estimated risk and 95% CI reported unless *p*-values are specified.
ABBREVIATIONS: HDL, High-density lipoprotein; NSE, no significant effect; PCDD, polychlorinated dibenzodioxins; TCP, trichlorophenol; VLDL, very low density lipoprotein.

also seen in TCDD-exposed rhesus monkeys. The mechanism underlying altered lipid metabolism has not been elucidated.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and lipid and lipoprotein disorders, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

GASTROINTESTINAL AND DIGESTIVE DISEASE, INCLUDING LIVER TOXICITY

This section discusses a variety of conditions encompassed by *International Classification of Diseases*, Ninth Edition (ICD-9) codes 520–579—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 functions of the gastrointestinal tract are to absorb nutrients and eliminate waste. Those complex tasks involve numerous chemical and molecular interactions on the mucosal surface and complex local and distant neural and endocrine activity. One common condition of the gastrointestinal tract is motility disorder, which could be present in 15% of adults. The most convenient way to categorize diseases that affect the gastrointestinal system is by the

affected anatomic segment. Esophageal disorders predominantly affect swallowing; gastric disorders are related to acid secretion; and conditions that affect the small and large intestines are reflected in alterations in nutrition, mucosal integrity, and motility. Some systemic disorders also affect the gastrointestinal system (inflammatory, vascular, infectious, 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 or duodenal ulcer, depending on the 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 at some period in life; a similar percentage is 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 recurrence. About 60% of healed duodenal ulcers recur in the first year; 80–90% recur within 2 years.

Increasing evidence indicates that the bacterium *Helicobacter pylori* is linked to peptic ulcer disease (duodenal and gastric). *H. pylori* colonizes the gastric mucosa in 95–100% of patients with duodenal ulcer and in 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 ulcers 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 also has been linked to duodenal ulcer prevalence and mortality. Finally, psychological factors, particularly chronic anxiety and stress, could 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—aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase (GGT)—are commonly noted in liver dis-

orders. The relative sensitivity and specificity of those enzymes for diagnosing liver disease vary, and several tests can 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 that 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 use (often associated with increased GGT) make interpretation of changes in GGT in exposed people difficult (Calvert et al., 1992). An increase in GGT can be considered a normal biologic adaptation to chemical, drug, or hormone exposure.

Cirrhosis is the most commonly reported liver disease in epidemiologic studies of herbicide or TCDD exposure. 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 can lead to portal hypertension with associated gastroesophageal varices, enlarged spleen, abdominal swelling attributable to ascites; and, ultimately, to hepatic encephalopathy that can progress to coma. It generally is impossible to distinguish the various causes of cirrhosis by clinical signs and symptoms or by pathological characteristics. The most common cause of cirrhosis in North America and many parts of Western Europe and South America is excessive alcohol consumption. Other causes include chronic viral 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*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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*, *Update 2000*, and *Update 2002* did not change that finding. Reviews of the pertinent studies underlying are found in the earlier reports.

Update of the Scientific Literature

Occupational Studies

Hu et al. (2003) studied dioxin exposure on hepatic function in 133 male workers at municipal-waste incinerators in Taiwan. Mean GGT, total bilirubin,

and triglyceride concentrations in the high-exposure group were slightly but not statistically significantly higher than were those in the low-exposure group. There was no statistically significant interaction between dioxin exposure and hepatitis B surface antigen status on a liver function test result.

No relevant environmental or Vietnam-veteran studies have been published since *Update 2002*.

Synthesis

Evaluation of the effect of herbicide and TCDD exposure on non-cancer gastrointestinal ailments is more difficult than is 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 ailments, so incidence data are more problematic. The strong interdependence among the 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.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest 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 after sufficiently high 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 endpoints.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and gastrointestinal and digestive disease, including liver toxicity, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

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. Various methods have been used in morbidity studies 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, there are insufficient data 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*, *Update 2000*, and *Update 2002*

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and circulatory disorders. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* did not change that finding. Reviews of the relevant studies are published in the earlier reports.

Update of the Scientific Literature

Occupational Studies

Swaen et al. (2004) presented results for a 21-year follow-up of mortality in a cohort of 1,341 licensed herbicide applicators working for government agencies in the Netherlands. The study period was from January 1980 to January 2001; information was available on the types and amounts of herbicides used in all municipal spraying projects in 1980, but those data could not be linked to the work of individual applicators. No data were available on any potential risk factors other than age. Standardized mortality ratios (SMRs) were calculated

based on the age and calendar-year, cause-specific mortality rates of the general population of the Netherlands. The SMR for deaths attributable to circulatory conditions was 0.68 (70 deaths observed; 102.6 deaths expected; 95% CI, 0.53–0.86).

Environmental Studies

Fukuda et al. (2003) conducted an ecological study of 590 municipalities in Japan to examine the relationship between several indexes of dioxin emissions from incineration plants and cause-specific mortality among residents. The Ministry of Health and Welfare provided data from 1996 and 1997 on total dioxin emissions from incineration plants. Data on dioxin concentrations, population size, and land area were used to compute four indexes for 426 municipalities: concentration of dioxins, amount of dioxins per unit of population, cumulative amount of dioxin from year of plant construction, and cumulative amount of dioxin per unit of land area. Several socioeconomic indicators also were created from census data on income, unemployment, education, size of residence, and amount of parkland. Mortality data were for 1994–1996. No analyses assessed the effects of different assumptions regarding latency and patterns of mortality.

Municipalities with and municipalities without incineration plants had similar age-adjusted mortality from ischemic heart disease ($p = 0.77$ and $p = 0.24$ for men and women, respectively). Stroke mortality did not vary by the presence of incineration plants for men but was slightly higher among women in municipalities with incineration plants (73.3/100,000 among women in municipalities with plants vs. 70.5/100,000 among women in municipalities without plants; $p = 0.04$). In analyses restricted to municipalities with incineration plants, there was a positive and statistically significant ($p < 0.05$) correlation between stroke mortality and 2 dioxin indexes (the concentration of dioxins released and the amount of dioxins released per population) for men and women. The sex-specific correlations between the dioxin indexes and stroke mortality were modest, ranging from 0.13 to 0.15. In addition, the value of the correlations declined to 0.03–0.05 ($p < 0.05$) after adjustment for socioeconomic characteristics.

Vietnam-Veteran Studies

Kim J-S et al. (2003) reported the results of a cross-sectional study of exposure to Agent Orange and the prevalence of a large number of health outcomes in Korean veterans who had served in Vietnam. The researchers recruited male veterans in 1995 from a list of those who had served in Vietnam and had contacted the Korean government to be examined for possible medical care and compensation for service-related conditions. In all, 4,432 veterans were on this list; 1,514 (34.2%) agreed to participate in the study. Of that group, 1,224 (27.6%) who were age-eligible for the study (45–64 years) completed the study interview

and examination. A comparison group consisted of 2,682 male non-Vietnam veterans who received military pensions and lived near Seoul. Only 154 (5.7%) agreed to participate in the study and provide complete data.

Kim J-S et al. (2003) created an index to estimate exposure to Agent Orange in the Vietnam veterans. The index included information about time spent in different regions of Vietnam and on their reports of exposure to Agent Orange on the skin, in the air, or through drinking or swimming in contaminated water. Quartiles were formed from scores on the index and the categories of exposure were compared with lipid-adjusted TCDD concentrations from blood samples drawn at the 1995 examination. The mean values of TCDD by quartile of the overall exposure index in the Vietnam veterans were 0.6, 0.62, 0.78, and 0.87 pg/g, respectively. The non-Vietnam veterans had an average serum TCDD of 0.3 pg/g. The assessment of health outcomes was done without knowledge of exposure status, but the researchers did not provide specific information on the definition of any health outcomes.

Hypertension was diagnosed in 27.9% (43/154) of non-Vietnam veterans and in 31.3% (383/1,224) of Vietnam veterans. The age-adjusted probability value for the difference in prevalence of hypertension was 0.01; among the Vietnam veterans, the prevalence of hypertension did not vary by quartile of Agent Orange exposure index ($p = 0.90$). In a logistic regression model that adjusted for age, body mass index, education, marital status, alcohol consumption, and tobacco use, the odds ratio for hypertension in Vietnam versus non-Vietnam veterans was 2.29 (95% CI, 1.33–3.95).

None of the other cardiovascular conditions was present in the sample of 154 non-Vietnam veterans. There was a statistically significant variation in the age-adjusted prevalence of disease in the Vietnam veterans, by exposure to Agent Orange for valvular heart disease ($p = 0.002$) and ischemic heart disease ($p = 0.03$). The result for valvular heart disease was attributable to a highly skewed distribution of a few cases—only 8 cases were diagnosed, and 6 were in the third quartile (with single cases in the first and fourth quartiles). Ischemic heart disease was more prevalent in the highest exposure quartile (10.1%) than in the lower quartiles (2%, 3.3%, and 1.6%, respectively). There was no adjustment for potential confounders other than age.

