Review of Literature on Herbicides, Including Phenoxy Herbicides and Associated Dioxins

Volume XIII
Analysis of Recent Literature on Health Effects
and
Volume XIV
Annotated Bibliography of Recent Literature on Health Effects
REVIEW OF LITERATURE ON HERBICIDES,
INCLUDING PHENOXY HERBICIDES
AND ASSOCIATED DIOXINS

Volume XIII: Analysis of Literature on
Health Effects Published in 1988
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Public Law 96-151 enacted December 20, 1979, mandated the Veterans Administration to conduct "a comprehensive review and scientific analysis" of the worldwide literature on Agent Orange and other phenoxy herbicides. This mandate was in response to an increasing awareness among veterans, the Congress, and the public of the potential long-term health consequences of exposure to these herbicides and the contaminant dioxin. In October 1981, the Veterans Administration published a two-volume "Review of Literature on Herbicides, Including Phenoxy Herbicides and Associated Dioxins." Because of continued active research, a two-volume update covering the literature through December 1983 was published in 1983. Each year since that time the review has been updated. The present two-volume update covers literature that became available during 1988.

This report was prepared by Clement Associates, Inc. It is an independent assessment of the current state of knowledge about the health effects of phenoxy herbicides, their contaminating polychlorinated dibenzo-p-dioxin impurities, and two other herbicides (picloram and cacodylic acid) that were used in Vietnam. The publication of this document by the Veterans Administration does not signify that the contents necessarily reflect the views and policies of the Veterans Administration.

The project director for Clement Associates, Inc., was Wayne D. Reichardt. The technical director and principal author of the review was Carl O. Schulz, Ph.D. Other contributing authors were Sharon Segal, Ph.D., and Sanjivani Diwan, Ph.D.
I. INTRODUCTION

This report consists of a bibliography and critical review of scientific literature that became available during 1988 on the health effects of the herbicides (including impurities) used as defoliants in the Vietnam conflict. This update comprises Volumes XIII and XIV of an ongoing series of publications entitled, Review of Literature on Herbicides, and Associated Dioxins." Volumes I and II were prepared by JRB Associates and published by the Veterans Administration in October 1981. Volumes III and IV, covering literature published from 1981 through 1983, were prepared by Clement Associates, Inc., and published by the Veterans Administration in June 1984. Volumes V and VI, covering literature that became available during 1984, were published by the Veterans Administration in 1985. Volumes VII and VIII, covering literature that became available in 1985, were published in 1986. Volumes IX and X covering literature that became available during 1986 were published in 1987, and Volumes XI and XII covering literature that became available in 1987 were published in 1988. In this report, Clement Associates, Inc., has identified and reviewed information that became available during 1988 in order to update the available data base on the potential adverse health effects of exposure to these herbicides and their contaminating impurities.

An attempt has been made to identify all scientific literature (including unpublished reports) relevant to the potential human health effects of the herbicidal preparation commonly referred to as Agent Orange, the herbicidal active ingredients 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichloroacetic acid and their esters, as well as polychlorinated dibenzo-p-dioxins (primarily 2,3,7,8-tetrachlorodibenzo-p-dioxin, henceforth referred to as TCDD) known to be contaminating impurities of some phenoxy herbicide preparations, and the herbicides, picloram and cacodylic acid.
The scope of this review does not include literature dealing exclusively with the chemistry, analysis, or environmental fate and effects of these compounds.

In order to identify relevant literature, Clement searched on-line data bases for literature published since late 1987. These searches covered the MEDLINE, TOXLINE, and SDILINE data bases of the MEDLARS on-line information system of the National Library of Medicine. Also, the on-line version of Current Contents (ISI, Philadelphia) covering all of 1988 was searched in January 1989. Print-outs of these searches were screened to identify relevant resources not already included in the earlier reviews.

Searching of on-line data bases is of limited usefulness because of a lag time of approximately 6 months between publication of scientific reports and their entry into on-line data bases. Therefore, Clement personnel manually screened weekly issues of Current Contents, Life Sciences published by the Institute for Scientific Information, Philadelphia, Pennsylvania, and biweekly issues of CA Selects: Carcinogens, Mutagens, and Teratogens published by Chemical Abstracts Service, Columbus, Ohio. Clement personnel also screened approximately 20 scientific journals and current awareness publications available in the Clement library.

Clement personnel obtained additional information at scientific meetings, including the 1988 Annual Meeting of the Society of Toxicology in Dallas, Texas, and at the regular meetings of the Veterans Administration Advisory Committee on Health-Related Effects of Herbicides. Also, in order to identify any unpublished documents or documents published in unconventional resources, Clement personnel contacted scientists who have been active in research on the health effects of phenoxy herbicides and dioxins.
II. SUMMARY AND CONCLUSIONS

The annotated bibliography that accompanies this critical review contains just over 300 citations to literature relevant to the human health effects of herbicides used in Vietnam. These documents became available during 1988. This year was very similar to the preceding three years in both the volume and nature of the literature that was found. Just less than half of the citations are abstracts or secondary sources, e.g., review articles. A large percentage of the full-length documents are reports of studies of the mechanism of action of chlorinated dibenzo-p-dioxin (dioxins) and related compounds in experimental animals.

A unique aspect of this update is that no documents were found relevant to the health effects of the herbicidal active ingredients, picloram and cacodylic acid. These were components of herbicides used in Vietnam and known by their color codes white and blue, respectively. Few studies relevant to the human health effects of these herbicides have been published in the ensuing years. All of the literature reviewed in this update of the review is relevant to the health effects of the phenoxy herbicides, i.e., 2,4-D and 2,4,5-T, and their contaminating dioxin impurities.

A. Health Studies Among Vietnam Veterans

Perhaps the most significant new scientific information that became available during 1988 was the result of three major studies of the health status of Vietnam veterans. One of these was a mortality study conducted among Vietnam veterans in Wisconsin. The others were morbidity studies among Vietnam veterans; one conducted by the U.S. Centers for Disease Control (CDC) and known as the Vietnam Experience Study (VES), and the other was sponsored by the American Legion. Of the three studies, only the American Legion study was designed to relate
health status to herbicide exposure. The other two studies were designed to examine possible health consequences of military service in Vietnam. Vital status and causes of death were ascertained in the Wisconsin study using state and federal veterans' records and death certificates. The health status of veterans in the American Legion study was determined using a self-administered questionnaire. In the VES, health status was determined by telephone interview. A random sample of those interviewed underwent a complete medical and physical examination.

Despite their differences in objectives and methods, these studies complement each other and, taken together, provide valuable additional information on the potential long-term consequences of military service in Vietnam and the possible interrelationships between herbicide exposure and other aspects of the Vietnam experience. The Wisconsin mortality study provided additional evidence for the "healthy veteran effect," i.e., the preinduction medical and physical screening of military recruits selects for individuals who are less likely to die from chronic diseases such as heart disease and diabetes. This study also suggested that the mortality patterns among all Vietnam-era veterans, whether they served in Vietnam or not, are different from those in the general population and from those among military veterans from other eras. The excess mortality among Vietnam-era veterans was due to causes that reflect habits that are detrimental to health, including smoking and alcohol abuse. When Vietnam veterans were compared to veterans of the same era who did not serve in Vietnam, there was excess mortality from external causes, including motor vehicle accidents and suicides, among the Vietnam veterans. There were too few deaths of cancer within the study population to allow comparison for specific types of cancer between Vietnam and non-Vietnam veterans. There was excess mortality from soft-tissue sarcoma (STS) among Vietnam veterans when compared to the non-veteran population, but this
could not be attributed to herbicide exposure in Vietnam because mortality from STS was also elevated among non-Vietnam veterans.

Both the VES and the American Legion study convincingly demonstrated that Vietnam veterans report more current and past health problems than non-Vietnam veterans. They also report more adverse reproductive effects and more health problems in their children. In the VES, reported adverse health consequences correlated with recall of herbicide exposure; the health status of Vietnam veterans who did not report herbicide exposure was comparable to that of non-Vietnam veterans. The physical and medical examination component of the VES failed to verify many of the differences in health status that were suggested by the results of the telephone interviews. The only differences between Vietnam and non-Vietnam veterans were hearing loss, the presence of occult blood in stools, lower sperm counts, and altered sperm morphology. This is not to say that reported adverse health effects among Vietnam veterans are "all in their heads." The perception of impaired health by Vietnam veterans is important of and by itself. It is also not possible to rule out a biochemical basis for this effect.

The individuals who conducted the American Legion study attempted to determine herbicide exposure objectively among the study cohort. This study showed that the distribution of reported herbicide exposure among Vietnam veterans was highly skewed with many veterans experiencing relatively little exposure. The analysis of data also indicated that reported herbicide exposure was highly correlated with combat involvement, to the extent that studies of the health effects of herbicide exposure that do not control for combat exposure must be interpreted with caution. Nevertheless, the American Legion study showed that many perceived health effects were independently related to reported herbicide exposure when combat exposure was controlled for.
B. Cancer

No new studies of the relationship between herbicide exposure in Vietnam and cancer became available during 1988. Studies of the relationship between specific cancers and occupational exposure to herbicides in agriculture or forestry gave inconsistent results. Responding to persistent criticism of earlier studies that showed statistically significant associations between occupational exposure to phenoxy herbicides and STS and **non-Hodgkin's lymphoma (NHL)**, Hardell and coworkers published the results of a new case-control study of STS in Sweden. This new study also showed a statistically significant association between STS and herbicide exposure. However, the odds ratio (OR) was smaller than that in the earlier study, possibly reflecting reduction of bias attributable to the use of controls who had not been diagnosed as having cancer in the earlier study. Both a cohort mortality study and a case-control study of NHL in Sweden failed to reveal any association between this form of cancer and phenoxy herbicide exposure. The case-control study did, however, show an association between this cancer and exposure to organic solvents, and it suggested that NHL localized in cutaneous tissues might be related to phenoxy herbicide exposure. A cohort mortality study of NHL in Yorkshire, England, that was not designed to ascertain phenoxy herbicide exposure suggested that NHL might be associated with a variety of different environmental factors whose only common linkage may be interference with immune function.

Two cohort mortality studies conducted among employees of the Dow Chemical Company in Midland, Michigan, found no cases of STS or NHL among employees engaged in the manufacture of 2,4-D or among employees with a history of **chloracne**, presumably resulting from exposure to chlorinated dioxins and related compounds. The study of workers engaged in the manufacture of 2,4-D found an increased risk of cancer at ill-defined sites, but the significance of this catch-all category of cancers is not clear.
The study of workers with a history of chloracne revealed an excess of deaths due to strokes.

A mortality study was conducted among individuals in Japan who had a history of chloracne and related symptoms caused by the ingestion of cooking oil contaminated with PCBs and contaminating chlorinated dibenzofurans. A preliminary report issued in 1968 suggested an increased risk of liver cancer among these individuals when compared to the population of Japan as a whole and to the population of the two prefectures in which the subjects resided. There was also an increased risk of chronic nonmalignant liver disease in this group. Further study to examine the relationship between the amount of contaminated oil consumed and cancer risk is underway. These results are of particular interest in view of animal studies that show increased incidence of liver cancer after oral administration of dioxins and furans.

Animal studies that were described in the last year indicated that 2,3,7,8-TCDD has the potential to cause cancer in hamsters, a species that is relatively resistant to the acute lethal effects of this compound. A secondary report of a study of the chronic toxicity of 2,4-D in rats indicated that there was a significantly increased incidence of astrocytomas in rats given the highest dose but that this finding was "not biologically significant." A published report of this study was not available for review.

Several epidemiologic studies that may contain relevant information about the relationship between cancer and exposure to phenoxy herbicides and/or dioxins are in their final stages and should be published in the near future. One of these is the Selected Cancers study being conducted by the CDC. This is a case-control study designed to study the association between specific cancers such as STS and NHL with military service in Vietnam. Another is a study of health effects among workers who are or were exposed to chlorinated dioxins in the United States.
being conducted by the National Institute for Occupational Safety and Health (NIOSH).

C. Genetic Effects

No new studies on the potential genetic effects of phenoxy herbicides or their dioxin impurities became available during 1988. Assays for mutational activity in bacteria and mammalian cells in culture were negative for 2,4-D, 2,4,5-T, and TCDD. These studies add to a substantial body of evidence that indicates that none of these compounds is directly mutagenic. An in vivo study indicated that TCDD was not mutagenic or clastogenic in liver cells in mice. A single study of the in vivo effect of 2,4-D on bone marrow cells in rats suggested that 2,4-D causes chromosomal aberrations. The significance of this finding for human health is unclear.

D. Reproductive and Teratogenic Effects

The results of the VES indicated that Vietnam veterans reported more adverse reproductive and child health outcomes in telephone interviews than did non-Vietnam veterans. A review of hospital birth records, however, indicated that Vietnam veterans were not at increased risk of fathering children with birth defects. A subsample of Vietnam veterans in this study did have a lower mean sperm count and altered sperm morphology compared with a subsample of non-Vietnam veterans. The significance of this finding is not clear. In the American Legion study, responses to a self-administered questionnaire indicated that miscarriage rates were independently and significantly associated with reported herbicide exposure in Vietnam. No questions designed to identify birth defects were included in the questionnaire. There was no association between reported herbicide exposure and difficulty in conception, time of conception of first child, birth weight, or sex ratio of
offspring. The reporting of miscarriages must be interpreted with caution in view of possible recall bias.