Synthesis

The new occupational and environmental studies of circulatory conditions do not support an association for exposure to herbicides, but they also do not represent compelling evidence for the lack of an association. The study of herbicide applicators (Swaen et al., 2004) uses mortality rates from the general population as the basis for comparison. The relatively low cardiovascular mortality observed for the herbicide applicators can be attributed to the "healthy worker effect," rather than to any health-related benefit of herbicides per se. Comparing

the herbicide applicators with a similar (but non-exposed) group of employed individuals would lead to a more definitive test of the association. The ecological study by Fukuda et al. (2003) reported no association between measures of dioxin emissions and cardiovascular or cerebrovascular mortality after adjustment for socioeconomic correlates of dioxin emissions. However, the study design precludes inferences about the relationship between exposure and disease among individuals. Furthermore, the aggregate measure of dioxins prevents conclusions regarding any specific congeners. For those reasons, the reports by Swaen et al. (2004) and Fukuda et al. (2003) cannot be interpreted as important evidence of *no* association.

The study of Korean veterans (Kim J-S et al., 2003) shows an elevated prevalence of hypertension in Vietnam veterans compared with that for veterans who served elsewhere. This difference is not the result of confounding by important risk factors for hypertension, and the Vietnam veterans did have higher serum TCDD concentrations than did the comparison veterans. Ischemic heart disease was not diagnosed in any of the non-Vietnam veterans, but it was found in Vietnam veterans and was most prevalent among veterans in the highest quartile of the Agent Orange exposure index. However, some of the weaknesses noted in previous reviews of this literature are also present in this study. There is no information on the measurement of disease, and therefore no opportunity to comment on the quality of measurement. For example, is hypertension based on more than one measurement of blood pressure or measurement on more than one occasion? Is ischemic heart disease diagnosed from direct examination or from self-report of a previous diagnosis? If the latter, is the self-report confirmed by medical records that reveal clinical symptoms, ECG changes, and cardiac enzyme concentrations? Confounding has been addressed for hypertension but not for ischemic heart disease, despite the availability of the data.

The most serious concern with the data is the possibility of selection bias in the formation of the study population. A law in Korea to support medical care and compensation for herbicides victims became effective in May 1993, and the sample of Vietnam veterans for the study apparently was drawn from a list of veterans who applied to a government agency for health examinations and potential compensation for health conditions. Therefore, increased participation might be predicted for Vietnam veterans with chronic health conditions, and the selective participation could vary by exposure status (both the "external" and the "internal" comparisons could be biased). The relatively low participation in each group (28% and 6% in Vietnam and non-Vietnam veterans, respectively) underscores the need for additional data on the influence of selection bias in the study.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and specific circulatory disorders (coronary artery disease, myocardial infarction, stroke, 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

Many studies have indicated that TCDD can affect the developing cardiovascular system, but there is only limited evidence that the cardiovascular system is a target of TCDD in adult animals. There have been reports of developmental defects in the cardiovascular systems of TCDD-treated birds and fish. Recently, a dose-dependent increase in myocardial fibrosis has been observed in marmosets acutely exposed to TCDD at relatively low doses. Subchronic treatment with TCDD of hyperlipidemic ApoE-deficient mice 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 for 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 their relevance to humans.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and circulatory disorders, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

AL AMYLOIDOSIS

Amyloidosis (ICD-9 code 277.3) is a group of related disorders that share the common feature of the deposition of insoluable, fibrous amyloid protein, mainly in the extracellular spaces of organs and tissues. Depending on the nature of the

amyloid precursor protein, amyloid fibrils can be deposited in one location or involve multiple organ systems. The deposition of amyeloid might have no clinical consequences or it can lead to severe compromise of organ function; the different consequences occur because some amyloid proteins are more fibrillogenic than others.

AL amyloidosis is the most common form of systemic amyloidosis. Amyloidosis is classified according to the biochemical properties of the fibril-forming protein: the letter "A" (for amyloid) is the first designation; "L" is the protein designation (light chain). Annual incidence is estimated at 1/100,000, or more than 2,000 new cases annually in the United States. Amyloidosis occurs mainly in people 50–70 years old and more often in males than in females (Solomon, 1999).

AL amyloid proteins are derived from immunoglobin light chains. The proteins are common to primary amyloidosis and amyloidosis associated with other disorders, such as multiple myeloma, B-cell lymphomas, or other plasma cell dyscrasias. AL amyloidosis results from a monoclonal population of plasma cells that consistently produce immunoglobulin light chains. Clinical findings can include monoclonal immunoglobulin or immunoglobulin fragment in the urine or serum, nephrotic syndrome, hepatomegaly, carpal tunnel syndrome, macroglossia, peripheral neuropathy, and cardiomegaly. The diagnosis is usually made late in the course of the disease (Buxbaum, 2004).

AL amyloidosis can occur in the setting of other diseases that are associated with exposure to compounds of interest, such as multiple myeloma and B-cell lymphomas. However, most often patients diagnosed with AL amyloidosis do not have multiple myeloma or any other B-cell neoplasm. A common finding is of a modest increase in the number of plasma cells in the bone marrow. The most common disorder associated with AL amyloidosis is multiple myeloma; AL amyloidosis occurs in 15–20% of patients with multiple myeloma. Other associated disorders include monoclonal gammopathies, light chain disease, and agammaglobulinemia (producing light chains but not intact immunoglobulins).

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

The VA identified AL amyloidoisis as a concern after the publication of *Update 1998*. AL amyloidosis was examined specifically by the committees responsible for *Update 2000* and *Update 2002*. Those committees concluded that there was inadequate or insufficient evidence to determine an association between exposure to 2,4-D, 2,4,5-T, 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 2002*.

Synthesis

There is insufficient evidence of an association with AL amyloidosis and the compounds of interest. Although AL amyloidosis and multiple myeloma are similar—they both result from a clonal proliferation of plasma cells—there is insufficient evidence to link the increased risk for multiple myeloma from exposure to the compounds of interest with a possible increased risk in AL amyloidosis.

Conclusions

Strength of Evidence from Epidemiologic Studies

On the basis of its evaluation of the epidemiologic evidence reviewed here and in previous *VAO* reports, the committee concludes that there is inadequate or insufficient evidence to determine an association between exposure to the compounds of interest and AL amyloidosis.

Biologic Plausibility

An association was reported between primary AL amyloidosis and TCDD exposure in a single study in mice (Toth et al., 1979). However, the TCDD exposure was relatively high (0.007–7.0 μg/kg per week for 1 year) and the amyloidosis was regarded as occurring only secondary to chronic skin lesions.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and AL amyloidosis, coupled with the lack of exposure information on Vietnam veterans, precludes quantification of any possible increase in their risk.

ENDOMETRIOSIS

Endometriosis (ICD-9 code 617) is a disease that affects 5.5 million women in the United States and Canada (NICHD, 2004). The endometrium is the tissue that lines the inside of the uterus that 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 the result is inflammation, internal bleeding,

and degeneration of blood and tissue from the growth that can cause scarring, pain, infertility, adhesions, and intestinal problems.

There are several theories about the etiology of endometriosis, including genetics, but the exact 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 but only those with immune system or hormonal problems experience the tissue growth associated with endometriosis. Despite numerous symptoms that can indicate endometriosis, diagnosis of the disease is possible 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 began after the observation that the incidence of endometriosis was higher in monkeys treated with low doses of TCDD than in control monkeys (Rier et al., 1993). Experimental and epidemiologic studies have been conducted. Several epidemiologic studies have investigated non-dioxin-like PCBs, and some have examined a possible association with TCDD or dioxin-like compounds.

Summary of *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002*

Endometriosis was first reviewed in this series of reports in *Update 2002*, which identified two relevant studies. Both were small and showed elevated odds ratios, but for both the confidence intervals were very wide and included 1.0. Relevant studies reviewed earlier and in this update are summarized in Table 9-3.