An epidemiologic study of reproductive outcomes in New Brunswick, Canada, suggested a possible association between birth defects and stillbirths with exposure to agricultural chemicals. However, the method of determining exposure and the lack of information regarding specific agricultural chemicals to which people in the province might have been exposed render these findings irrelevant to the question of health effects of phenoxy herbicides. Two surveys of reproductive outcomes in areas that were contaminated with dioxins failed to reveal any associations between this contamination and adverse reproductive effects. A study of children born to women who consumed cooking oil contaminated with PCBs and chlorinated dibenzofurans in the Yucheng incident in 1979 revealed lower birth weights, hyperpigmentation, conjunctivitis, neurologic changes, and abnormalities of the gingiva, nails, teeth, and lungs when compared to the children of unexposed mothers. Indications of delayed development and behavioral alterations were also evident. Comparison of these children to children of mothers with similar body burdens of PCBs indicated that the effects were probably the result of exposure to dibenzofurans. The findings are important in that they are consistent with experimental evidence indicating that exposure of pregnant animals to polychlorinated dibenzo-p-dioxins and furans cause structural and development defects in their offspring.

The results of studies in experimental animals indicate that prenatal exposure to relatively large amounts of 2,4-D and 2,4,5-T produces neurochemical and behavioral alterations in neonates. A number of studies of the effects of TCDD in experimental animals indicates that this compound and structurally similar compounds interact with steroid hormone receptors including estrogen and androgen receptors leading to complex and not yet fully understood responses. It is clear from
these studies, however, that the endocrine system may be a sensitive target for these compounds.

E. Effects on the Immune System

There were no differences between Vietnam veterans and non-Vietnam veterans with regard to indicators of immune status or function in the VES. This study was not designed to assess the role of herbicide exposure. Studies of human populations who may have been exposed to environmental dioxin contamination suggest an association between such exposure and altered populations of T-cell subsets, but these are not reflected in measures of immune function. The clinical significance of these findings is not clear. Recent animal studies have been designed to elucidate the mechanism by which TCDD and related compounds alter immune function in experimental animals. No clear mechanism has been established.

F. Neurobehavioral Effects

Studies of Vietnam veterans revealed significant differences in indicators of psychological well-being when compared to non-Vietnam veterans. However, there is no objective evidence that this is associated with herbicide exposure. Measures of neurologic function failed to reveal significant differences. Studies of neurologic function in a population exposed to dioxin when it was accidentally released in Seveso, Italy, showed no significant differences when compared to a control population. Studies in experimental animals indicate that the central nervous system is an important target for the acute toxic effects of 2,4-D, but the significance of these effects to humans experiencing chronic exposure to relatively low concentrations of this compound is not known.
G. Other Toxic Effects

Three case reports of acute intoxication resulting from the ingestion of large quantities of commercial formulations of phenoxy herbicides resulted in remarkably similar clinical descriptions. The course of poisoning was consistent with severe central nervous system depression and rhabdomyolysis leading to respiratory failure and acute kidney failure.

Studies of hepatic function in individuals who were exposed to dioxins or furans suggested that these may result in subtle alterations of some enzyme activities, including those involved in porphyrin metabolism and in the metabolism of aryl hydrocarbons. There is some evidence that susceptibility to these effects is genetically controlled.

A brief abstract indicated that occupational exposure to dioxins might be associated with changes in cerebral blood flow. This report is intriguing in light of findings in an epidemiologic study that workers with exposure to dioxins were more likely to die of strokes. Studies of the effects of dioxin in experimental animals indicated that relatively large single doses of TCDD alter the \( \beta \)-adrenergic responsiveness of the heart. An additional study indicated that subchronic exposure of rats to 2,4-D resulted in impaired kidney function.

H. Tissue Residues of Chlorinated Dibenzo-p-dioxins

The results of several studies during the past few years have indicated that concentrations of dioxins in human blood and adipose tissues may be reliable qualitative indicators of relatively heavy exposure to these compounds or to phenoxy herbicides contaminated with these compounds in the past. Results of studies that were published in 1988 confirmed preliminary reports that the concentrations of dioxins in blood samples from Vietnam veterans were not different from those in blood samples from non-Vietnam veterans or the general population. While a few individuals who may have been heavily
exposed to phenoxy herbicides in Vietnam have elevated concentrations of dioxin in their blood up to 20 years after exposure, this approach is not useful for assessing relative herbicide exposure among a sufficiently large population of Vietnam-era veterans for a definitive epidemiologic study of association between herbicide exposure and health effects. Furthermore, limited results from individuals exposed to dioxins in Seveso, Italy, suggest that present tissue levels of dioxins are not strictly correlated with the severity of signs and symptoms of the exposure in the past. This indicates that there is individual variability in either the rate of elimination of these compounds from the body or in sensitivity to the toxic effects of these compounds.
A. Studies of the Health Status of Vietnam Veterans

During 1988 the results of three major epidemiologic studies among Vietnam veterans became available. Together they contribute a great deal of information relevant to the long-term health effects of participation in that military action. The first of the studies discussed in this section is the Vietnam Experience Study (VES), which was conducted under the direction of the United States Centers for Disease Control (USCDC 1988a,b,c, 1989). The second study was sponsored by the American Legion (Stellman et al. 1988a,b,c). The third study (the Wisconsin study) was actually completed in 1986, but the final report was not published in the open literature and only became available in 1988 (Anderson et al. 1986).

While the studies had certain common aspects, i.e., they compared Vietnam veterans to veterans of the same era who did not serve in Vietnam, it is important to recognize that each had different objectives and differed in approach. The first two studies (VES and American Legion) were studies of veterans' health at the time of the study (morbidity studies), while the Wisconsin study was a mortality study. The American Legion study relied solely on a self-administered questionnaire to ascertain health effects and exposure, whereas a subsample of subjects in the VES underwent medical and physical examinations in addition to being interviewed by telephone. The VES was conducted using a random sample of military records to identify enlisted men who served one tour in Vietnam. The American Legion study was conducted among a sample of American Legion members in six states. Both the VES and the Wisconsin study were designed to relate health outcomes to military service in Vietnam and made no effort to relate observed effects to herbicide exposure per se. The American Legion study, on the other hand, was designed to study the relationship of health status to both herbicide and
combat exposure. These studies are described and reviewed in
detail in this section, and the results pertinent to specific
health effects, i.e., psychological and social status and
reproductive outcomes, are summarized in those sections of this
chapter that deal with those end points.

1. The Vietnam Experience Study (VES),

In 1979 and 1981 Congress passed legislation that directed
that appropriate studies be conducted to ascertain the health
status of Vietnam veterans because of concern that aspects of the
Vietnam experience may have had lasting adverse health
consequences among these veterans. One result of that
legislation has been a series of epidemiologic investigations
conducted by the CDC referred to collectively as the veterans' health studies.

As originally designed the veterans' health studies had
three components. The first of these was the Agent Orange
Exposure Study. This study was intended to ascertain exposure of
Vietnam veterans to Agent Orange and other herbicides and to
relate that exposure to potential adverse health effects.
Initial attempts to ascertain herbicide exposure involved the use
of military service records combined with records of when and
where herbicide spray missions took place (the HERBS tapes).
These records were deemed inadequate for this purpose primarily
because military service records were not sufficiently detailed
to pinpoint the location of specific servicemen at specific
times. Also, investigators found little correlation between
individual recall of exposure and evidence of such exposure from
military records and the HERBS tapes. With the development of
analytical techniques that permitted the detection of minute
quantities of chlorinated dibenzo-p-dioxins in the blood,
investigators at CDC conducted a pilot study to determine whether
concentrations of these compounds in the blood of Vietnam
veterans might serve as a reliable indicator of herbicide
exposure (Marshall et al. 1987, USCDC 1988d). The results of
this pilot study revealed no correlation between serum levels and any other evidence of herbicide exposure. The results of the extensive attempts by CDC to ascertain herbicide exposure have been reviewed by a number of experts from all branches of the U.S. government. Based on a concurrence that it is not possible to ascertain herbicide exposure among Vietnam veterans reliably and objectively, the Agent Orange Exposure Study has been cancelled (USCDC 1989).

The second component of the veterans' health studies, the Selected Cancers study, is a case-control study of several specific types of cancer that have been potentially linked to phenoxy herbicide exposure. This study is scheduled to be completed in 1989 and the results reported in 1990 (USCDC 1989).

The third component of the veterans' health studies is the VES. The VES is a historical cohort study designed to compare the health status of veterans who served in Vietnam with that of veterans of the same era who did not. The VES has both a mortality component and a morbidity component. The results of the mortality study were published in 1987 (USCDC 1987) and were critically reviewed in Volume XI of this review (Clement 1988). Briefly, this study indicated that Vietnam veterans had significantly lower mortality from diseases of the circulatory system and significantly higher mortality from external causes than did non-Vietnam veterans. Mortality due to cancer was similar in the two groups, but there were too few cancer deaths in either cohort to allow meaningful statistical analyses for any specific type of cancer.

The results of the morbidity study portion of the VES were published in 1988 and 1989 (USCDC 1988a,b,c, 1989). The criteria for inclusion in the VES is that an individual must be a male veteran who entered military service for the first time during the Vietnam era (1965-1971) and have served a single term of enlistment with a minimum of 16 weeks of active duty. Only veterans who were in pay grades E-1 through E-5 at discharge were
included. Individuals who were classified as "duty soldier" or "trainee" were not included in the study. The goal of the study was to interview 6,000 Vietnam and 6,000 non-Vietnam veterans and to conduct complete medical examinations of 2,000 in each group.

The actual numbers of individuals included in this study are shown in Figure 1. The sample of 48,513 records that were initially screened was identified by computer using a random number generating program. The telephone interviews were conducted by trained interviewers working at computer terminals. Responses were entered directly into a computerized data base. The interviewers were not aware of whether the individual they were interviewing had served in Vietnam or not until late in the interview. Interviews lasted 32 minutes on average and contained questions about past and present health status, past and present psychological and behavioral status, and demographic descriptors.

A random sample of interview subjects was invited to undergo a full medical and psychological evaluation at the Lovelace Medical Foundation in Albuquerque, New Mexico. This examination, which lasted for 2 days, included a complete medical history, a general medical examination, a dermatologic examination, chest X-ray, pulmonary function test, skin hypersensitivity test using seven recall antigens, a series of neurodiagnostic tests, blood and urinalysis including determination of T and B cell subset populations, and a series of psychological diagnostic tests. Semen samples were collected from a small subgroup of each cohort for analysis.

The final report of the VES morbidity study fills five volumes and two supplements (USCDC 1989). The results have been summarized in three full-length journal articles (USCDC 1988a,b,c). A complete description of the results is beyond the scope of this review. In their summary of the study results, the authors reported that the Vietnam and non-Vietnam veterans were quite similar in current demographic and social characteristics. In the interviews the Vietnam veterans reported more current use of prescription drugs and more current and past diseases and
VIETNAM-ERA ARMY PERSONNEL
RECORDS FILED AT NPRC
APPROX 4,900,000

RANDOM SAMPLE
48613

QUALIFIED FOR STUDY

VIETNAM
9.568

NON-VIETNAM
9.023

DIED ON ACTIVE DUTY
234

DIED BETWEEN DISCHARGE AND 12/31/83
246

STUDY COHORT
9.324

STUDY COHORT
8.989

MORTALITY STUDY

ELIGIBLE FOR INTERVIEW
9.078

ELIGIBLE FOR INTERVIEW
8.789

INTERVIEWED
7.924

INTERVIEWED
7.364

INTERVIEWED
7.924

INTERVIEWED
7.364

RANDOM SAMPLE

INVITED FOR EXAM
3.317

INVITED FOR EXAM
3.126

EXAMINED
1.972

EXAMINED
2.490

VIETNAM

NON-VIETNAM

FIGURE 1. Design of the Vietnam Experience Study

symptoms than did the non-Vietnam veterans. These diseases and symptoms included hypertension, benign growths, chloracne and other skin conditions, gastrointestinal ulcers, liver conditions, and impaired ability to father children. The Vietnam veterans also reported more health problems among their children.

The physical and laboratory examination portion of the study revealed few differences between the two cohorts and failed to confirm most of the differences reported in the telephone interviews. Vietnam veterans did have more hearing loss and more Vietnam veterans had occult blood in their stools compared to non-Vietnam veterans. Semen analysis revealed lower mean sperm counts and lower proportions of morphologically normal sperm cells in Vietnam veterans. The reproductive portion of the study showed no difference in the number of children fathered by men in the two cohorts, and there was no difference in the rate of birth defects. Among Vietnam veterans, 15% reported symptoms consistent with post-traumatic stress disorder, and current psychological problems were more prevalent among Vietnam veterans than among non-Vietnam veterans. The study results showed that Vietnam veterans who reported herbicide exposure reported more postservice symptoms and diseases and more birth defects among their children than did Vietnam veterans who did not report herbicide exposure. Vietnam veterans who did not report exposure to herbicides reported diseases and symptoms at a rate very similar to that of non-Vietnam veterans. As described above, herbicide exposure could not be objectively confirmed.

The results of the Vietnam experience study for psychological and reproductive end points are discussed in those sections of this chapter. The VES is a carefully designed and conducted study and is relatively powerful because of the large numbers in both cohorts. The authors did point out several inherent limitations, however. First, the long elapsed time since the Vietnam experience may have affected the accuracy of recall of specific exposures and situations. Second, a larger
proportion of the Vietnam veterans accepted the invitation to participate in the medical examination phase than did non-Vietnam veterans. Third, there was no indirect measure of herbicide exposure. Finally, the "Vietnam experience" encompasses a wide variety of different and complex individual situations, and the cohort is probably quite heterogeneous with regard to major factors such as exposure to combat and chemicals. The authors performed numerous analyses designed to reveal systematic bias in the study and found no evidence of such bias. The major conclusions of the study are consistent with those of similar studies.

2. The American Legion Study

The results of a cross-sectional survey of the psychological and physical well-being of Vietnam-era veterans who were members of the American Legion were published as a series of articles in 1988 (Stellman et al. 1988a,b,c). The objective of this study was to examine possible associations between psychological and physical health and exposure to combat and/or phenoxy herbicides in Vietnam. The cohort of Vietnam-era veterans was constructed by creating a computer file of all American Legion members in six states (Colorado, Ohio, Maryland, Pennsylvania, Indiana, and Minnesota) with less than 20 years of continuous membership as of October 15, 1983. A sample of one-seventh of these names was selected at random. Returnable postcards were mailed to the sample to ascertain whether their dates of military service fell within the Vietnam era (1964-1975). Non-Vietnam-era veterans were eliminated from the sample. The remaining sample was broken down by state, and a list of names was sent to the six state Departments of the American Legion. "Volunteer researchers" in each state were then asked to contact the local Post members in the sample to determine whether they were Vietnam era veterans and whether they had served in Southeast Asia during the Vietnam era. Each volunteer was asked to contact individuals on his list until he had identified 15 Southeast Asia veterans and 15 non-
Southeast Asia veterans who were willing to participate in the study. Detailed questionnaires were mailed to all Vietnam era veterans identified by the researchers.

The sole instrument used for this survey was a self-administered questionnaire, which took from 45 minutes to 1 hour to complete. Among other purposes, the questionnaire was designed to determine relative exposures to herbicides and to combat. The methodology for determining herbicide exposure has been described in an earlier paper (Stellman and Stellman 1986, see Volume X of this review). The questionnaire contained a list of about 100 locations in Vietnam where troop activity was known to have centered. Respondents were asked to give the dates at which they served at any of these locations. Using the HERBS tapes showing the dates and locations of all herbicide spray missions in Vietnam, the authors developed an integrated herbicide exposure index for each respondent based on all spray missions that occurred within a 15-kilometer radius of the locations where the veteran had served, weighted inversely according to distance and exponentially according to elapsed time from the spray mission. Combat exposure was determined from responses to eight questions about extent of enemy fire and life-threatening situations.

The questionnaires were also designed to obtain information on demographic characteristics; smoking, drinking, and drug use; social and behavioral outcomes; and health status. The questions on health status were designed to assess general and reproductive health status as well as ascertain the prevalence of certain specific symptoms. Respondents were asked to identify physician-diagnosed health problems as well as a number of other conditions and symptoms, e.g., skin discoloration or acne, that have been associated with exposure to phenoxy herbicides or their dioxin contaminants. Questions about reproductive health were designed to evaluate potential difficulties with conception, specific birth outcomes, and maternal smoking and alcohol use.
Responses to questions regarding physical and psychological status among Vietnam veterans were compared to those among Vietnam-era veterans who served in areas other than Southeast Asia; odds ratios were computed to determine the relative risks for each condition or reported symptom. In addition, respondents who served in Vietnam were classified into categories (three each) reflecting relative exposure to herbicides and combat, and the responses were analyzed for correlations with either of these variables. Joint effects of combat and herbicide exposure were analyzed using multiple regression, logistic regression, and analysis of variance.

A total of 6,810 men completed the questionnaires. Of these, 2,858 (42.0%) had served in Vietnam. The area of service could not be determined for 19 respondents. Combat scores ranged from 8 to 40. There were 1,209 Vietnam veterans in the low (8–15) category, 1,084 in the medium (16–25) category, and 552 in the high (26–40) combat category. Herbicide exposure indices computed for 2,087 Vietnam veterans ranged from 0.0 to 9.9. There were 947 men in the low (0.0–0.097), 583 men in the medium (0.098–0.308), and 557 men in the high (0.308–9.9) herbicide exposure categories.

When the responses of Vietnam veterans were compared to those of Vietnam-era veterans who served elsewhere, the odds ratios for medically diagnosed heart disease, venereal diseases, and benign fatty tumors were statistically greater than 1.0. This was also true for the self-reported conditions of "skin rash with blisters," "change in skin color," and "increased sensitivity to light." Among Vietnam veterans whose exposure to herbicides could be categorized, there was a statistically significant trend of increasing odds ratios with exposure for the diagnosis of benign fatty tumors and the self-reported symptoms of adult acne, skin rash with blisters, and increased sensitivity to light. Combat exposure was significantly associated with the reported diagnoses of high blood pressure, stomach or duodenal
ulcer, benign fatty tumors, arthritis or rheumatism, hepatitis, genitourinary problems, nervous system disease, and major injuries. Because benign fatty tumors were associated with both herbicide exposure and combat, this response was analyzed further. Both exposures were found to be independent predictors of the diagnosis.

In addition to specific diagnoses and symptoms, groups of self-reported symptoms were combined into five separate "symptom scales" referred to as "faint," "fatigue," "aches," "colds," and "skin" and combined responses within each of these scales were analyzed. The odds ratios for all five of these "symptom scales" were significantly greater than 1.0 when Vietnam veterans were compared to non-Vietnam veterans. Also each of the five "symptom scales" was significantly associated with both herbicide and combat exposure among Vietnam veterans. Analysis of variance indicated that herbicide and combat exposure were independent predictors of each "symptom scale." The results of analyses of the responses pertaining to psychological outcomes and to reproductive effects are discussed in the sections devoted to those effects below. It should be noted that only 11 medical conditions were mentioned by at least 0.5% of the respondents. Medical conditions that had too few responses to tabulate and analyze included cancer.

Great care must be exercised in interpreting the results of this study. The authors recognized and discussed many of the limitations that are inherent in both the design and the conduct of the study (Stellman and Stellman 1986, Stellman et al. 1988a). The study is a cross-sectional, self-reported survey of health status as of 1983. Although past medical problems were addressed in the questionnaire, there is the possibility of bias in the recall of past health problems. There was no attempt to verify any reported diseases or conditions by examining medical records or conducting medical examinations. By 1983, Vietnam veterans, especially those associated with veterans' organizations, had
ample opportunities to be informed of many potential long-term consequences of combat experience and/or exposure to phenoxy herbicides. Thus, they might be more likely to recall related symptoms or problems in their own experience. It is also possible that their recollection might be inaccurate of the time and location of military service as much as 20 years in the past. This inaccuracy would not be expected, however, to result in a systematic bias. The authors also addressed the questions of representativeness and bias in selection of the ultimate study population. They pointed out that American Legion members are probably not representative of Vietnam-era veterans in general. Potential bias in the selection of Vietnam veterans by the "volunteer researcher" in each of the posts is impossible to evaluate. Furthermore, the response rates among those to whom the questionnaire was mailed ranged from 52.5% in Pennsylvania to 64.1% in Minnesota. The authors attempted to analyze whether the low response rate might bias the results by comparing responses from posts where the response rates were very low to responses from posts where there were high response rates. No significant differences were found in either measures of health status measures or measures of combat and herbicide exposure, suggesting, but not confirming, that the status of those who did not respond was similar to those who did.

Little significance can be attached to reported correlations between health effects and herbicide exposure in the study. The methodology used to assess herbicide exposure was less rigorous than that attempted and abandoned as unreliable by the CDC. The use of only 100 locations in Vietnam allows wide latitude for exposure. The strong correlation between herbicide exposure index and combat exposure seen in this study is not remarkable in that these may not be independent variables and both may be subject to recall bias despite the authors' assertions to the contrary. At best this study adds support to findings of the VES that veterans who recall more herbicide exposure report more
adverse health effects. In the American Legion study neither the exposure nor the health consequences were objectively verified.

Other epidemiologic investigations have equated military service in Vietnam with exposure to herbicides. Two aspects of the American Legion study are of interest in interpreting these studies. First, possible exposure of American troops to herbicides shows a very skewed distribution with the vast majority of troops reporting little or no exposure. Second, herbicide exposure was very highly correlated with combat experience. Therefore, sophisticated statistical analysis was required to investigate the independent influence of these two variables on subsequent health status. Both of these facts tend to support criticisms of earlier epidemiologic studies of Vietnam veterans. It is possible that causal relationships between adverse health effects and exposure to Agent Orange in Vietnam in "negative" studies are obscured because of the inclusion of large numbers of subjects with little or no herbicide exposure. On the other hand, borderline positive findings such as increased risks of NHL and lung cancer among Marines who served in Vietnam (Breslin et al. 1987, 1988) might be attributable to the confounding variable of combat-related stress.

3. The Wisconsin Study

An extensive epidemiologic investigation of mortality patterns among Vietnam-era veterans in Wisconsin was completed by Anderson et al. in 1986, but the results of this study were not published in the open literature. Using the Wisconsin Department of Veterans Affairs DD-214 Military Service Separation File, the authors identified a study population of 122,238 veterans who had served a minimum of 6 months on active duty in the period from January 1, 1964 until December 31, 1975 (the Vietnam era). The same data base was also used to subdivide the study population into two categories, Vietnam veterans and non-Vietnam veterans. The vital status of the veterans in the study population was ascertained using the Wisconsin Department of Veterans Affairs
Graves Registration File (which also contained information on service dates, theater of service, and branch of service), Wisconsin Center for Health Statistics Death Index Tapes (which also indicated veteran status), and the U.S. Veterans Administration BIRLS data base. Causes of death were determined from abstracts of death certificates contained in the Death Index Tapes. Standardized mortality ratios (SMRs) for causes of death were calculated using four different referent populations. These were the U.S. population as a whole, the entire Wisconsin population, all Wisconsin non-veterans, and all Wisconsin veterans. SMRs were calculated for all Wisconsin Vietnam-era veterans, Vietnam veterans, and non-Vietnam veterans compared to each of these referent groups. Also, SMRs for seven causes of death were calculated by comparing Vietnam veterans to non-Vietnam veterans.

Comparison of Wisconsin Vietnam veterans with the U.S. population as a whole and the entire Wisconsin population revealed no statistically significant excess SMRs for any cause of death. SMRs were significantly lower than 100 for all causes of death and for all cancers as well as for a number of specific causes of death, including cancer of the respiratory system, lung cancer, and cardiovascular diseases. When Vietnam veterans were compared to Wisconsin non-veterans, there was a significant excess of deaths from suicide (SMR = 118). When Vietnam veterans were compared to all Wisconsin veterans, there was a significantly elevated SMR (289) for cancers of other lymphatic tissues. Comparisons between Vietnam veterans and non-Vietnam veterans were made only for seven cause-of-death categories. These were all causes, and all cancers, all cardiovascular diseases, all external causes of death, all accidents, all motor vehicle accidents, and suicide. The SMRs for all external causes of death, all accidents, and all motor vehicle accidents were significantly elevated for Vietnam veterans in this comparison,
while there were no differences between the groups in deaths from all causes and from cancer, cardiovascular disease, or suicide.

An interesting aspect of this study was that mortality patterns were similar among all Vietnam-era veterans in Wisconsin whether or not they had served in Vietnam. Mortality patterns among all Vietnam-era veterans were quite different from those in any of the four referent populations, however. These differences for the most part reflected the "healthy veteran" effect as manifested by deficits in the SMRs for all causes of death, all cancers, all respiratory and cardiovascular diseases, all metabolic and nutritional diseases, and many other causes. There were no statistically significant excess SMRs in any of these comparisons. The authors pointed out that the healthy veteran effect was always slightly more pronounced in non-Vietnam veterans than in Vietnam veterans. This aspect of the study provided no evidence that Vietnam veterans were more likely to die of STS or NHL than were non-Vietnam veterans or the population at large. There were only nine deaths due to STS in the Vietnam-era cohort, four in Vietnam veterans and five in non-Vietnam veterans.

As part of the same epidemiologic investigation, the authors conducted a proportionate mortality study to look for associations between veteran status and any specific causes of death (Anderson et al. 1986). All deaths from 1960 through 1979 were identified from the Wisconsin Center for Health Statistics. The veteran status of each deceased individual was determined from the same records as those used in the SMR study described above. All Wisconsin veterans' deaths were compared to Wisconsin non-veterans' deaths by sex and race. Also, deaths among Wisconsin Vietnam veterans and Wisconsin non-Vietnam veterans were compared to Wisconsin non-veterans and to Wisconsin veterans from other eras.

When white male Vietnam veterans were compared to non-veterans, proportionate mortality ratios (PMRs) were
significantly elevated for cancer of the pancreas \((\text{PMR} = 280)\), all external causes of death \((125)\), all accidents \((127)\), motor vehicle accidents \((140)\), and suicide \((122)\). There were also a number of PMR deficits including those for all lymphopoietic cancers \((61)\). When white male Vietnam veterans were compared to all other white male veterans, PMRs were significantly elevated for STS \((\text{PMR} = 1530)\), diseases of the genitourinary system \((261)\), all external causes of death \((105)\), all accidents \((108)\), and motor vehicle accidents \((117)\). When white male Vietnam veterans were compared to white male veterans of the Vietnam-era who did not serve in Vietnam (non-Vietnam veterans), PMRs were significantly elevated for cancer of the pancreas \((554)\), all genitourinary diseases \((292)\), and pneumonia \((204)\). The PMR for STS in this comparison was 147, which was not significantly different from 100. The total number of deaths due to STS among Wisconsin Vietnam veterans was five.