Update of the Scientific Literature

Three environmental studies have been conducted since *Update 2002* that examined the relationship between exposures to some of the compounds of interest and endometriosis. Eskenazi et al. (2002) investigated the development of endometriosis among participants of the Seveso Women's Health Study. The cohort consisted of women who had lived in proximity to the Seveso accident site in 1976 and had TCDD serum measurements in blood collected between 1976 and 1980. Among 601 participants examined 20 years after the accident, 19 had confirmed endometriosis cases; 277 were non-diseased; 305 had symptoms but unconfirmed diagnoses and were classified as "uncertain." Women in the highest exposure group (TCDD >100 parts per trillion [ppt]) showed a doubling in the risk of endometriosis compared with the lowest exposure group (TCDD ≤20 ppt), although the increase was not statistically significant (relative risk [RR] = 2.2, 95% CI, 0.5–8.0), possibly because of the small number of confirmed cases ($N = 9$). No increase was found among the highly exposed uncertain group or among the mid-exposure cases (20.1–100 ppt). Grouping women in the uncertain

TABLE 9-3 Selected Epidemiologic Studies—Endometriosis

Reference	Study Population	Study Results
ENVIRONMENTAL		
New Studies		
De Felip et al., 2004	Pilot study of Italian and Belgian women of reproductive age; compared concentrations of TCDD and total TEQ in pooled blood samples from women diagnosed with endometriosis to controls	*Mean Concentration TCDD (pg/g lipid)* Italy: Controls (10 pooled samples) = 1.6; Cases (2 sets of 6 pooled samples) = 2.1, 1.3 Belgium: Controls (7 pooled samples) = 2.5; Cases (Set I, 5 pooled samples; Set II, 6 pooled samples) = 2.3,2.3 *Mean Concentration (pg TEQ/g lipid)* Italy: Controls (10 pooled samples) = 8.9±1.3 (99% CI, 7.2–11); Cases (2 sets of 6 pooled samples) = 10.7±1.6; 10.1±1.5 Belgium: Controls (7 pooled samples) = 24.7±3.7 (99% CI, 20–29); Cases (Set I, 5 pooled samples; Set II, 6 pooled samples) = 18.1±2.7; 27.1±4.0
Fierens et al., 2003	Belgian women with environmental exposure to PCDDs/PCDFs or dioxins; compared analyte concentrations[a] in cases vs controls	Mean concentrations (pg TEQ/g lipid): Cases (n = 10) = 26.2 (95% CI, 18.2–37.7) Controls (n = 132) = 25.6 (95% CI, 24.3–28.9) No significant difference
Eskenazi et al., 2002	Residents of Seveso Zones A and B ≤30 years of age in 1976; compared incidence of endometriosis across serum TCDD concentrations	Serum TCDD 20.1–100 ppt: 8 exposed cases; OR = 1.2 (90% CI, 0.3–4.5) Serum TCDD >100 ppt: 9 exposed cases; OR = 2.1 (90% CI, 0.5–8.0)
Studies Reviewed in *Update 2002*		
Pauwels et al., 2001	Patients undergoing infertility treatment in Belgium; compared number of women with endometriosis and without endometriosis who had serum dioxin levels ≥100 pg TEQ/g serum lipid	6 exposed cases; OR = 4.6 (95% CI, 0.5–43.6)

continues

TABLE 9-3 *Continued*

Reference	Study Population	Study Results
Mayani et al., 1997	Residents of Jerusalem being evaluated for infertility; compared number of women with elevated TCDD concentrations in diagnosed with endometriosis (n=44) with subjects not diagnosed with endometriosis (n=35)	8 exposed cases; OR = 7.6 (95% CI, 0.9–169.7)

[a] Dioxin TEQs calculated using the WHO (1998) Toxic Equivalency Factor methodology.
ABBREVIATIONS: PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofurans; TCDD, tetrachlorodibenzo-*p*-dioxin; TEQ, toxicity equivalents.

category with the non-diseased women resulted in an increasing trend with exposure (RR, 1.6, and RR, 2.8 for the mid- and high-exposure groups, respectively), but the findings were not significant. A major limitation of the study was the inability to confirm with laparoscopy the disease state of the largest group, those with an uncertain diagnosis. All participants had resided in the two zones closest to the Seveso plant, so no truly unexposed control group was included in the study for comparison.

Fierens et al. (2003) completed a population-based cross-sectional study of residents in several Belgian towns in the vicinity of industrial sites or municipal solid-waste incinerators and a control group with no known exposures to dioxins or PCBs. The research included an assessment of the association between serum dioxin concentration and the prevalence of endometriosis. Self-administered questionnaires identified 10 cases of endometriosis in 142 females responding. Mean analyte concentrations were determined based on measurements of seventeen 2,3,7,8-polychlorinated dibenzodioxins or dibenzofurans. There was no difference in mean TEQ concentrations between the 10 cases and 132 controls (26.2 pg TEQ/g lipid [95% CI, 18.2–37.7] and 25.6 pg TEQ/g lipid, respectively). The study's usefulness is compromised because of reliance on self-reports and because of the small number of cases.

De Felip et al. (2004) conducted a pilot case–control study of women of reproductive age in Italy (N = 22) and Belgium (N = 18) to determine whether there is a correlation between blood concentrations of dioxin-like compounds and endometriosis. Subjects were selected from patients at the gynecological department of one hospital each in Italy and in Belgium. Selection criteria included nulliparity, absence of a chronic disease, and absence of an ascertained professional exposure to environmental contaminants. Controls were those patients

suspected of having a benign adnexal mass; cases were suspected of having endometriosis. Dietary habits were documented by a questionnaire. All patients underwent a 10 mm laparoscopy, and the presence of endometriosis was confirmed by histologic examination of lesions. Of the subjects, 12 Italian and 11 Belgian women had endometriosis (cases). Blood samples were pooled for analyte analyses in sets (all including women ranging in age from 18 to 40 years), as follows: For Italian subjects, one set from controls ($N = 10$) and two sets from the cases (each set, $N = 6$). For Belgian subjects, one set from the controls ($N = 7$) and two sets from the cases ($N = 5$ and $N = 6$). Congener-specific concentrations were reported for 2,3,7,8-TCDD. The pooled blood concentrations, expressed in pg/g lipid, were as follows: In Italy, 1.6 for controls, 2.1 and 1.3 for the case sets; in Belgium, 2.5 for controls, 2.3 and 2.3 for the case sets. The data, therefore, do not indicate that the concentration of 2,3,7,8-TCDD was elevated in women with endometriosis. Looking at total TEQ (from PCDDs, PCDFs, and dioxin-like PCBs), the mean concentration was 8.9 ± 1.3 pg TEQ/g lb (99% CI, 7.2–11) in Italian controls, and 10.7 ± 1.6 and 10.1 ± 1.5 pg TEQ/g lb in Italian cases. In Belgian women the corresponding values were 24.7 ± 3.7 pg TEQ/g lb (99% CI, 20–29) in controls and 18.1 ± 2.7 and 27.1 ± 4.0 pg TEQ/g lb in cases. Overall, the study did not show that women with endometriosis had higher 2,3,7,8-TCDD or total TEQ than did controls. The study is limited in its ability to detect differences, however, by the small number of subjects. The selection criteria, which allowed all women with suspected gynecological abnormality, also introduced bias. The study did show that blood concentration of dioxin tend to be higher among Belgian women than in Italian women; concentrations in the Belgian group were comparable to those in the Belgian subjects in the study by Fierens et al. (2003).

No relevant occupational or Vietnam-veteran studies have been published since *Update 2002.*

Synthesis

None of the three studies discussed in this report demonstrated an increased risk for endometriosis with exposure to dioxin or dioxin-like compounds. Those results are consistent with the two case–control studies reviewed previously (IOM, 2003), both of which showed elevated odds ratios, but for which the confidence intervals were very wide and included 1.0. It should be noted, however, that all those studies were limited in their ability to detect an increase in endometriosis with exposure to dioxin or dioxin-like compounds, especially considering the small number of confirmed cases of endometriosis in most of the studies.

Conclusions

Strength of Evidence from Epidemiologic Studies

There is inadequate or insufficient evidence to determine an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and endometriosis.

Biologic Plausibility

There is evidence from animal studies that TCDD can exacerbate or cause endometriosis. Other evidence demonstrates that TCDD inhibits progesterone-associated transforming growth factor b_2 expression and endometrial matrix metalloproteinase suppression; those effects have been suggested as mechanisms that underlie an association between TCDD and endometriosis. The ability of TCDD to alter the expression of several growth factors, cytokines, and hormones also could mediate the promotion of endometriosis. Notably, the AhR and several AhR target genes are expressed in human endometriotic tissues. Functional AhRs are present in endometrial and endometriotic stromal cells and TCDD up-regulates RANTES (regulated on activation, normal T expressed and secreted) expression on those cells—also a possible mechanism in dioxin's activity in endometriosis. Other data, however, do not support the hypothesis that dioxin may lead to the development of endometriosis. Because some animal data and the sparse human data support the biologic plausibility of an association between TCDD exposure and endometriosis, the investigations should continue.