The authors of these studies concluded in their overall summary that the studies were consistent with the findings of other veteran studies in that qualifying for military service (passing the preinduction medical screening) is reflected in reduced risk of mortality due to many chronic diseases (the healthy veteran effect). The findings also reflected excess risk among veterans for causes of death that reflect lifestyles that are detrimental to health, namely lung cancer, emphysema, and cirrhosis of the liver. Vietnam veterans showed increased risks for death from external causes, including motor vehicle accidents and suicide, over any other group. The authors suggested that these causes of mortality might be indicative of increased stress experienced by the veteran population. The increased risk of STS seen in Vietnam veterans could not be attributed to service in Vietnam or exposure to Agent Orange because the same increase was seen in non-Vietnam veterans.

This study is similar in design to studies conducted among Vietnam veterans in New York State (Lawrence et al. 1985), in
Massachusetts (Kogan and Clapp 1985, 1988), and among all U.S. veterans (Breslin et al. 1987, 1988). Like those studies, the validity of this study depends on the completeness and accuracy of the records that were used to establish the cause of death and military service records. Causes of death in this Wisconsin study were taken from death certificates and, therefore, are subject to error especially for causes of death that are difficult to diagnose and/or classify. It appears that the identification of veteran status and whether or not veterans served in Vietnam was based on better data in this study than in either the Massachusetts or the New York studies. The statistical power of this study (as of the others) is limited because of the small number of deaths attributable to specific causes. Thus, for example, in the SMR portion of this study comparisons between Vietnam and non-Vietnam veterans could only be accomplished for seven relatively common causes of death. These studies are not likely to detect small but important excesses of mortality from relatively rare diseases.

4. Summary and Conclusions

Three major epidemiologic studies of veterans of the Vietnam era became available for review in 1988. Two were morbidity studies, and one was a mortality study. These studies differed from each other in both objectives and design, but there was enough overlap that certain general conclusions can be drawn from them. First, it is quite clear from both the VES and the American Legion study that Vietnam veterans perceive that they are less healthy than veterans of the same era who did not serve in Vietnam. Vietnam veterans report more symptoms of impaired physical and psychological status, seek more medical care, and report more medical and psychological problems than do non-Vietnam veterans. The results of the VES indicate, however, that medical and physical examination reveals few differences between Vietnam and non-Vietnam veterans. The American Legion study did not have a medical examination component.
The VES was not designed to relate health effects to exposure to herbicides while serving in Vietnam. The American Legion study attempted to assess herbicide exposure and its relation to adverse health effects and found a number of correlations between herbicide exposure index and reported health status. However, there is no evidence that the herbicide index is a valid objective measure of exposure. Neither study provided any suggestive evidence that there were increased incidences of cancer among Vietnam veterans or individuals exposed to herbicides.

The Wisconsin mortality study was not inconsistent with other mortality studies among Vietnam veterans. This study showed that veterans of the Vietnam era were less likely to die of common causes than non-veterans (the healthy veteran effect), although this effect was not as great in Vietnam veterans as in veterans who did not serve in Vietnam. Veterans of the Vietnam era were more likely to die of external causes than were veterans of other eras and non-veterans. Comparisons between Vietnam veterans and veterans of the same era who did not serve in Vietnam could only be made for several common causes of death. Vietnam veterans were more likely to die of external causes including accidents than non-Vietnam veterans. In the proportionate mortality portion of the study Vietnam veterans were more likely to die from accidents and suicide than any other comparison group. This study did not provide any evidence of excess mortality from cancer in Vietnam veterans compared to non-Vietnam veterans but the numbers of deaths from cancers of specific sites were too small to allow meaningful comparisons. Also there was no attempt in this study to ascertain exposure to herbicides.

Taken together these studies are consistent with the results of earlier studies of Vietnam veterans. These individuals report more health problems, and their psychological well-being is worse than non-Vietnam veterans. However, objective measures of health
status do not reveal many significant differences. There is little evidence that any lasting health consequences can be related reliably to herbicide exposure. The suggestion that herbicide exposure and combat exposure may be closely related implies that this is an important source of confounding in any epidemiologic study of Vietnam veterans in which combat exposure was not assessed.
Anderson, H.E., Hanrahan, L.P., Jensen, M., Laurin, D., Yick, W-Y., and Wiegman, P. 1986. Wisconsin Vietnam veteran mortality study—Proportionate mortality ratio results—Standardized mortality ratio results (Final report). State of Wisconsin Department of Health and Social Services, Division of Health and Social Services, Division of Health, Section of Environmental and Chronic Disease Epidemiology, Madison, WI. March 1986, 64 pages


B. Cancer

No new studies of the relationship between cancer and military service in Vietnam other than those discussed in Section A above became available during 1988. The VES, American Legion, and Wisconsin studies were not specifically designed to look at cancer. In the Wisconsin study, there were five deaths attributed to STS among Vietnam veterans, but the PMR for this cancer was not significantly different from 100 when compared to non-Vietnam veterans and to non-veterans. In none of these studies was there a significant difference between Vietnam and non-Vietnam veterans in the reporting, diagnosis, or mortality of cancer. The remaining studies of cancer in humans that became available in 1988 were of populations exposed either to phenoxy herbicides during their manufacture or use, or to chlorinated dioxins and related compounds as a result of environmental contamination. These and animal studies relevant to the carcinogenic potential of phenoxy herbicides and/or their dioxin contaminants are discussed in the remainder of this section.

1. Studies of Humans

Epidemiologic studies conducted in Sweden in the late 1970s suggested an association between STS and exposure to phenoxy herbicides (Hardell and Sandstrom 1979; Eriksson et al. 1981). Although several other reports have suggested increased incidence of this classification of cancer among people exposed to phenoxy herbicides, a number of carefully designed and conducted epidemiologic investigations published in the intervening years have failed to support such an association (Balarajan and Acheson 1984; Smith et al. 1984; Hoar et al. 1986; Wiklund and Holm 1986; Woods et al. 1987). The possibility of such an association has been the subject of ongoing debate. The authors of the original Swedish studies decided to conduct a new case-control study in order to see if they could reproduce their earlier findings (Eriksson and Hardell 1988; Hardell and Eriksson 1988a).
All cases of STS that were diagnosed from 1978 through 1983 in males 25–80 years of age were identified from the Regional Cancer Registry in Umea, Sweden. Only histopathologically diagnosed cases were included. Diagnoses were not confirmed at the time of the study. Each case was matched to four living referents and two deceased referents by age and by county of residence at the time of diagnosis. A second referent group was created by identifying 190 individuals from the same Regional Cancer Registry who had been diagnosed as having some form of cancer other than STS, malignant lymphoma, or nasopharyngeal cancer during the same time period as the STS cases were diagnosed. All living cases and referents were asked to complete a mailed questionnaire. For deceased cases and referents, the questionnaire was mailed to next-of-kin. Telephone interviews were conducted by blinded interviewers to complete incomplete responses or clarify unclear responses. The criterion for classifying an individual as having been exposed to phenoxyacetic acid herbicides was at least 1 day of exposure at least 5 years before diagnosis of cancer or the time of the interview.

Of the 55 cases of STS identified from the Cancer Registry, 18 were alive and 37 were deceased at the time of the study. Among the referent group with other types of cancer, 112 were living and 78 were deceased. Questionnaires were completed for 54 of the 55 cases of STS, 208 of 220 living referents, 103 of 110 deceased referents, and 179 of the 190 referents with other forms of cancer. Nine (16.7%) of the cases met the criteria for exposure to phenoxy herbicides compared to 18 (8.7%) of the living referents, 4 (3.9%) of the deceased referents, and 14 (7.8%) of the referents with other forms of cancer. There were no differences between living and deceased cases and cancer referents in the percentage that reported exposure to phenoxy herbicides. The relative risks for STS among individuals who were exposed to phenoxy herbicides were 3.3 [95% confidence limits (CL) = 1.4–8.1] when cases were compared to living and
deceased population referents and 2.2 (0.9—5.3) when cases were compared to referents with other forms of cancer. If eight cases of "uncertain diagnosis" were removed from the analyses, the relative risks increased to 3.7 (1.5—9.1) and 2.4 (1.0—5.9), respectively. The authors reported no association between STS and exposure to chlorinated phenols although very few cases of referents were exposed to this group of chemicals.

The authors concluded that this investigation confirmed their previous findings of an association between exposure to phenoxyacetic acids and STS. It is interesting to note that the relative risks found in this study are considerably lower than those found in the earlier studies, i.e., 5.3 (2.4—11.5) in Hardell and Sandstrom (1979) and 5.1 (2.5—10.4) in Eriksson et al. (1981). Furthermore, the relative risk for STS when cases were compared to referents with some other form of cancer (2.2) is not statistically significant and is comparable to the relative risk of 1.6 (0.7—3.3) reported by Smith et al. (1984) who also used a referent group comprising individuals with other forms of cancer. The rationale for using referents with other forms of cancer is to reduce the possibility of recall bias because individuals with cancer may be more likely to recall chemical exposures that might be responsible for their disease.

The decrease in relative risk observed by Hardell and Eriksson in their recent study when cases are controlled to referents with cancer instead of general population referents suggests that recall bias might play some role in this and their earlier studies, but it does not fully account for all of the association. Hardell and Eriksson did not include specific information on the design or content of the questionnaire that they used to ascertain exposure. This lack of detail, which was also characteristic of their earlier publications, precludes the independent evaluation of other potential sources of recall bias or of potential confounding variables.
An epidemiologic study of STS among agricultural and forestry workers in Sweden was critically reviewed in Volume IX of this review (Wiklund and Holm 1986). That record-linkage study was subsequently published (Wiklund et al. 1987b). The authors have expanded the study to include an analysis of the incidence of malignant lymphoma in the cohort (Wiklund et al. 1988). The design is identical to the earlier study of STS except that the tumors included were Hodgkin's disease (HD) and NHL. The cohort consisted of 354,620 Swedish men who were employed in agricultural or forestry occupations at the time of the 1960 Swedish census. The cohort was subdivided into six subclassifications identified as land/animal husbandry, horticulture, other agricultural occupations, silviculture, timber cutting, and other forestry occupations. The reference cohort consisted of 1,725,845 Swedish men of the same age who were employed in economic activities other than agriculture or forestry in 1960.

The relative risks for NHL in the total study cohort and in the six subcohorts ranged from 0.66 to 1.11 and did not differ significantly from 1.0. Furthermore, there was no time-related trend in relative risk in the total cohort or in any of the subcohorts. There were statistically significant elevations in the relative risks (RR) of HD in two of the subcohorts, namely other agricultural occupations and silviculture, RR = 1.74 (1.08-2.61) and 2.26 (1.25-3.69), respectively. A statistically significant increase in risk with time was also observed in these two subcohorts but not in the total cohort.

The subcohort, other agricultural occupations, is a miscellaneous category so this subgroup was further broken down into reindeer husbandry, fur farming, other animal care (not livestock), veterinary work, other relevant occupations, and other agriculture. When broken down in this manner, the relative risk for HD was statistically elevated only among those employed in fur farming. A total of 36 cases of HD were reviewed more
carefully in order to seek clues as to the cause of the disease. Of the 15 cases of HD among men employed in silviculture, eight were employed in management or administration. All five cases in fur farmers were among men engaged in the raising of mink. All four workers with HD who were classified as employed in other animal care were poultry farmers. The remaining 12 cases of HD had miscellaneous occupations such as cemetery worker and landscape gardener.

The results of this study do not support an association between exposure to phenoxy herbicides and an increased risk of NHL. The increased risk of HD within two subcohorts is unlikely to be related to herbicide exposure as there is little reason to believe that the individuals in these subcohorts were more heavily exposed to herbicides than men in the other cohorts. If there is a common factor among individuals with an increased risk for HD in this study it might be the handling of animals other than livestock.

The design of this study was criticized in Volume IX of this review because of its classification of exposure on the basis of employment in 1960. The authors addressed this and other criticisms in the discussion section of the present paper. The authors stated that the loss of sensitivity due to the definition of the cohorts based on employment status in a single year was small because only 13% of those who were employed in agriculture/forestry in 1960 were not employed in those categories in 1950 and only 8% of the comparison cohort were employed in agriculture/forestry in 1950. Furthermore, a mail survey of a subgroup of the study cohort indicated that at least 25% of them had used phenoxy acid herbicides one day or more during the period from 1950 to 1980. However, actual herbicide exposure was not further characterized in either cohort. In conclusion, this study does not support an association between exposure to phenoxy herbicides and an increased risk for NHL. The increased risk for HD seen in two subcohorts in this study is
unlikely to be associated with exposure to herbicides. Because of the lack of detailed and specific information on herbicide exposure in both the study cohort and the reference cohort, this study cannot be considered to be definitive evidence for a lack of association between phenoxy herbicide exposure and malignant lymphoma.

A case-control study of NHL in Sweden was described by Olsson and Brandt (1988). The authors interviewed 167 adult males who were treated for NHL in the Department of Oncology at the University of Lund Hospital between 1978 and 1981. The interview was designed to obtain a lifetime occupational history with special emphasis on exposure to organic solvents, chlorophenoxyacetic acid herbicides, and chlorophenols. Two different control groups were used. One consisted of 50 men from the same geographical region as the NHL patients who had served as controls in a case-control study of Hodgkin's disease. The other consisted of 80 men from all areas of Sweden who had been identified as controls in a case-control study of STS.