Increased Risk of Disease Among Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and endometriosis, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

THYROID HOMEOSTASIS

The earlier volumes in this series of reports (IOM, 1994, 1996, 1998, and 2001) did not specifically address the thyrotoxic potential of TCDD and the herbicides used in Vietnam. The topic of thyroid homeostasis was first subjected to an integrated review in *Update 2002* (IOM, 2003).

The thyroid gland secretes the hormones thyroxine (T4) and triiodothyronine (T3), which stimulate metabolism. 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 operates in thyroid

physiology both as a constituent of thyroid hormones and as a regulator of glandular function. Control of circulating concentrations of those 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 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 hamper the development of many organ systems, including the nervous and reproductive 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 not usually associated with either hyperthyroidism or hypothyroidism.

TCDD affects the concentrations of thyroid hormones; the effects appear to be species dependent and could 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.

Summary of *Update 2002*

The thyrotoxic potential of the compounds of interest was first addressed in this series of reports in *Update 2002* (IOM, 2003). The committee responsible for *Update 2002* concluded that there was inadequate or insufficient information to determine an association between exposure to 2,4-D, 2,4,5-T, 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.

Update of the Scientific Literature

Occupational Studies

Johnson et al. (2001) measured serum hormone and TCDD concentration in 37 men who had sprayed 2,4,5-T in Victoria, Australia. In correlation analysis,

TCDD concentrations were inversely related to T3 and TSH. The association was strongest when historical, but not current, serum TCDD concentrations were considered. In a paper reviewed in *Update 1998*, Zober et al. (1994) examined 138 BASF workers exposed to TCDD in a 1953 industrial incident. The researchers reported that thyroid disease was increased ($p < 0.05$) in the exposed population.

Environmental Studies

No relevant environmental studies have been published since *Update 2002*.

Vietnam-Veteran Studies

Pavuk et al. (2003) examined thyroid hormone status in the AFHS cohort. At each examination (1982, 1985, 1987, 1992, 1997) 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 did 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 in the high-exposure Ranch Hand group than in the comparison population, but the confidence intervals were wide and included 1 at each examination (1982: OR, 1.8, 95% CI, 0.7–5.9; 1985: OR, 1.4, 95% CI, 0.7–3.2; 1987: OR, 1.9, 95% CI, 0.8–4.5; 1992: OR, 1.7, 95% CI, 0.8–3.9; 1997: OR, 1.8, 95% CI, 0.9–3.4).

An earlier study of the Ranch Hand cohort (AFHS, 1991) was reviewed in *VAO*, but not reconsidered in *Update 2002*. The assessment of endocrine function in that study included a series of thyroid function tests, which showed no difference in thyroid function between exposed and control veterans.

Synthesis

Numerous animal experiments and several epidemiologic studies show that TCDD and dioxin-like compounds appear to exert an influence on thyroid homeostasis. The effects are hypothesized to provide a mechanism by which TCDD may affect early development of neurologic and sensory organs and of motor function when exposure occurs in utero or during lactation. However, clear effects of TCDD on thyroid homeostasis have also been observed when adult animals were exposed. Several human studies observed increases in TSH without evidence of increases in T4, indicating that the infants (selected for uncompli-

cated gestation, labor, and delivery) and the Ranch Hand Air Force personnel were able to adapt to the changes in thyroid status that might have been induced by the higher body burdens of TCDD and TEQ.

Conclusions

Strength of Evidence from Epidemiologic Studies

There is inadequate or insufficient evidence of an association between exposure to the compounds of interest and adverse effects on thyroid homeostasis. Some effects have been observed in humans, but the functional importance of those changes is unclear because adaptive capacity could be adequate to accommodate them.

Biologic Plausibility

TCDD affects concentrations of T4, T3, and TSH in experimental animals, but the effects lack 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. The National Toxicology Program reported that female rats exposed chronically to TCDD demonstrated altered thyroid follicles. This was further characterized as being attributable to a reversible hypertrophic response of the follicular cell. Chapter 3 discusses recent toxicologic studies relevant to the biologic plausibility of the effects of TCDD and the herbicides on the thyroid gland.

Increased Risk of Disease in Vietnam Veterans

The lack of data on the association between exposure to the chemicals of interest and adverse effects on thyroid homeostasis, coupled with the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

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 an association between exposure to 2,4-D, 2,4,5-T, 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 that were used in *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, and *Update 2002* to categorize diseases by the strength of the evidence.

Health Outcomes with Sufficient Evidence of an Association

For diseases in this category, a positive association between exposure and outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. The committee regarded evidence from several small studies that are free 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.

For *VAO*, *Update 1996*, *Update 1998*, *Update 2000,* and *Update 2002*, the committees concluded that there was sufficient evidence of an association between exposure to at least one compound of interest 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 exposure and outcome, it can 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.

For *Update 1996, Update 1998*, *Update 2000,* and *Update 2002,* the committees determined there was limited or suggestive evidence of an association between exposure to at least one compound of interest 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 concluded that there was limited or suggestive evidence of an association between exposure to at least one compound of interest and type 2 diabetes. The committee responsible for *Update 2002* reached that same conclusion. Evidence reviewed in this 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 Association

The scientific data on many of the health effects reviewed by the committee were inadequate or insufficient to determine an association between exposure to the compounds 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 association. Some studies failed to control for confounding or used inadequate exposure assessment. This category includes non-malignant 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 known human exposure must be consistent in not showing a positive association between exposure and outcome at any magnitude of exposure. The studies also must 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. 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*, *Update 2000*, and *Update 2002* 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, 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 the compounds of interest and various non-cancer 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 elicits 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 alters 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's effects mediate non-cancer endpoints is not clear. TCDD has been shown to inhibit hepatocyte DNA synthesis, alter vitamin A homeostasis, 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 (differential regulation of genes) that alter cell function. Although structural differences in the AhR have been identified in various species, the receptor operates similarly in animals and humans. Animal data support a biologic basis for 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.

There is little evidence that the cardiovascular system is a sensitive target of TCDD toxicity in adult animals. It has been proposed however, that dioxin may increase the incidence of ischemic heart disease by exacerbating its severity. For example, treatment of rats with dioxin-like chemicals was found to affect several cardiovascular risk factors including heart weight, serum cholesterol, and blood pressure.

The immune system is particularly 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, and that results in a decrease in the number and response some types of T cells. Some recent evidence suggests that TCDD directly affects T cells and their ability to undergo activation. TCDD could 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, also could be affected by TCDD. The function and commitment patterns of hematopoietic stem cells may also be affected by TCDD. As with other effects of TCDD, the immunotoxic effects are species 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. Despite considerable laboratory research, the mechanisms that underlie 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 also could 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.

Although data on the health effects of the herbicides discussed in this report are not extensive, effects have been seen in several 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 also has 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 examined the potential immunotoxicity of the herbicides used in Vietnam. Effects on the immune systems of mice were reported for 2,4-D administered at doses that were high enough to produce clinical toxicity; the 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; by the lack of strong evidence of organ-specific effects among species; and by differences in route, dose, duration, and timing of exposure. Investigating the biologic mechanisms that underlie TCDD's toxic effects continues to be an area of active research, and future updates of this report might be able to present more and better information on which to base conclusions, at least for that compound.

Increased Risk of Disease Among Vietnam Veterans

Although there are data to suggest an association between exposure to the chemicals of interest and some of these health effects, the lack of exposure information on Vietnam veterans precludes quantification of any possible increase in their risk.

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10

Research Recommendations

As part of its charge, the committee makes recommendations concerning the need, if any, for additional scientific studies to resolve uncertainties concerning the health effects of the chemicals of interest 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 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 of interest and in elucidating the mechanisms underlying those effects, there are still important gaps in our knowledge. The scope of potential research on these chemicals is wide, and this is not an exhaustive list of future research that might have value.

VIETNAM VETERAN STUDIES

As in prior reports (IOM, 1994, 1996, 1999, 2000, 2001, 2003) that reviewed the epidemiologic evidence and evaluated the quality of available information, this committee recommends continuation of epidemiologic studies of existing veterans cohorts. This is especially important as the veteran populations grow older and the incidence of many health outcomes (e.g., cancer, neurological disorders) increases with age. The same reasoning is applicable to other established cohorts (such as NIOSH, Seveso, and IARC). The committee considers such continued research essential for addressing diseases with long latency or

age-related incidence. In addition, a high priority should be placed on clarifying the role of genetic factors, in particular the role of aryl-hydrocarbon receptor (AhR) polymorphisms, in determining individual susceptibility to disease.