Daily exposure to organic solvents for a minimum of 1 year was significantly associated with an increased risk of NHL in this study (OR = 3.3, 95% CI = 1.9–5.8). There was a significant trend toward increasing risk with increased duration of exposure. The authors found no association between risk of NHL and exposure to either phenoxy herbicides or chlorophenols. However, there was a significant association between localized skin lymphoma and exposure to phenoxy herbicides (OR = 10.0).

The authors noted that the interviewers were not blinded as to the case or control status of interviewees. Also, the use of healthy controls raised the possibility of recall bias regarding exposure. Nevertheless, the authors felt that limiting the definition of solvent exposure to a minimum of one year would minimize errors in recall. The authors also felt that the conclusions of the study were strengthened by an apparent dose-response relationship and by the observation of a median latency
of 21 years. These results may be relevant to interpretation of other studies of a possible association between NHL and exposure to phenoxy herbicides in that exposure to organic solvents may be a confounding variable in those studies.

Cartwright et al. (1988) described the results of a case-control study of NHL in Yorkshire, England. The objective of this study was to examine a wide variety of factors, including lifestyle, familial connections, and medical and occupational histories, for potential involvement in the etiology of this class of cancers. It was not a specific study objective to look at the association of this form of cancer with agriculture, in general, or with exposure to phenoxy herbicides and their associated dioxins, in particular. All cases of NHL that were diagnosed in Yorkshire hospitals with histopathology laboratories between October 1979 and December 1984 served at the basis for the identification of the cases for this study. Individuals who had died at the time of the study or for whom the diagnosis of NHL could not be confirmed from a review of diagnostic material by a panel of pathologists were excluded from the study. The controls for this study were selected from a population of patients from the same hospitals who had been diagnosed with some disease or condition other than NHL during the same time period. Controls were matched to cases on the basis of residential health district, sex, and age (±3 years). Subjects with cancer and control subjects were interviewed by trained interviewers using standardized questionnaires. Interviewers were aware of the diagnosis of the individual being interviewed.

A total of 437 subjects with cancer and 724 control subjects were interviewed. There was a slightly increased risk (OR = 1.13) for NHL among individuals who had worked in agriculture but this increase was not statistically significant. The odds ratio for NHL among individuals who had been exposed to fertilizers, pesticides, and/or herbicides bordered upon statistical significance (OR = 1.3, 95% CL = 1.0–1.8). However, the
questionnaire was not designed to break out herbicide exposure from the other agricultural chemicals in this category. Thus, the most that can be said about this study is that it is not inconsistent with other epidemiologic studies that have suggested possible associations between NHL and agricultural professions and exposure to phenoxy herbicides. Perhaps of more potential interest was the finding of associations between a number of diverse exposures and previous medical history and NHL. These included exposures to wood dust and epoxy glues. The authors suggested that a common aspect of these factors was the possibility of disturbed or aberrant immune function. This hypothesis is intriguing in view of the extensive animal evidence that chlorinated dibenzo-p-dioxins are potent immune suppressors. However, it is equally likely that the random associations seen in this study are simply the result of multiple comparisons.

Hardell et al. (1987) published a report of a case-control study of Kaposi sarcoma in AIDS patients. The study was designed to investigate a potential etiologic role for dioxin-contaminated products, including phenoxy herbicides, in AIDS. They found no significant difference in exposure to dioxin-containing products between AIDS patients with Kaposi sarcoma and controls. In contrast to this study, Vineis and Zahm (1988) published a letter to the editor suggesting that risks of Kaposi sarcoma may be elevated in individuals exposed to phenoxy herbicides and calling for further epidemiologic investigation of such an association.

Bond et al. (1988) have published the results of a mortality study among 878 workers who were employed in the manufacture, esterification, amination, formulation, and/or packaging of 2,4-D herbicides. The cohort was identified from the employment records of the Dow Chemical Company facility in Midland, Michigan, and consisted of all employees who worked in any of four different plants where these operations were conducted during the period from 1945 through 1982. The employment records for each individual in the cohort were reviewed by industrial
Hygienists who assigned an exposure score to each based on job classification and the months employed in each job. Each job classification was assigned to one of three exposure categories, high, medium, or low.

Mortality by specific causes of death in the cohort was determined from Dow's vital status registry, which contains causes of death as listed on death certificates for all of Dow's current and former employees. Mortality rates within the cohort were compared to rates within the U.S. white male population as a whole and also to mortality rates among all other male employees of Dow Chemical Company. The mortality data were also analyzed by production area, duration of exposure, and cumulative exposure indices. A latency period of 15 years was assumed for the purpose of analysis.

For the entire cohort, 1963 was the median year of first exposure to 2,4-D. Over half of the cohort was first exposed more than 15 years before the study. The mean duration of exposure was 3 years, and one-third of the cohort held jobs classified in the high exposure category at one time or another. The total person-years at risk was 16,297 with an average follow-up of 18.6 years. There were 111 deaths among the cohort by the end of 1982. The standardized mortality ratio for all causes of death was 100 when the cohort was compared to the U.S. white male population. There were 26 deaths due to cancer in the cohort compared to 22.6 expected (SMR = 115, 95% CL = 75-168). There were no deaths due to STS or nasopharyngeal, stomach, liver, or brain cancer within the cohort. The SMRs for cancer of the large intestine (212) and for cancers of the lymphatic and hematopoietic systems (202) were elevated but were not significantly different from 100. There was one death attributable to HD (SMR = 265, 3-1472). There were no deaths attributable to NHL. The cancers of the lymphatic and hematopoietic system were leukemia and lymphosarcoma or reticulosarcoma. The only SMR that was significantly different
from 100 in the cohort was that for cancer of other and unspecified sites (SMR = 306, 101-729). This category is a catch-all category for rare, miscellaneous cancers. A review of the death certificates for the five individuals who died with this classification of cancer revealed no misclassification of cancers that might have been brain cancer, HD, or NHL.

For causes of death other than cancer, there were elevated SMRs for cerebrovascular disease (SMR = 132), gastric and duodenal ulcer (SMR = 151), diseases of the genitourinary system (SMR = 158), and accidents (SMR = 136). None of these was statistically significant. When the cohort was compared to other male workers at the Dow facility, the relative risks were 1.24 (95% CL = 0.84-1.83) for all cancers, 1.21 (0.62-2.34) for lymphatic and hematopoietic cancers, and 2.01 (0.84-4.80) for cancer of other and ill-defined sites. All of the men who died of lymphatic and hematopoietic cancers or cancers of other or ill-defined sites had worked at one time in a plant known as the 2,4-D plant. However, the authors found no trends of increasing risk with increasing duration or intensity of exposure.

This study shows no statistically significant excess risk for any cause of death except cancer of other and ill-defined sites. This finding is difficult to interpret because this classification is a catch-all category for a variety of poorly characterized cancers. It is unlikely that these five cancer cases had a common etiology. The study gives no evidence for an increased risk of STS, NHL, stomach cancer, or brain cancer among workers exposed to 2,4-D. Though not statistically significant, the elevated SMRs for cancer of the large intestine, lymphatic and hematopoietic cancer, and cerebrovascular disease are noteworthy. Also, it is interesting to note that the so-called "healthy worker effect" is not apparent in this study. The mortality rates in workers exposed to 2,4-D are comparable to those in the U.S. population at large and higher than those in the other male employees of Dow. With 87% of this cohort still
In another mortality study conducted among employees of the Dow Chemical Company in Midland, Michigan, Bond et al. (1987) reviewed the medical records of 2,192 men who may have been exposed to chlorinated dibenzo-p-dioxins during their employment. Each employee was placed in one of four categories reflecting whether or not they had ever had chloracne since their first exposure. The categories were "definite," "probable," "possible," and "none." The mortality experience among those who probably or possibly had chloracne was compared to mortality patterns among those who possibly or never had chloracne and to mortality patterns within the U.S. white male population as a whole.

There were 322 employees categorized as definitely or probably having had chloracne. Among these there were 33 deaths compared to 39.5 expected on the basis of the U.S. white male population. There were six deaths from cancer versus 8.9 expected. None of the cancers were of the nasopharynx, stomach, liver, soft tissues, or lymphatic or hematopoietic tissues. There were four deaths from vascular lesions of the central nervous system (stroke) in the chloracne group compared to 1.9 expected. This cause of death was not elevated in the group without chloracne (10 observed vs. 15.6 expected). However, the SMR was not statistically different from 100. Cancers of the lymphatic and hematopoietic tissues and cancers of other and ill-defined sites were slightly elevated in the group without chloracne, but these SMRs (177 and 192, respectively) were not significantly different from 100.

This study contributes significant new information to the question of the relationship between exposure to chlorinated dioxins and health outcomes.
dioxins and cancer. There is little doubt that the workers with chloracne experienced significant exposure to dioxins at some point in the past. Since this type of exposure has been carefully controlled in recent years, it is also likely that a number of years have passed since these exposures took place. Thus, two common criticisms of epidemiologic studies of the association between cancer and dioxins, namely inadequate evidence of exposure and insufficient latency, are probably not applicable to this study. Nevertheless, there were no cases of STS, or lymphatic or hematopoietic cancers within this cohort. Continued surveillance of this cohort is important both for monitoring additional cancer mortality and for continued monitoring of the incidence of strokes.

Green (1987) published a brief preliminary report of a cohort mortality study of forestry workers employed by a Canadian public utility from 1959 to 1982. The cohort consisted of 1,222 men employed for 6 months or more who were exposed to phenoxy herbicides including 2,4-D and 2,4,5-T during brush clearing and vegetation control activities along power lines and at power stations. The identity of the control population that was used to calculate SMRs for this cohort was not stated in this brief report. Mortality due to cancer in this cohort was not different from the reference population (18 deaths vs. 16.4 expected, SMR = 110). There were no deaths in the cohort due to either STS or NHL. Mortality due to diseases of the circulatory system, other causes, and accidents was reduced in the cohort relative to the reference population, but these differences were not statistically significant. The reason that the author published a preliminary report, however, was that mortality due to suicide was significantly increased in the cohort (SMR = 212, C.L. = 126–298). When the cohort was broken down into categories based on duration of exposure, the SMRs for suicide were significantly elevated among workers with relatively short (less than 5 years)
and with medium (5—14 years) durations of exposure but not among workers with long (more than 16 years) exposure.

While this study indicates no association between exposure to phenoxy herbicides and increased mortality due to cancer, the lack of detail regarding the ascertainment of exposure, the formation of the cohort, and the nature of the reference population precludes independent evaluation. The author pointed out that the cohort was still relatively young with 96% being under 55 years of age. The finding of excess deaths due to suicide in the cohort is of interest in view of similar findings of excess mortality due to suicide among Vietnam veterans (see Breslin et al. 1986 in Volume X of this review and Anderson et al. 1986).

Kuratsune et al. (1987) provided a preliminary report on the results of a mortality study among victims of Yusho disease in Japan. The population consisted of 1,821 people who were classified as having consumed rice oil that was contaminated with polychlorinated biphenyls (PCBs) in 1968. Subsequent research has suggested that many of the signs and symptoms of acute poisoning in these people were attributable to chlorinated dibenzofurans that resulted from the thermal decomposition of PCBs during heating. Chlorinated dibenzofurans are structurally related to chlorinated dibenzo-p-dioxins and cause a similar spectrum of toxic effects in experimental animals.

The authors of this study were able to determine the vital status of 1,761 of these Yusho victims as of the end of 1983. The underlying causes of death for the 120 people who had died were obtained from death certificates. Cause-specific death rates within the cohort were compared to national age- and sex-specific death rates for the same causes in 1970, 1975, and 1983. The number of deaths from all causes was slightly elevated in males and slightly depressed in females compared to the Japanese population at large. These differences were not statistically significant. There were significantly more deaths than expected.
from all cancers, those due to liver cancer, and those due to cancer of the respiratory system among males in the cohort. Cancer of the liver as a cause of death was also elevated among women (2 observed vs. 0.66 expected), but this difference was not statistically significant. There were fewer deaths attributable to cerebrovascular disease than expected among both males and females in the cohort. Deaths due to cancers at sites other than those mentioned and due to diabetes, cardiovascular diseases, respiratory diseases, kidney disease, and gastrointestinal diseases were comparable to expected death rates from these causes. There were 6 deaths due to chronic liver diseases and cirrhosis among males in the cohort compared to 2.26 expected. This difference was not statistically significant.

The authors further analyzed the mortality due to liver cancer in males by comparing it to mortality from this cancer in the two prefectures of Japan where the victims resided. Also, only those deaths that occurred at least 9 years after the poisoning were included in the analyses. The increased odds ratio for this cause of death continued to display statistical significance in these two analyses.

The authors emphasized the preliminary nature of their findings. All of the excess liver cancers occurred in males from one of the two prefectures in which the poisonings occurred. The authors had not yet attempted to relate cancer death rates to the amount of contaminated rice oil that was consumed. Certainly, the cumulative doses of chlorinated dibenzofurans to which the victims of Yusho disease were exposed were orders of magnitude higher than potential doses experienced by Vietnam veterans exposed to Agent Orange. The observed increased risks of both malignant and non-malignant liver disease in this cohort is consistent with studies in experimental animals that show that the liver is a primary target for the toxic and carcinogenic effects of chlorinated dibenzo-p-dioxins (Kociba et al. 1978, NTP 1982).
The National Institute for Occupational Safety and Health (NIOSH) has been identifying and studying mortality and morbidity among workers in the United States who have been exposed to chemicals contaminated with chlorinated dioxins. To date no results of these studies have been published despite announcements that such results were imminent during the past 4 years. During 1988 two reports and an abstract describing exposure information and selection of subjects for the study became available but these contained no results (Marlow and Fingerhut 1986, Marlow et al. 1987, Sweeney et al. 1988). Furthermore, the results of a study of dioxin-exposed workers from the W.G. Krummrich plant in Sauget, Illinois, that was described in 1986 have never been published (see Hryhorczuk et al. 1986 in Clement 1987). The results of these studies could be extremely useful in resolving questions about possible relationships between exposure to dioxin and long-term health effects.

2. Animal Studies

The results of a 2-year feeding study of 2,4-D in rats have been described in a toxicology review (Mullison 1986), but a published report of this study has not been identified. Mullison also indicates that a similar study in mice was completed in 1987. It is not clear why these studies have not been published or reported upon at scientific meetings. In the rat study, 2,4-D was incorporated into the diet of male and female Fischer 344 rats for 2 years. The concentrations resulted in nominal daily average doses of 1, 5, 15, and 45 mg/kg. Mullison indicated that the 1 mg/kg daily dose was a no-observed-effect level based on the occurrence of histopathological changes in the kidneys of rats at the 5 mg/kg and higher dose levels. A "slight but statistically significant" increase in the incidence of astrocytomas (brain tumors) was observed among male rats in the highest dose group. Mullison reported that, based upon a thorough biological evaluation and analysis of study results, Dr.
Adalbert Koestner concluded that 2,4-D was not the cause of these astrocytomas. No additional information is available in Mullison's brief summary.

Rao et al. (1988) described a study of the carcinogenic potential of 2,3,7,8-TCDD in Syrian golden hamsters. Male hamsters were given either intraperitoneal or subcutaneous injections of TCDD dissolved in dioxane once each month for 6 months. Control animals were injected with the dioxane vehicle alone. The doses tested were 50 and 100 $\mu$g/kg by the subcutaneous route and 100 $\mu$g/kg by the intraperitoneal route. Four of 18 hamsters treated with 100 $\mu$g/kg by the intraperitoneal route and 3/14 hamsters treated with 100 $\mu$g/kg by the subcutaneous route developed squamous cell carcinomas of the facial skin. The earliest tumor appeared 8 months after treatment began. None of the hamsters treated with vehicle or with 50 $\mu$g/kg TCDD developed tumors.

That the tumors seen in this study were all of the same type, occurred at a site remote from the site of injection, were malignant, and only appeared in the high-dose animals strongly supports the authors' conclusion that 2,3,7,8-TCDD is carcinogenic in hamsters. Earlier studies have already shown TCDD to be carcinogenic in mice and rats when administered orally. Hamsters are relatively resistant to the acute lethal toxic effects of 2,3,7,8-TCDD when compared to mice and rats. The site of cancer incidence in the hamsters is quite different from mice and rats for whom the liver is the primary target.

Several authors reported the results of investigations relevant to the mechanism by which TCDD elicits a carcinogenic response in experimental animals. Lincoln et al. (1987) compared the activity of TCDD with that of a well-known tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), in an assay for intercellular communication in cultured Chinese hamster V79 cells. Unlike TPA, TCDD did not inhibit metabolic cooperation in this system. This is consistent with earlier studies that
indicated that these compounds promote cancer by different mechanisms. Wahba et al. (1988b,c) found an increase of single strand breaks in hepatic DNA when rats were treated with a single dose of TCDD. These authors speculated that the enhanced formation of DNA single-strand breaks plays a role in hepatic tumor formation and that this effect is secondary to the stimulation of lipid peroxidation by TCDD. Randerath et al. (1988) looked for evidence of covalent adducts of TCDD with DNA in the livers and kidneys of rats treated with TCDD and found none. They did find that TCDD inhibited the formation of age-related foci of increased DNA synthesis. How this effect is related to the carcinogenic effect of TCDD is unknown.

3. Summary and Conclusions

Two morbidity studies and one mortality study among Vietnam veterans failed to reveal any association between military service in Vietnam and cancer, but these studies were not designed to examine such an association. A case-control study of soft-tissue sarcoma in Sweden showed a statistically significant increase in risk of STS associated with occupational exposure to phenoxy herbicides in agreement with an earlier study by the same authors. The relative risk was lower in this study, however, than in the earlier study. Four epidemiologic studies of agricultural populations in Sweden, England, and Canada revealed no associations between employment or place of residence and STS or NHL. One of these studies indicated that NHL was associated with occupational exposure to organic solvents but not with exposure to phenoxy herbicides or chlorophenol. In the other three studies there was no attempt to characterize herbicide exposure per se, and the proportions of the cohorts with relatively heavy herbicide exposure probably were quite small. An apparent increase in risk for HD in one of these studies was unlikely to be associated with herbicide exposure and was more likely to be associated with contact with animals. Studies of cancer mortality among Dow Chemical employees with exposure to
2,4-D during manufacturing or with a history of chloracne revealed no increased risk of cancer or any specific form of cancer. However, there did appear to be an excess of deaths due to cardiovascular diseases of the central nervous system (strokes) among men with a history of chloracne. These studies, although limited in statistical power, add to a growing body of evidence that industrial exposure to dioxins does not result in greatly increased risks of cancer.

A cancer study in hamsters shows that TCDD is carcinogenic in this species as well as in mice and rats, even though hamsters are relatively insensitive to the acute lethal toxicity of TCDD. That TCDD is such a universal carcinogen in experimental animals continues to have implications for human health. A brief secondary report indicates that rats fed 2,4-D for 2 years exhibited an increased incidence of neural astrocytomas, but the biological significance of this finding is open to question. The results of a companion study in mice are not available. Studies of the mechanism by which TCDD causes increased incidence of cancer in animals are inconclusive. It is hoped that the results of several ongoing studies of the health status of dioxin-exposed workers will be published in the near future. These should help resolve the outstanding questions regarding the human carcinogenic potential of dioxins.
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C. Genetic Effects

No new reports of genotoxic effects of phenoxy acetic acids or of TCDD in humans were found. The available in vivo and in vitro studies are discussed below.

1. In Vivo Studies

a. 2,4-D. Adhikari and Grover (1988) tested the genotoxic potential of 2,4-D in rat bone marrow cells. Dose levels selected for the study were based on the LD$_{50}$ value for rats. Male Wistar rats received 2,4-D by intraperitoneal injection at doses of 17.5, 35, or 70 mg/kg (0.2 ml each) twice at intervals of 24 hours. To arrest mitosis, colchicine (4 mg/kg) was administered intraperitoneally 2 hours before the animals were sacrificed. Ethyl methane sulfonate (EMS) and distilled water-treated groups served as positive and negative controls, respectively. The metaphase preparations revealed that 2,4-D induced chromatid breaks, chromatid fragments, ring chromosomes, and dicentric chromosomes in rat bone marrow cells. Pulverized chromosomes, polyploid cells, and a few cells with despiralized chromosomes were also noticed. The frequency of aberrant cells ranged from 4.46% to 6.66% (versus 8.60% to 17.90% in EMS controls and 1.8% to 2.3% in negative controls). The frequency of breaks and percentage of aberrant cells were statistically significant ($p<0.05$) at the intermediate and high doses. The lowest effective dose of 35 mg/kg body weight/day was 1/20 LD$_{50}$. This study, conducted according to the standard procedures, supports the earlier findings of Turkula and Jalal (1987) reported by Clement (1988) (see Volume XI of this review) and provides sufficient evidence that 2,4-D induces a considerable frequency of chromosomal aberrations in rat bone marrow cells. Occurrence of the observed chromosomal aberrations correlates well with the administration of known mutagens to animals.

b. TCDD. TCDD is a known potent carcinogen in a variety of animal species and a suspect carcinogen in humans. However, results of most cytogenetic studies have been negative. The
cytogenetic effects of TCDD in vivo on liver cells of mice exposed to high concentrations were evaluated by Brooks and his coworkers (1988). Male C57BL/6J mice were given a single intraperitoneal injection of 25, 37.5, 75, or 150 μg of TCDD/kg body weight or corn oil carrier alone. This strain was selected for its high level of cytosolic protein receptors for TCDD transport to the nucleus. Liver sections from the nonhepatectomized mice were studied for TCDD-induced hepatotoxicity at 1, 7, and 30 days after injection. Two-thirds partial hepatectomies were performed at 1, 7, and 30 days after the treatment, and chromosomal aberrations and mitotic indexes were scored 54 hours after hepatectomy. The mice were injected with 4 mg colchicine/kg body weight 4 hours before sacrifice.

The results of the study showed that the degree of liver damage in nonhepatectomized mice was both dose- and time-dependent. Significant liver damage including necrosis and architectural collapse was seen at three high-dose levels of TCDD 30 days after injection. In all exposure groups except 25 μg/kg, TCDD treatment produced a graded dose-dependent decrease (p<0.05) in mitotic index of regenerating hepatocytes. The mitotic index in partially hepatectomized animals at 1 and 7 days after TCDD injection decreased as the dose of TCDD increased. Because of low mitotic index observed at higher TCDD doses, evaluation of frequency of chromosomal aberrations in animals from the 75 and 150 μg/kg TCDD dose groups could not be made. The cytogenetic analysis of the partially hepatectomized animals from the 25 and 37.5 μg TCDD/kg dose groups and the control group at 1 or 7 days after exposure revealed no rings or dicentric aberrations, chromatid type deletions, or exchanges in any of the cells scored. The frequency of chromosomal aberrations was not significantly elevated above that in controls for any dose of TCDD. This well-conducted study clearly indicates that TCDD is not a clastogen for mouse hepatocytes, even at high doses that cause marked hepatocellular necrosis. These findings are
consistent with the findings in nonhepatic murine tissue
process of cancer induction involves both genotoxic and
nongenotoxic modes of action, these negative cytogenetic effects
of TCDD exposure in mice cannot be directly extrapolated to its
possible effects in humans.

2. In Vitro Studies
a. 2,4-D, 2,4,5-T, and TCDD. Components of Agent Orange
(2,4-D, 2,4,5-T, and their esters in addition to the contaminant TCDD) were tested for mutagenicity using Salmonella/arabinose-resistant (Ara\(^+\)) assay system (Soler-Niedziela et al. 1988). Preincubation tests were performed using arabinose-sensitive (Ara\(^-\)) strain SV50 of Salmonella typhimurium both with and without metabolic activation from rat or hamster S9 mix. Each compound was tested using triplicate plates at three- to six-dose concentrations separated by half-log intervals. Ten milligrams per plate was the highest concentration used for each compound, except for TCDD, which was tested at 1 mg/plate. A 2-amino-anthracene-treated group (2.5 µg/plate) was used as a positive control; a DMSO-treated group served as a negative control. The concentration of the compounds used were as follows: 33, 333, and 3,333 µg/plate for 2,4,5-T and 2,4-D and its isoocetyl ester; 0.33, 3.3, 33, 100, 1,000, and 10,000 µg/plate for 2,4-D n-butyl ester; 100, 1,000, and 10,000 µg/plate for 2,4,5-T isobutyl, isoocetyl, and n-butyl esters; and 10, 100, and 1,000 µg/plate for TCDD. A compound was defined as mutagenic when the average number of mutants obtained was twice the solvent control and showed a dose-response relationship. None of the compounds showed mutagenic activity as defined by these criteria. All chemicals except TCDD showed variable toxicity at the higher dose levels of 3,333 and 10,000 µg/plate. Positive controls responded strongly to both rat and hamster S9 activation while solvent control gave a negative response. The validity of the system was
demonstrated with positive controls, but none of the compounds gave any indication of mutagenicity.

Both 2,4-D and 2,4,5-T were nonmutagenic in *S. typhimurium* strains TA97, TA98, TA100, and TA102 when tested with or without metabolic activation at concentrations of 100 or 1,000 \( \mu g/plate \) (Mersch-Sundermann et al. 1988). These compounds failed to increase the number of revertants per plate over that of the spontaneous rate.

Three chlorophenoxy herbicides, namely, 2,4-D, 2,4-(dichlorophenoxy)butyric acid (2,4-DB), and 4-chloro-2-methylphenoxyacetic acid (MCPA), were tested in *S. typhimurium* strains for point mutations and in *Aspergillus nidulans* for mitotic recombination (Kappas 1988). 2,4-DB was nonmutagenic with or without metabolic activation in both the Ames and the Aspergillus tests. 2,4-D and MCPA were nonmutagenic in *Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538 when tested at concentrations ranging from 10 to 1,000 \( \mu g/plate \) with or without metabolic activation from rat S9 mix. However, both compounds were weakly mutagenic in strain TA97a at concentrations between 250 and 750 \( \mu g/plate \); the induction of frameshift mutations occurred only in the presence of S9 mix. The weak mutagenic response observed may have resulted from an unknown high sensitivity of this strain to mutagen. The overall results of the *Salmonella* assays support the findings reported in a previous Clement report (Clement 1985) that 2,4-D is not a point mutagen. On the contrary, both 2,4-D and MCPA increased the frequency of mitotic segregants in *A. nidulans* colonies at concentrations of 4-48 \( \mu M \) and 1,500-3,000 \( \mu M \), respectively. At least 100 colonies were tested at each concentration of the chemical. In the presence of S9 mix, 2,4-D increased the number of mitotic segregants more than three times and MCPA alone more than twice the control level. The mitotic segregation occurred mainly by mitotic crossing over. Although no statistical analysis of data was provided by the author, the results clearly
indicate that 2,4-D is an indirect mutagen, while MCPA can produce genetic change by causing direct effect on the chromosomes in *A. nidulans*.