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 who conducted aerial spray missions (Operation Ranch Hand). A baseline morbidity study and a matched comparison cohort study were initiated in 1982, with follow-up assessments in 1985, 1987, 1992, and 1997. In accordance with the study protocol, one additional assessment was completed in 2003, and a final report will be issued in May, 2005 (personal communication, Joel Michalek, Brooks Air Force Base, September 24, 2004).

The AFHS is one of the few primary sources of information on the health of Vietnam veterans known to have been exposed to Agent Orange and other herbicides, and the study is coming to its scheduled end. A congressionally-mandated Institute of Medicine (IOM) committee has been recently formed to evaluate the future of the AFHS. That IOM committee is charged with evaluating the scientific merit of maintaining the records and samples, and how those specimens could be made available for independent researchers. That committee also will evaluate the merit of extending that study beyond its scheduled end and, if extended, what oversight would be most appropriate. Previous *VAO* committees have recommended extending the AFHS, and this committee encourages the newly appointed AFHS review committee to consider those recommendations in the course of its deliberation.

Army Chemical Corps Study

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 have demonstrated increased TCDD concentrations in Chemical Corps veterans who reported spraying herbicides as part of their duties. Information on health outcomes from that cohort is expected to provide insight into the effects of the chemicals of interest on the entire population of Vietnam veteran. The committee has long awaited publication of more data from this study, and reasons why it has not been forthcoming are not apparent. Issues concerning continuation similar to those being evaluated by the IOM committee reviewing the AFHS should be assessed as well for the study of the Army Chemical Corps veterans.

National Institute of Occupational Safety and Health (NIOSH) Cohort

Starting in 1978, NIOSH began to study US workers potentially exposed to TCDD. In total, 5,132 workers from 12 large manufacturing companies were included in this cohort. The NIOSH cohort has been an extremely valuable source of data in assessing the health effects associated with TCDD exposure. The studies have included high quality exposure assessment, and evaluations of a wide range of health outcomes have been published. Given its value as an important source of epidemiological data, the committee recommends that studies of the NIOSH cohort be extended.

Exposure Reconstruction Study

The IOM Committee on the Assessment of Wartime Exposure to Herbicides in Vietnam oversaw the development of an herbicide exposure model for Vietnam veterans (the "Stellman model"), which was described in detail in a recent publication (IOM, 2003b,c). That committee concluded the model was adequate for use in epidemiology studies. This committee recommends that the model be validated using appropriate measures of exposure (e.g., serum TCDD concentrations) in appropriate study populations.

STUDIES OF VIETNAMESE

As discussed in earlier updates, the Vietnamese represent an understudied population. Although there are likely to be significant logistical challenges, the many people with substantial exposure within the Vietnamese population represent a potentially informative study sample. It will be important to include appropriate exposure measures, such as TCDD levels in tissues, when studying Vietnamese residents. 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.

OTHER RESEARCH

The committee recognizes that the study of the possible diseases associated with exposure to the chemicals of interest is complicated by the latency period related to many health outcomes. Appropriate analytic techniques are available for addressing issues of latency, as reviewed in the recent IOM report on such issues (IOM, 2004). However, most studies to date have used relatively simple approaches. Approaches such as using a predetermined lag period or assessing cumulative exposure could potentially lead to erroneous conclusions about true dose-response relationships, thereby precluding detection of important health

effects. Hence, more detailed and sophisticated latency analyses should be used in future research efforts.

The committee believes that experimental research into the mechanisms that underlie human health outcomes can provide valuable information related to risk of disease in Vietnam veterans. The central role of the AhR in animal models is clear, and AhR gene differences in animals clearly affect susceptibility to TCDD. Although work to date on the AhR in humans has been limited, variations in this specific genetic factor alone are likely to affect human susceptibility to the toxic effects of TCDD, other dioxin-like chemicals, and herbicide formulations containing these chemicals. However, in addition to the AhR, it is also clear from recent research that variations in the genetics regulating the expression or activity or other factors, including proteins interacting with the AhR as well as the gene products regulated by the AhR, are critical in determining susceptibility to TCDD and the type of toxic effect observed. Studies addressing the identification, distribution, and functional consequences of polymorphisms of the AhR and these other cofactors in human populations should be pursued.

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APPENDIX A

Epidemiologic Tables for Chapter 4

In Tables A-1, A-2, and A-3, respectively, studies are grouped according to whether their subjects had occupational exposures, had environmental exposures, or were specifically veterans. The tables provide an overview of design aspects of those epidemiologic studies reviewed in this and earlier reports that presented results on more than one health outcome or that investigated populations that have been repeatedly studied. The summaries include the study's design type, the numbers of subjects in the study and comparison populations, and a synopsis of how subjects were selected, how data were collected, what inclusion criteria were used, and how exposure was determined. Results were discussed in the appropriate health outcome chapter of the *Veterans and Agent Orange* document in which the publication was reviewed. The references for this section are at the end of Chapter 4.

TABLE A-1 Epidemiologic Studies—Occupational Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
PRODUCTION WORKERS				
***NIOSH Studies Reviewed in* Update 2002**				
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 Hand subjects	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. Endpoints reported are all cancers, ischemic heart disease, and diabetes	5,132 (3,538 with exposure data divided into septiles of cumulative exposure; 608 with 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 A-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 A-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				
Bodner et al., 2003	Cohort	Additional 10-year follow-up of cohort studied by Cook et al. (1986), through 1995; Dow cohort findings compared with IARC International Study and NIOSH Dioxin Registry.	2,187	—
***Dow Studies Reviewed in* Update 2002**				
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	40,600 non-exposed 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 non-exposed

***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 non-exposed 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	—

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Sobel et al., 1987	Case-control	Study of STS among Dow chemical employees (1940–1979) compared to employees without STS for possible association with several chemical exposures	14	126
Cook et al., 1986	Cohort	Mortality experience (1940–1979) of men manufacturing chlorinated phenols compared to 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

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***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)	—
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	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 non-exposed 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	—

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Coggon et al., 1991	Cohort	Mortality experience among four cohorts of workers potentially exposed (1963–1985) to phenoxy herbicides and chlorophenols compared to national (England and Wales) expected numbers and to the local population where factory is located	1,104 Factory A 271 Factory B 345 Factory C 519 Factory D	—
Manz et al., 1991	Cohort	Mortality experience of workers (1952–1984) at Hamburg plant of Boehringer exposed to TCDD compared to national mortality and workers from another company	1,184 men 399 women	(a) population (b) 3,120 gas workers
Saracci et al., 1991	Cohort	Study of mortality experience of 20 international cohorts of herbicide sprayers and production workers compared to mortality experience expected for the nation	16,863 men 1,527 women	—
Coggon et al., 1986	Cohort	Study of mortality experience (through 1983) among workers manufacturing and spraying MCPA (1947–1975) compared to expected numbers of deaths among men of England and Wales and for rural areas	5,754	—

Lynge, 1985	Cohort	Study of cancer incidence among Danish workers exposed to phenoxyherbicides compared to expected results from the general population	3,390 men 1,069 women	—
***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)

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***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
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 non-exposed 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				
***Cohort Studies of Agricultural Workers Reviewed in* Update 2002**				
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

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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
***Cohort Studies of Forestry Workers Reviewed in* Update 2002**				
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	—
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 non-exposed workers	54	54
New Cohort Studies of Herbicide and Pesticide Appliers				
Flower et al., 2004	Prospective cohort	Parental pesticide application and cancer risk in offspring of pesticide appliers in AHS cohort	20,625 appliers and spouses; 21,375 children born during or after 1975	—
Swaen et al., 2004	Cohort	Mortality follow-up in Dutch male herbicide appliers	1,341	—
Alavanja et al., 2003	Prospective cohort	Correlation between pesticide exposure (including 2,4-D and 2,4,5-T) and prostate cancer in pesticide appliers	55,332	—
***Cohort Studies of Herbicide and Pesticide Appliers Reviewed in* Update 2002**				
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 Appliers 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 and Wilkund 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	—

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***Cohort Studies of Herbicide and Pesticide Appliers 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)	—
***Cohort Studies of Herbicide and Pesticide Appliers 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 Appliers 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	—

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

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

García 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
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	130,420 death certificates	