Little is known about the chemical changes that may occur in the cell membranes of 2,4-D-treated cells that would influence the growth of cells. de Duffard and coworkers (1988) studied the *in vitro* toxicity of 2,4-D on the growth of Chinese hamster ovary (CHO) cells and determined their phospholipid, cholesterol, glycolipid, ganglioside, and protein contents following marginally cytotoxic concentrations. Failure of the cellular adhesiveness and cell monolayer behavior were the criteria of toxicity used. 2,4-D at concentrations of $10^{-3}$ M reduced the total cell number to approximately 10%-20% of controls. When 2,4-D was removed after 4 days of treatment, a rapid resumption of cell growth rate occurred. At $10^{-5}$ M 2,4-D, a significant delay of the growth rate was observed, and glycolipid and ganglioside contents increased in both logarithmic and stationary phases of culture. No changes in protein, cholesterol, and phospholipids were noted. Although 2,4-D affected the growth of cells, no morphological changes were seen at both $10^{-5}$ M and $10^{-3}$ M 2,4-D doses. It has been suggested that glycolipids may be involved in membrane-mediated phenomena such as cell-to-cell recognition, contact inhibition, control of growth, and cellular morphology (Tadaro and Green 1963, Hakomari and Murakari 1968, Hakomori 1975). The authors suggested that the reduced growth rate of CHO cells following 2,4-D treatment may have been the result of augmented levels of membrane glycolipid and ganglioside contents in both phases of cell growth. The results of the study provide good evidence that 2,4-D affects the growth of CHO cells by altering the levels of complex glycolipids in their cell membranes.

3. **Conclusions**

No new information became available on the potential genotoxic effects of phenoxy herbicides and associated dioxins in
exposed humans. 2,4-D was reported to cause damage to chromosomes in rat bone marrow cells. New experimental studies in animals provided additional evidence that TCDD does not induce mutations. Studies in cells in culture showed no genetic effects in bacteria following exposure to TCDD, 2,4-D, 2,4,5-T, and their esters, although 2,4-D produced genetic damage in fungi. The relevance of these findings to humans is not known.


Turkula, T.E., and Jalal, S.M. 1987. Induced clastogenicity in white rats by the herbicide 2,4-D. Cytologia 52:275-281
D. Reproductive and Teratogenic Effects


Two studies were published in 1988 that assessed to some degree the possible association between paternal exposure to Agent Orange and the occurrence of birth defects in children (USCDC 1988c, 1989; Stellman et al. 1988c). These studies are reviewed below.

The CDC recently reported a health survey of Vietnam veterans referred to as the VES (USCDC 1988c). This study has been described in detail in Section A above. An interim analysis of VES interview data showed differences in the rates of total birth defects and cerebrospinal malformations between the children of the two cohorts.

Three substudies were subsequently conducted. The first examined the occurrence of total birth defects in the two cohorts to verify the reported cohort differences, the second examined the occurrence of cerebrospinal malformations in the offspring of the two cohorts, and the third involved analysis of semen from subsamples of the cohorts. In the first substudy, birth weight was also examined. Of the 4,462 veterans who were included in both the interview and medical records examination portion of the VES, 2,282 veterans reported a total of 4,122 offspring. The eligible study population (which excluded adult children, miscarriages, and children conceived before Vietnam assignment, etc.) consisted of a total of 3,683 children with birth defects (1,945 offspring of Vietnam veterans and 1,738 offspring of non-Vietnam veterans). The second substudy assessing the occurrence of cerebrospinal malformations in the children of Vietnam veterans included a total of 403 eligible children. Records of 127 eligible children (82.5%) of the Vietnam veterans and 94 eligible children (67.1%) of non-Vietnam veterans were obtained and the birth defects were analyzed. The odds ratio was used as a primary measure to assess the association of Vietnam experience with reproductive outcomes. These ratios were adjusted by using...
multiple logistic regression methods for various veteran characteristics such as age of veteran at the birth of the child, race, primary military occupation specialty, etc. Vietnam veterans reported significantly more birth defects in their children than did non-Vietnam veterans; the rates of each cohort were 64.6 and 49.5 per 1,000 total births, respectively, and the adjusted odds ratio was 1.3 (95% CI, 1.2–1.4). Odds ratios for central nervous system anomalies such as hydrocephalus, anomalies of the integument, and musculoskeletal deformities were significantly greater than 1.0. In the substudy assessing total birth defects, the rates of total birth defects were similar in the two cohorts (72.6 and 71.1, respectively); the adjusted odds ratio was 1.0 (95% CI, 0.8–1.4). The rates of total, major, minor, and suspected defects were similar among children of Vietnam and non-Vietnam veterans (adjusted ORs: 1.0, 1.1, 1.0, and 0.9, respectively). The odds ratios varied considerably when the major and minor defects were stratified by race. The adjusted odds ratios for total defects among offspring of black veterans was 3.3 (95% CI, 1.5–7.5) compared with 0.9 (95% CI, 0.7–1.3) for offspring of white veterans and 0.4 (95% CI, 0.2–1.3) for offspring of Hispanic veterans and veterans of other races. The increased odds ratio in blacks was probably due to their genetic susceptibility. For all races combined, there were no differences between children of Vietnam and non-Vietnam veterans in prevalence of total, major, minor, or suspected birth defects documented in records. The rates of low birth weight (<2,500 g) were similar in offspring of Vietnam (5.6%) and non-Vietnam veterans (5.5%; the adjusted OR = 1.1; 95% CI, 0.8–1.4).

In the semen analysis substudy, semen samples were collected from 571 participants (324 Vietnam veterans and 247 non-Vietnam veterans) during the medical examination phase of the VES. The Vietnam veterans had significantly lower sperm concentrations and significantly fewer sperm cells with "normal" morphology than did
non-Vietnam veterans. Furthermore, the percentage of Vietnam veterans with sperm concentrations below the normal range was nearly twice that of the non-Vietnam veterans (15.9% vs. 8.1%). Even after adjustment for smoking behavior and alcohol, drug, and medication uses, these differences were still statistically significant. There were no differences between the cohorts for sperm motility and other measures of sperm movement. The fertility record, however, was the same for the two veterans' groups.

In conclusion, although Vietnam veterans reported more adverse reproductive and child health outcomes in the telephone interview than did non-Vietnam veterans, the hospital birth records of the children showed that Vietnam veterans were not at increased risk of fathering children with obvious birth defects. However, the hospital records may not have reported the potential functional deficits in children that could develop at a later stage. The study design and analysis did not take into account recall biases and interview biases that could have confounded the results of the study. Moreover, the study did not attempt to identify the various agents and their exposure levels to which the Vietnam veterans were exposed. Therefore, although the findings of the study were equivocal, the data are of limited value in assessing the reproductive outcomes of Vietnam veterans who were exposed to Agent Orange.

The self-administered questionnaire that was the survey instrument in the American Legion study of health effects of combat and herbicide exposure in Vietnam veterans contained a series of questions designed to assess reproductive health and outcomes among the veterans (Stellman et al. 1988c). These questions sought information on childbearing attempts, number of pregnancies fathered, miscarriages in resulting pregnancies, sex of live-born children, and birth weight. Unlike the VES, no questions designed to identify birth defects were included.
There were no apparent associations between either reported herbicide or combat exposure and difficulty in conception, time to conception of first child, birth weight, or sex ratio of offspring. More of the spouses' pregnancies resulted in miscarriage for Vietnam veterans than for non-Vietnam veterans (7.6% vs. 5.5%). This difference was statistically significant. Logistic regression analysis indicated that miscarriage rates were independently and significantly associated with reported herbicide exposure and maternal smoking behavior in a dose-related manner. Miscarriage rates were not associated with combat exposure.

The authors of this study considered the finding of an association between miscarriages and herbicide exposure to be very significant. However, the identification of miscarriages is totally dependent on recall and is therefore subject to recall bias. The results of the VES discussed above indicate that Vietnam veterans reported adverse reproductive health outcomes more frequently than non-Vietnam veterans, but review of hospital birth records failed to substantiate these differences.

Examination of possible adverse effects of pesticides on human reproductive outcomes has proven to be complex and controversial. White et al. (1988) reported on a series of epidemiological investigations that assessed the proposed association between exposure to some pesticides used in agriculture and forestry, including chlorinated and nonchlorinated phenoxy herbicides, and the occurrence of stillbirths and birth defects in children born in New Brunswick, Canada, from 1971 to 1981. Data on stillbirths were obtained from Statistics Canada, and data on birth defects were obtained from the Canadian Congenital Anomalies Surveillance System (CCASS). Three types of anomalies were identified: neural tube defects, facial clefts, and bilateral agenesis. Combined categories of these birth defects and of 40 other abnormalities documented by CCASS and Statistics Canada were also included in
the analysis. Exposure to pesticides was estimated by the use of an agricultural chemical exposure opportunity index and information from forestry spraying records. Crude estimates of potential exposure to agricultural chemicals were obtained from an agricultural chemical exposure opportunity (ACEO) index, which was developed from maps of soil capability that graded areas in terms of their suitability for agricultural production. Information on chemicals used in forestry was obtained from maps and records of spray application companies and the New Brunswick Department of the Environment.

Geographic and temporal analyses and case-control studies using data obtained from vital statistics, hospital records, and the CCASS revealed no association between exposure during the periconceptional period to pesticides used in forestry and adverse reproductive outcomes. Statistically significant associations were observed between exposure to agricultural chemicals, as estimated by the ACEO index, and the occurrence of spina bifida without hydrocephalus \((p=0.01)\) and also the occurrence of stillbirths during the second trimester \((p=0.0322)\). These associations could not be linked to specific pesticide use because of the general nature of the ACEO index. This study, in general, is limited by the lack of data on parental pesticide exposure, i.e., whether the source is environmental or occupational. Many statistical tests were performed that could have resulted in a multiple-testing bias. This study suggests the need for a more detailed study of specific parental exposures to agricultural chemicals that may be associated with adverse reproductive outcomes. No association between exposure to phenoxy herbicides and adverse reproductive outcomes could be assessed because of the lack of specific exposure information.

There has been continued discussion of the findings from Seveso, Italy, that indicate adverse reproductive effects of TCDD in humans (Clement 1985). Earlier studies reported an apparent elevation in the rate of spontaneous abortion and suggestive
increases in the rates of certain birth defects (angioma, central nervous system effects, hypospadias, hip dislocation, and defects of the digestive tract) in areas of TCDD contamination during July 1976 through December 1980 (Anonymous 1983). There was no indication of an effect on gestational age, birth weight, pathological conditions of pregnancy, or the rate of perinatal or neonatal death (Anonymous 1983, Bianco et al. 1984).

Mastroiacovo et al. (1988) recently reported on an investigation of the frequency of congenital malformations in children born in Seveso, Italy, between January 1, 1977, and December 31, 1982. Certain areas around Seveso were contaminated with TCDD released during the production of trichlorophenol in July 1976. Three areas of decreasing contamination levels, Zones A, B, and R, were identified. The contaminated areas were defined on the basis of soil concentrations of TCDD. The authors used the population residing in the surrounding area as a control. Data were collected from an ad hoc birth defects registry that identified a total of 15,291 (live and stillborn) births; 742 malformed children were identified in this group. The malformations reported included major defects (central nervous system defects, heart, kidney defects, etc.), multiple defects, and mild defects such as hemangioma. There were 26 births in Zone A; none of these had any major structural defects. Two infants had mild defects, one with hemangioma and another with a small periurethral cyst. The frequencies of major defects detected in Zone B, with moderate TCDD contamination, and in Zone R, with low TCDD contamination, were 29.9 and 22.1 per 1,000 total births, respectively. The frequency of major birth defects in the control population was 27.7 per 1,000 births. The relative risk of specific categories of birth defects and for groups of malformations showed no increased risk for any type of birth defect.
The study by Mastroiacovo et al. (1988) had several design weaknesses that limited its interpretation. These limitations included:

1. A possible *misclassification* of exposure status occurred because Zone A was evacuated less than a month after the accident. The people were moved to Zone B or R, and some of the women left the area for the duration of their pregnancy.

2. An undetermined number of women in all exposure areas who were pregnant at the time of the accident underwent therapeutic abortions on the advice of health officials. This action could have significantly reduced the ascertainment of malformations related to exposure. It is possible that some of the women who chose to abort their pregnancies would have had children born before the beginning of the study (January 1, 1977). Any malformations occurring in these infants would have been missed.

3. The malformation registry may have missed a substantial number of cases of spontaneous abortion because the focus of the registry is on birth defects in infants and children.

4. The lack of specific data on TCDD exposure or body burden restricted the interpretation of the study results. These are particularly important because the population was obviously mobile. Therefore, the exposure categories chosen did not accurately reflect the exposure of the population.

5. Another major limitation was the small sample size available in the most contaminated areas. Although the exposed population was one of the largest identified as living in an area contaminated by TCDD, it was not large enough for the identification of low and specific teratogenic risks associated with exposure. Thus, the overall reproductive risk related to TCDD exposure may be small or may be limited to very rare and specific birth defects.