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
LaVecchia et al., 1989	Case-control	Study of Italian men and women with HD, NHL, and MM (1983–1988), compared to population of Italy for association with occupations and herbicide use	69 HD 153 NHL 110 MM	396
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	(1) 330 (2) 190
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 A-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 A-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

continues

TABLE A-1 *Continued*

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

continues

TABLE A-1 *Continued*

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

TABLE A-1 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 US 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:2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic; AHS, Agricultural Health Study; CDC, Centers for Disease Control and Prevention; CLL, Chronic lymphocytic leukemia; COPD, chronic obstructive pulmonary disease; FRG, Federal Republic of Germany; HD, Hodgkin's disease; IARC, International Agency for Research on Cancer; ICD, International Classification of Diseases; MCPA, methyl-4 chlorophenoxyacetic acid; MM, multiple myeloma; NIOSH, National Institute for Occupational Safety and Health; NHL, non-Hodgkin's lymphoma; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofurons; PD, Parkinson's disease; PCP, pentachlorophenol; PCT, porphyria cutanea tarda; PMR, proportionate mortality ratio; SEER, surveillance, epidemiology, and end results; SES, socioeconomic status; SIR, standardized incidence ratio; SMR, standardized mortality ratio; STS, soft-tissue sarcoma; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCP, 2,4,5-trichlorophenol; TSH, thyroid-stimulating hormone; T3, triiodothryonine; T4, thyroxine; USDA, US Department of Agriculture; Update *2000,* Veterans and Agent Orange: *Update 2000* (IOM, 2001); *Update 1998, Veterans and Agent Orange: Update 1998* (IOM, 1998); *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 A-2 Epidemiologic Studies—Environmental Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
New Studies from Seveso				
Baccarelli et al., 2004	Population based	mRNA concentrations, AhR, ARNT, CYP1A1, CYP1B1 genes, and EROD activity in peripheral blood lymphocytes in a Seveso, Italy cohort	62	59
Eskenazi et al., 2004	Cohort	Relationship between serum TCDD concentration and age at exposure in SWHS participatants	899	None
Eskenazi et al., 2003a	Cohort	Association between maternal serum TCDD and birth outcome SWHS participants	888	None
Landi et al., 2003	Population based	Effect of TCDD-mediated alterations in AhR-dependent pathway in Seveso zone A and B residents	62	59
Baccarelli et al., 2002	Population based	Immunologic effects in Seveso residents, compared with previous published results	62	59
Eskenazi et al., 2002a	Cohort	Association between menstrual cycle characteristics and serum TCDD in SWHS participatants	381	None
Eskenazi et al., 2002b	Cohort	Association between endometriosis and serum TCDD concentration in SWHS participants	601	None
***Studies from Seveso Reviewed in* Update 2002**				
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 SWHS	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
Pesatori et al., 1998	Cohort	Mortality (through 1991) study of residents in industrial accident exposure-related geographic regions	805 zone A 5,943 zone B 38,625 zone R	232,747
***Studies from Seveso Reviewed in* Update 1998**				
Bertazzi et al., 1997, 1998	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

continues

TABLE A-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 non-exposed 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 non-exposed residents in neighboring towns	556 zone A 3,920 zone B 26,227 zone R	167,391

Barbieri et al., 1988	Cohort	Comparison of prevalence of peripheral nervous system involvement among Seveso residents with chloracne, compared to residents 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

continues

TABLE A-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Filippini et al., 1981	Cohort	Comparison of prevalence of peripheral neuropathy on two screening examinations among Seveso residents, compared to residents in non-exposed 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
Stehr-Green et al., 1986; Hoffman et al., 1987	Cohort	Health effects (1971–1984) in Quail Run Mobile Home Park residents compared with residents of noncontaminated mobile home parks	154	155
Webb et al., 1987	Cross-sectional	Pilot study of Missouri residents exposed to TCDD in the environment (1971) for health effects, comparing potentially high-exposed to low-exposed residents	68 (high exposure)	36 (low exposure)

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

continues

TABLE A-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Constable and Hatch, 1985	Review	Summaries of reproductive outcomes among Vietnamese populations, includes nine unpublished studies		
Other New Environmental Studies				
Fierens et al., 2003	Population-based, cross-sectional	Association between serum dioxin, prevalence of diabetes, endometriosis in several Belgian towns	194	63
Fukuda et al., 2003	Ecologic cohort	Correlation between incinerator dioxin emissions and mortality in 803 Japanese municipalities	426 Municipalities with plants	164 Municipalities without plants
***Other Environmental Studies Reviewed in* Update 2002**				
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
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

continues

TABLE A-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 non-exposed 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
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)	—	—

continues

TABLE A-2 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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: 2,4,5-T, 2,4,5-trichlorophenoxyacetic; AhR, arylhydrocarbon receptor; AML, acute myelogenous leukemia; ARNT, arylhydrocarbon receptor nuclear transporter; BCC, basal cell carcinoma; CYP1A1, cytochrome p450 1A1; CYP1B1, cytochrome p450 1B1; EROD, 7-ethoxyresorufun-*O*-deethylase; HD, Hodgkin's disease; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; PCB, polychlorinated biphenyls; PD, Parkinson's disease; SCC, squamous cell carcinoma; SWHS, Seveso Women's Health Study; STS, soft-tissue sarcoma; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; *Update 2000, Veterans and Agent Orange: Update 2000 (*IOM, 2001); *Update 1998, Veterans and Agent Orange: Update 1998 (*IOM, 1998); *Update 1996, Veterans and Agent Orange: Update 1996* (IOM, 1996); US EPA, United States Environmental Protection Agency; and *VAO, Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (IOM, 1994).

TABLE A-3 Epidemiologic Studies—Veterans' Exposure

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
UNITED STATES STUDIES				
New Ranch Hand Studies				
Akhtar et al., 2004	Cohort	Follow-up to Ketchum et al. (1999), comparing cancer incidence among Ranch Hands with Vietnam veterans who served in Southeast Asia but did not spray herbicides and with US national cancer rates	1,189 Ranch Hands for external analysis; 1,009 Ranch Hands for internal analysis	1,776 Comparison subjects for external analysis; 1,429 comparison subjects for internal analysis
Barrett et al., 2003	Cohort	Serum TCDD measurement and psychological functioning among Ranch Hand veterans	1,109	1,493
Michalek et al., 2003	Cohort	Correlation for TCDD elimination and Ranch Hands with diabetes	343	No comparison group
Pavuk et al., 2003	Cohort	Study to examine the relationship between serum TCDD and thyroid function in Ranch Hand veterans	1,009	1,429
***Ranch Hand Studies Reviewed in* Update 2002**				
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 among Ranch Hand veterans	937	1,052
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 among Ranch Hand veterans	1,109	1,493

continues

TABLE A-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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 among Ranch Hand veterans	761	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 hematologic function among Ranch Hand veterans	953	1,280
Steenland et al., 2001	Cohort	A study to reexamine and compare diabetes data from the NIOSH cohort and the US 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 among Ranch Hand veterans	980	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 among Ranch Hand veterans	871	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	914 372 (lymphocyte counts conducted)	1,186 491 (lymphocyte counts conducted)
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 among Ranch Hand veterans	930	1,200

continues

TABLE A-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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	859	1,223
***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)

continues

TABLE A-3 *Continued*

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

continues

TABLE A-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
***Department of Veterans Affairs (VA) Studies Reviewed in* Update 2002**				
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
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
Kang et al., 2000b	Cohort	Study of gynecologic cancers among female Vietnam veterans compared to femae veteran controls	484	5,946
***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

continues

TABLE A-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Dalager et al., 1991	Case-control	Cases of NHL diagnosed 1969–1985 among Vietnam-era veterans compared to cases of other malignancies among Vietnam-era veterans for association with Vietnam service	201	358
Eisen et al., 1991	Cohort	Health effects of male monozygotic twins serving in the armed forces during Vietnam era (1965–1975)	2,260	2,260
Thomas et al., 1991	Cohort	Mortality experience (1973–1987) among women Vietnam veterans compared to women non-Vietnam veterans and for each cohort compared to 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 STET/OIC (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

continues

TABLE A-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
Stellman SD et al., 1988	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 JM et al., 1988	Cohort	Assessment of social and behavioral outcomes among American Legionnaires who served in Southeast Asia (1961–1975) for association with combat and herbicide exposure	2,858	3,933
***State Studies 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 non-veteran 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

continues

TABLE A-3 *Continued*

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

continues

TABLE A-3 *Continued*

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

continues

TABLE A-3 *Continued*

Reference	Study Design	Description	Study Group (*N*)	Comparison Group (*N*)[a]
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				
Other New Vietnam-Veterans' Studies				
Kim H-A et al., 2003	Cohort	Immunotoxicologic effects of Agent Orange exposure on Korean Vietnam veterans	51 (24 Veterans–patient; 27 veterans–normal)	36
Kim J-S et al., 2003	Cross–sectional	Agent Orange exposure and Korean Vietnam veterans	1,224	154
Mo et al., 2002	Cohort	Skin and general disease patterns among Korean Vietnam veterans	332	None
***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: 2,4,5-T, 2,4,5-trichlorophenoxyacetic; AFHS, Air Force Health Study; CDC, Centers for Disease Control and Prevention; CDVA, Commonwealth Department of Veterans' Affairs; HD, Hodgkin's disease; MVA, mother vehicle accidents; NIOSH, National Institute for Occupational Safety and Health; NHL, non-Hodgkin's lymphoma; NMES; National Medical Expenditure Survey; PTSD, posttraumatic stress disorder; SMR, standardized mortality ratio; STS, soft-tissue sarcoma; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; *Update 2000, Veterans and Agent Orange: Update 2000* (IOM, 2001); *Update 1998, Veterans and Agent Orange: Update 1998* (IOM, 1998); *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).

APPENDIX B

Agendas of Public Meetings Held by the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Fifth Biennial Update)

FIRST PUBLIC MEETING

Wednesday, May 19, 2004
Room 109, Keck Building,
Washington, D.C.

Presentations

- **Welcome, Opening Remarks, and Introductions**
  John Stegeman, Committee Chair

- **Charge to the Committee**
  Mark Brown, Department of Veterans Affairs
  Director, Environmental Agents Service
  Department of Veterans Affairs
  Washington, DC

- **Veterans' Concerns**
  Rick Weidman, Vietnam Veterans of America

- **Veterans' Concerns Regarding Acute Myelogenous Leukemia**
  Marion Gillespie, Wife of Vietnam Veteran

SECOND PUBLIC MEETING

Wednesday, July 7, 2004
House Room, Hyatt on Capitol Square
75 East State Street
Columbus, Ohio

Presentations

- **Opening Remarks**
  John Stegeman, Committee Chair

- **Esophageal Carcinoma**
  Delvin Plonka, Veteran Service Officer, St. Joseph County, Indiana

- **Concerns Regarding Agent Orange and Cancers**
  Vicki Noyes, Wife of Vietnam Veteran

- **Concerns of Veterans in Their 50s and 60s Due to Agent Orange**
  John Stoner, Veterans' Representative, State of Ohio

- **Concerns of Veterans**
  David Barker, Ohio AMVETS

- **Health Concerns from Agent Orange**
  Richard Rollins, Vietnam Veteran

- **Recent Work on the Ranch Hand Study**
  Joel Michalek, Brooks Air Force Base, Texas

APPENDIX C

International Classification of Diseases (ICD) Codes for Cancers of Interest

The International Classification of Diseases (ICD) 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 or were written at a time when the Ninth Edition of the classifications (ICD-9) was in place. Accordingly, ICD references in this report use that scheme. The Tenth Edition (ICD-10) began to be implemented in the United States in 1999. It differs from ICD-9 in the level of detail (~8,000 categories versus ~5,000) and nomenclature (alphanumeric versus numeric codes); additions and modifications were also made to some coding rules and the rules for selecting the underlying cause of death (Anderson et al., 2001).

Table C-1 lists the ICD-9 and ICD-10 codes for the various forms of cancer addressed in the report. The listed codes are for malignant neoplasms. In situ neoplasms, benign neoplasms, neoplasms of uncertain behavior, and neoplasms of unspecified behavior have separate codes in both schemes.

TABLE C-1 Surveillance, Epidemiology, and End Results (SEER) Program Malignant Neoplasm Site Groupings for ICD-9 and ICD-10; National Center for Health Statistics (NCHS) Data

Cancer Site	ICD-9 codes	ICD-10 codes
Buccal cavity and pharynx		
Lip	140.0–140.9	C00.0–C00.9
Tongue	141.0–141.9	C01; C02.1–C02.9
Salivary glands	142.0–142.9	C07; C08.0–C08.9
Floor of mouth	144.0–144.9	C04.0–C04.9
Gum and other mouth	143.0–143.9, 145.0-145.6, 145.8-145.9	C03.0–C03.9; C05.0–C05.9; C06.0–C06.9
Nasopharynx	147.0–147.9	C11.1–C11.9
Tonsil	146.0–146.2	C09.0–C09.9
Oropharynx	146.3–146.9	C10.1–C10.9
Hypopharynx	148.0–148.9	C12; C13.0–C13.9
Other buccal cavity and pharynx	149.0–149.9	C14.0–C14.9
Digestive system		
Esophagus	150.0–150.9	C15.0–C15.9
Stomach	151.0–151.9	C16.0–C16.9
Small intestine	152.0–152.9	C17.0–C17.9
Colon excluding rectum	153.0–153.9, 159.0	C18.0–C18.9; C26.0
Rectum and rectosigmoid junction	154.0–154.1	C19; C20
Anus, anal canal, and anorectum	154.2–154.3, 154.8	C21.0–C21.9
Liver and intrahepatic bile duct		
Liver	155.0,155.2	C22.0; C22.2–C22.4; C22.7–C22.9
Intrahepatic bile duct	155.1	C22.1
Gallbladder	156.0	C23
Other biliary	156.1–156.9	C24.0–C24.9
Pancreas	157.0–157.9	C25.0–C25.9
Retroperitoneum	158.0	C48.0
Peritoneum, omentum, and mesentery	158.8–158.9	C48.1–C48.2
Other digestive organs	159.8–159.9	C26.8–26.9; C48.8
Respiratory system		
Nasal cavity, middle ear, and accessory sinuses	160.0–160.9	C30.0, C30.1; C31.0–C31.9
Larynx	161.0–161.9	C32.0–C32.9
Lung and bronchus	162.2–162.9	C34.0–C34.9
Pleura	163.0–163.9	C38.4
Trachea, mediastinum, and other respiratory organs	162.0, 164.2–165.9	C33; C38.1–C38.3, C38.8; C39
Bones and joints	170.0–170.9	C40.0–C40.9; C41.0–C41.9
Soft tissue (including heart)	171.0–171.9, 164.1	C38.0; C47.0–C47.9; C49.0–C49.9
Skin		
Malignant melanomas	172.0–172.9	C43.0–C43.9
Other malignant skin neoplasms	173.0–173.9	C44.0–C44.9

TABLE C-1 *Continued*

Cancer Site	ICD-9 codes	ICD-10 codes
Breast (male and female)	174.0–174.9, 175	C50.0–C50.9
Female genital system		
Cervix	180.0–180.9	C53.0–C53.9
Corpus	182.0–182.1, 182.8	C54.0–C54.9
Uterus, not otherwise specified	179	C55
Ovary	183.0	C56.0–C56.9
Vagina	184.0	C52
Vulva	184.1–184.4	C51.0–C51.9
Other female genital organs	181, 183.2–183.9, 184.8, 184.9	C57.0–C57.9; C58
Male genital system		
Prostate	185	C61
Testis	186.0–186.9	C62.0–C62.9
Penis	187.1–187.4	C60.0–C60.9
Other male genital organs	187.5–187.9	C63.0–C63.9
Urinary system		
Urinary bladder	188.0–188.9	C67.0–C67.9
Kidney and renal pelvis	189.0, 189.1	C64.0–C64.9; C65.0–C65.9
Ureter	189.2	C66.0–C66.9
Other urinary organs	189.3–189.4, 189.8–189.9	C68.0–C68.9
Eye and orbit	190.0–190.9	C69.0–C69.9
Brain and other nervous system		
Brain	191.0–191.9	C71.0–C71.9
Meninges	192.1	C70.0–C70.9
Other nervous system[a]	192.0, 192.2–192.9	C72.0–C72.9
Endocrine system		
Thyroid	193	C73
Other endocrine (including thymus)	164.0, 194.0–194.9	C37; C74.00–C74.92; C75.0–C75.9
Lymphomas		
Hodgkin's disease	201.0–201.9	C81.0–81.9
Non-Hodgkin's lymphomas	200.0–200.8, 202.0–202.2, 202.8–202.9	C82.0–C82.9; C83.0–C83.9; C84.0–C84.5; C85.0–C85.9; C96.3
Multiple myeloma	203.0; 238.6	C90.0, C90.2
Leukemias		
Lymphocytic		
Acute lymphocytic	204.0	C91.0
Chronic lymphocytic	204.1	C91.1
Other lymphocytic	202.4; 204.2–204.9	C91.2-C91.4, C91.7, C91.9
Myeloid (granulocytic)		
Acute myeloid	205.0; 207.0, 207.2	C92.0, C92.4–C92.5; C94.0, C94.2
Chronic myeloid	205.1	C92.1
Other myeloid	205.2–205.3, 205.8–205.9	C92.2–C92.3, C92.7, C92.9

continues

TABLE C-1 *Continued*

Cancer Site	ICD-9 codes	ICD-10 codes
Monocytic		
Acute monocytic	206.0	C93.0
Chronic monocytic	206.1	C93.1
Other monocytic	206.2–206.9	C93.2, C93.7, C93.9
Other leukemia		
Other acute	208.0	C94.4, C94.5; C95.0
Other chronic	207.1, 208.1	C94.1; C95.1
Aleukemic, subleukemic and "not otherwise specified"	203.1, 207.2, 207.8, 208.2–208.9	C90.1; C91.5; C94.3, C94.7; C95.2, C95.7, C95.9
Miscellaneous malignant neoplasms	159.1, 195.0–195.8, 196.0–196.9, 199.0–199.1, 202.3, 202.5–202.6, 203.8	C26.1; C76.0–C76.8; C77.0–C77.9; C78.0–C78.8; C79.0–C79.8; C80; C88.0–C88.9; C96.0–C96.2, C96.7, C96.9; C97

[a] Cancers of the peripheral nerves and the autonomic nervous system are classified as "soft tissue" in the ICD.

Adapted from: Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Fay MP, Feuer EJ, Edwards BK (eds). *SEER Cancer Statistics Review, 1975-2000*, National Cancer Institute. Bethesda, MD, Table A-4.

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Anderson RN, Minino AM, Hoyert DL, Rosenberg HM. 2001. Comparability of cause of death between ICD-9 and ICD-10: preliminary estimates. National Vital Statistics Reports 49(2):1–32.

APPENDIX D

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

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Kiros T. Berhane, Ph.D., is Associate Professor in the Department of Preventive Medicine at the University of Southern California, Los Angeles. His research has considered longitudinal-time series analysis, flexible-modeling techniques, modeling time-to-event data, and latency. Dr. Berhane received his doctorate in biostatistics from the University of Toronto (Canada) and completed a postdoctoral fellowship in biostatistics at Johns Hopkins University (1994–1995). He currently serves as a member on a scientific advisory panel for an air pollution study under the auspices of the Western Interprovincial Scientific Studies Association in Canada. Dr. Berhane served on the *Veterans and Agent Orange: Update 2002* committee.

Paul F. Engstrom, M.D., is Senior Vice President of the Population Science Division and Medical Director of the Fox Chase Network at the Fox Chase Cancer Center. Dr. Engstrom's research interests include chemoprevention. He served on the *Veterans and Agent Orange: Update 2002* committee.

Richard A. Fenske, Ph.D., is Professor of Environmental and Occupational Health Sciences and Director of the Pacific Northwest Agricultural Safety and Health Center at the University of Washington in Seattle. Dr. Fenske's work has focused on the evaluation of environmental health risks in special populations. His specialty areas include health risks of pesticide exposures, development of new exposure assessment methods, and investigation of the role of skin exposure in workers and children. Dr. Fenske served on the *Veterans and Agent Orange: Update 2002* committee.

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Thomas A. Gasiewicz, Ph.D., is Professor and Chair of the Department of Environmental Medicine and Director of the Environmental Health Sciences Center in the Department of Environmental Medicine at the University of Rochester, School of Medicine. Dr. Gasiewicz has published extensively on the toxicokinetics of dioxin, dioxin toxicity, and the aryl hydrocarbon receptor in the molecular mechanism of dioxin toxicity. Dr. Gasiewicz served on the *Veterans and Agent Orange: Update 2000* and the *Veterans and Agent Orange: Update 2002* committees.

Claudia Hopenhayn, Ph.D., M.P.H., is Assistant Professor in the Department of Preventive Medicine at the University of Kentucky School for Public Health. Her primary research focuses on cancer and reproductive outcomes within the context of environmental and occupational epidemiology and cancer control. Dr. Hopenhyn's expertise combines toxicology, biologic markers of exposure and effect, statistics, risk factors, and assessment of intervention within a framework of epidemiology and multidisciplinary collaborations, both in the United States and abroad.

Nancy I. Kerkvliet, Ph.D., is Professor in the Department of Environmental and Molecular Toxicology at Oregon State University. Dr. Kerkvliet's research has focused on animal modeling to explain how specific chemicals alter immune function and how immune suppression caused by dioxin is mediated by the aryl hydorcarbon receptor, a transcription factor that is activated upon ligand binding. She previously served on the *Subcommittee of Jet Propulsion Fuel 8* and the *Committee on Toxicology*.

Loren D. Koller, D.V.M., Ph.D., has served in academia for nearly 30 years, the past 16 as Professor in the College of Veterinary Medicine, Oregon State University, Corvallis. Dr. Koller was dean of the college for 10 years. He was the first chair of the Society of Toxicology's Immunotoxicology Specialty Section. Dr. Koller serves on the *Committee on the Assessment of Wartime Exposure to Herbicides* and served on the *Veterans and Agent Orange: Update 2000* and the *Veterans and Agent Orange: Update 2002* committees.

Joel McCullough, M.D., M.P.H., M.S., is Medical Director of Environmental Health for the Chicago Department of Public Health. Dr. McCullough received his medical degree from the Vanderbilt University School of Medicine, a master's of science in preventive medicine from the University of Iowa Graduate School of Preventive Medicine, and a master's of public health from the University of Washington School of Public Health. He has worked for several organizations, including the National Center for Environmental Health and the National Institute for Occupational Safety and Health. Dr. McCullough has been in his current position since October of 2001.

David S. Strogatz, Ph.D., M.S.P.H., is Associate Professor and Chair of the Department of Epidemiology and Biostatistics at the State University of New York School of Public Health in Albany. Dr. Strogatz originally became involved with the Agent Orange studies when he served as a reviewer for the *Veterans and Agent Orange: Update 1998* report. He subsequently served as a member of the *Veterans and Agent Orange: Update 2000* and the *Veterans and Agent Orange: Update 2002* committees. His research examines the epidemiology of diseases including diabetes and cardiovascular disease, and the impact of socioeconomic status and race on health.

Staff Biographies

Michelle Catlin, Ph.D., is Senior Program Officer in the Institute of Medicine (IOM) Board on Health Promotion and Disease Prevention. Before joining IOM, she served as Program Officer with the Board on Environmental Studies and Toxicology of the National Research Council. She received a master's of science in pharmacology and toxicology from Queen's University, Canada, and a doctorate in environmental health (toxicology) 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*.

Mary Burr Paxton, Ph.D., is Senior Program Officer in the IOM Board on Health Promotion and Disease Prevention. Before joining IOM, she worked as a consultant on the regulation of toxic substances and managed the conduct and

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Jennifer A. Cohen is Senior Program Associate in the IOM Board on Health Promotion and Disease Prevention. She received her undergraduate degree in art history from the University of Maryland. She also has 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.*

Rose Marie Martinez, Sc.D., is Director of the IOM Board on Health Promotion and Disease Prevention. Before joining IOM, she was Senior Health Researcher at Mathematica Policy Research, where she studied the effects 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 former Assistant Director for Health Financing and Policy with the US General Accounting Office, for which she directed evaluations and policy analysis on national and public-health issues. Dr. Martinez received her doctorate from the Johns Hopkins School of Hygiene and Public Health.

David A. Butler, PhD, is a senior program officer in the IOM Board on Public Health. He received his BS and MS in engineering from the University of Rochester and PhD in public-policy analysis from Carnegie-Mellon University. Before joining IOM, Dr. Butler served as an analyst for the US Congress Office of Technology Assessment and was a research associate in the Department of Environmental Health at the Harvard School of Public Health. He has directed several IOM studies on environmental-health and risk-assessment topics, resulting in the reports *Veterans and Agent Orange: Update 1998*, and *…Update 2000*; the report series *Characterizing the Exposure of Veterans to Agent Orange and Other Herbicides Used in Vietnam*; *Clearing the Air: Asthma and Indoor Air Exposures;* and *Damp Indoor Spaces and Health.*

Sonia J. Cheruvillil is a senior program assistant in the IOM Board on Health Promotion and Disease Prevention. She is currently a graduate student at the George Washington University School of Public Health. She received her under-

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Joe A. Esparza worked as a project assistant in the IOM Board on Health Promotion and Disease Prevention. He attended Columbia University in the City of New York where he studied biochemistry. 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*; *Publicly Funded Agricultural Research and the Changing Structure of US Agriculture*; and *Veterans and Agent Orange: Update 2002*.

Peter James is a research assistant in the IOM Board on Health Promotion and Disease Prevention. He received his undergraduate degree in environmental science and the history and sociology of science from the University of Pennsylvania. He is a candidate for a Master of Health Science degree at the Johns Hopkins University Bloomberg School of Public Health in the Department of Environmental Health Sciences. In addition to his work with the *Veterans and Agent Orange* studies, he is involved with the *Gulf War and Health* studies.

Index

Note to the reader: This index contains entries for each of the six 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), *Veterans and Agent Orange: Update 2004* (VI). 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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