Stockbauer et al. (1988) conducted an epidemiological investigation of birth defects occurring in the population living in Times Beach, Missouri. Nine areas of this town were contaminated when waste oil containing high concentrations of TCDD was sprayed on dirt roads to reduce dust beginning in 1971.
These areas were identified as contaminated areas by the U.S. Environmental Protection Agency and the Missouri Department of Natural Resources. Exposure to TCDD was assumed if the mother's address on the birth or fetal death certificate corresponded to an area contaminated with TCDD at a level of 1 ppb or greater. A list of addresses was compared with records from Missouri Vital Statistics of live births and fetal deaths for the period of January 1, 1972, to December 31, 1982. A total of 410 births during the years 1972–1982 were identified among women potentially exposed to dioxin based on this protocol. A total of 820 births in uncontaminated areas was selected as a control group after being matched for maternal age and race, year of birth, hospital of birth, and plurality. The measures of reproductive outcome analyzed included all birth defects combined, specific grouping of birth defects, fetal deaths, infant deaths, body weight, and intrauterine growth retardation. No data on miscarriages and impaired conception were available for analysis. The statistical analysis of the data failed to reveal significant risk ratios between the dioxin-exposed births and the unexposed births for fetal deaths, infant deaths, perinatal deaths, intrauterine growth retardation, and low and very low birth weight. No evidence of a dose-response relationship was found for any of the outcomes measured. No statistically significant excess risk associated with dioxin exposure was noted for the categories of all birth defects combined.

The limitations of this study include absence of a precise biologic indicator of TCDD exposure, lack of information on maternal contact with soil or duration of such exposure before and during pregnancy, and possible case misclassification. Although TCDD may be harmful at the levels identified in the study, statistically significant adverse reproductive effects were not detected. Among the possible reasons for this failure are the rarity of the adverse reproductive outcomes, the small
size of the exposed population, exposure misclassification, diagnostic misclassification, and lack of information on confounding variables such as maternal alcohol consumption, medical condition of the parents, medication use, and variations in parental susceptibility to the effects of TCDD.

Rogan et al. (1988) reported congenital birth defects in children from Taiwan who were victims of transplacental and lactational exposure to PCBs. The epidemic was noted in May 1979. The cases were identified retrospectively as far back as December 1978. The registry reported about 2,000 persons who were exposed to contaminated cooking oil. Mothers who were poisoned from cooking oil contaminated by thermally degraded PCBs developed Yucheng or oil disease. They reported lower birth weight, hyperpigmentation, conjunctivitis, nail changes, and natal teeth in their children at the time of birth. In 1985, the physical examination of 117 children born to exposed women and 108 unexposed control women revealed neurologic, dysmorphologic, dermatologic, and dental changes in the offspring of exposed women. The exposed children were shorter (averaging 97% of control height), weighed less (averaging 93% of control weight), and also had abnormalities of the gingiva, skin, nails, teeth, and lungs more frequently than did the controls. These affected children also showed behavioral abnormalities and delay in reaching developmental milestones.

Similar observations were reported in the Japanese epidemic in 1968 where mothers of the affected children were exposed to thermally degraded PCBs via consumption of cooking oil. No estimates of the exposed population were reported by the authors. Although the toxic effects reported in the present study (Rogan et al. 1988) are consistent with those observed in the Japanese epidemic, the exposures are relatively low in comparison. The children of workers exposed to PCBs uncontaminated by polychlorinated dibenzofurans (PCDFs) did not present with as many symptoms of poisoning, but the mothers had body burdens of
PCBs that were comparable to those seen in the Taiwan epidemic. Qualitatively, PCBs and PCDFs may be similar in toxicity but PCDFs exert toxicity at much lower doses. The oil in Taiwan contained 100 ppm PCBs and about 0.1 ppm PCDFs. Therefore, the authors speculated that the toxic effects seen in the epidemics in Taiwan and Japan were at least in part due to the presence of PCDFs, which are closely related chemically to TCDD.

2. Studies of Experimental Animals

a. 2,4-D. The toxic effects of 2,4-D butyl ester on central nervous system development in chick embryos were reported by Duffard et al. (1987). Fertilized hen's eggs were treated externally (3.1 mg/egg) with a single dose of compound before incubation or at different times of incubation (5th, 10th, or 15th day). Controls were treated with ether alone. One day after hatching, chicks were decapitated, and brain and spinal cord tissue were removed and analyzed for myelin markers. Eggs that were intact on the 22nd day of incubation were examined for dead embryos. 2,4-D butyl ester permeated the egg-shell and produced approximately 50% mortality in groups treated on day 0, 5, or 10 of incubation. However, the treatment on the 15th day improved the hatchability to 71%. Chicks hatched from the 5th and 10th day treatment groups showed some postural and motor dysfunctions similar to those found in chicks treated prior to incubation. These defects consisted of fusion of neck vertebrae, articulatio-coxa dislocation, and curled claws. Chicks hatched from the 15th day treatment group were normal.

2,4-D butyl ester treatment produced hypomyelination in the cerebrum, brain stem, and spinal cord of chicks. The extent of hypomyelination depended on the time of treatment, i.e., during the proliferation and development of myelin forming oligodendroglia cells. In cerebrum and brain stem, the concentration of galactolipids and 2',3'-cyclic nucleotide 3'-phosphohydrolase activity were significantly reduced in the day 0, day 5, and day 10 treatment groups compared to controls,
but in the 15th day treatment group only sulphatides were decreased. Spinal cord only presented decreases in marker contents in the 5th day treatment group; cerebellum presented normal myelin markers in all four groups. UDP galactose-ceramide galactosyl transferase activity was reduced in whole brain of chicks hatched from eggs treated prior to incubation. The findings of the study indicate that among the central nervous system regions, cerebrum and brain stem appear to be most sensitive to the toxic action of 2,4-D butyl ester in the chick. The vulnerable period in central nervous system development includes proliferation and development of myelin forming cells. The relevance of these findings to humans cannot be determined.

b. \textit{2,4-D/2,4,5-T Mixture}. Prenatal exposure to a \textit{1:1} mixture of 2,4-D and 2,4,5-T produced behavioral and neurochemical alterations in neonates of rats (Mohammad and St. Omer 1988b). Pregnant rats were gavaged with a mixture at 0, 50, 100, or 125 mg/kg/day during days 6—15 of gestation. Dams from the two high-dose groups gained less weight ($p<0.05$). Sex ratios, birth weights, and the physical appearance of the pups at birth were unaffected by the treatment, although pup mortality in the 125-mg/kg/day group increased on postnatal day 1. By postnatal day 60, pups in this group showed normal growth and maturation but slightly reduced weight. Behavioral effects including delayed surface righting and negative geotaxis at 45° were noted in pups at all treatment levels. Additional effects such as olfactory discrimination and increased running wheel activity were noted with increasing dose levels. Biochemical changes in various brain areas included reduction in RNA levels, protein/DNA ratio, brain dopamine, serotonin, and 5-hydroxyindolacetate levels. These changes varied according to the dose, specific brain area, and postnatal day of examination. The data were available in abstract form and therefore assessment of the severity of the effect and dose-response relationship was not possible. Behavioral alterations are becoming more widely
accepted as sensitive indicators of injury to the fetus or the neonate, but it is not always clear how the affected animal behaviors correspond to human behavior.

Recently, Mohammed and St. Omer (1988a) investigated the neurochemical basis for the behavioral teratogenic effects of mixture of 2,4-D and 2,4,5-T in Sprague-Dawley rats following in utero exposure. Pregnant rats were gavaged once daily with 0, 50, or 125 mg/kg of mixture (1:1) in peanut oil. This mixture also contained 0.0125 ppm of TCDD. On postnatal days 1, 15, and 22, the brain tissues (cerebrum, cerebellum, neocortex, or thalamus-hypothalamus) from offspring were dissected and processed to analyze the DNA, RNA, protein, glutamate, and γ-aminobutyrate (GABA) contents. The overall effects of 2,4-D and 2,4,5-T mixture on the developing pattern of the ratios of protein and RNA contents of regional brain areas to DNA were not significant on postnatal day 1. However, the protein/DNA ratios in the neocortex were significantly reduced below control values in both treatment groups on postnatal day 22, thus suggesting a postweaning effect. Glutamate and GABA are putative excitatory and inhibiting neurotransmitters, respectively. Glutamate levels in the cerebrum of 1-day-old pups from 50- and 125-mg/kg/day groups were significantly lower than control values by 9% and 13%, respectively, and in the cerebellum, by 23% and 11%, respectively. No significant changes occurred in glutamate levels in any of the regional brain areas examined on postnatal day 15 and 22. The levels of GABA in various brain areas were not significantly affected by the treatments.

GABA is formed by decarboxylation of glutamate, but there is a significant lag in the development of the biosynthetic enzyme glutamic acid decarboxylase. It is therefore possible that the neonatal decrease in glutamate levels observed was not reflected as a change in neonatal GABA levels. These findings suggest that prenatal exposure of rats to nonteratogenic doses of 2,4-D and 2,4,5-T mixture produces specific rather than generalized changes
in the neurotransmitter system in the brains of preweaned offspring.

c. TCDD. In an earlier study, Umbreit et al. (1987) reported anti-estrogenic effects of TCDD in female C57BL/6 mice. Recently, the authors evaluated reproductive toxicity in male C57B/6 mice treated with contaminated soils from a 2,4,5-T manufacturing site in Newark, New Jersey, and a metal scrap yard where equipment from the manufacturing site was recycled (Umbreit et al. 1988). The soils contained a wide variety of contaminants including TCDD. Groups of 10 male mice were treated by gavage with 10% suspension of soil A (288 μg/kg total TCDD dose) or soil B (33 μg/kg total TCDD dose). In addition, three groups of males received either pure TCDD (90 μg/kg total TCDD dose) in corn oil and acetone (9:1), decontaminated soil (hydrocarbons removed), or recontaminated soil (90 μg/kg total TCDD dose) once weekly starting 6 weeks before mating and continuing for a total of 30 weeks. Fifty mice of each sex receiving decontaminated soil served as mating partners and negative controls. Females were treated three times a week 2 weeks before mating and throughout pregnancy and weaning for a total of 25 weeks. Analysis of mating performance of male F₀ mice showed a reduced number of pups per litter in the group treated with soil A. There was a significant decrease (p<0.05) in total live pups born and pups that survived until weaning. No such effects were observed in other treatment groups. Because of the earlier findings of lack of reproduction in F₀ females treated with TCDD-corn oil or recontaminated soil, the selected female mice from groups treated with the soils and controls as above (but dosed thrice weekly), were examined for estrous cycles. Females from both groups produced erratic estrous cycles or inhibited them completely after 6 weeks of receiving a cumulative dose of 108 μg/kg TCDD.

Based on the results of the study, no decrease in reproductive function of male mice was detected following TCDD treatment. Soil A, on the other hand, did cause a decrease in
survival of pups and litters. Analysis of soil A showed low extractability of dioxins and furans. Therefore the role of TCDD in the reproductive toxicity seen in mice exposed to soil A is uncertain. Lack of apparent toxicity in $F_0$ male mice contrasts with results observed in $F_0$ females given similar treatment but dosed three times a week. Female mice receiving soil A had smaller litters, fewer live pups were born per litter, and fewer litters survived weaning. The toxic effects observed were probably associated with the interaction of TCDD and other toxic compounds in soil A. The observations noted in the offspring of $F_0$ males treated with soil A may reflect subtle defects or mutations in the germinal cells and possibly reflected a dominant lethal effect mediated through disruption of sperm DNA. The interruption of the estrous cycle in TCDD-treated females was possibly due to an anti-estrogenic effect of TCDD. However, in TCDD-treated and recontaminated soil-treated groups, estrous cycles stopped after 6 weeks of treatment whereas mice were mated 2 weeks after treatment. Therefore, either the reduction in estrogen was sufficient to prevent pregnancy before its detection by the blockage of estrous cycling, or some other factor contributed to the lack of reproductive ability. Thus, the overall results of the study indicate that TCDD caused an androgenic or antiestrogenic effect on the treated mice.

One of the proposed mechanisms by which TCDD may produce reproductive toxicity involves down regulation of the estrogen receptor. This mechanism may play an important role by which TCDD reduces the occurrence of estrogen-mediated tumors in rats. Chronic administration of TCDD to Long Evans rats has been reported to lower the incidence of spontaneous mammary and uterine tumors (Kociba et al. 1978). Since the growth of some uterine and mammary tumors is estrogen-dependent, Romkes et al. (1987a,b) studied the role of Ah receptor in the down regulation of uterine and hepatic estrogen receptor (ER) levels in rats. The preliminary findings (Romkes et al. 1987b)
discussed the proposed mechanism in hepatic and uterine tissues of rats. This report was only an abstract. Romkes et al. (1987a) in a later publication discussed the results of their earlier study in detail as described below.

Single intraperitoneal administration of TCDD to 21-day-old female rats at doses of 80 or 400 $\mu$g/kg in corn oil (5 ml/kg) resulted in a dose-dependent decrease in uterine and hepatic ER levels. TCDD also inhibited the estrogenic effect of estradiol. Administration of 15 $\mu$g/kg estradiol resulted in an increase in cytosolic (194%) and uterine levels (155%) of ER and a 42% increase in uterine weight. On the contrary, pretreatment of rats with 80 $\mu$g/kg TCDD prior to estradiol (15 $\mu$g/kg) administration completely blocked the estrogenic effect of estradiol. The uterine and hepatic ER levels were 51% and 54% lower, respectively, than observed in the control group, and uterine net weight was 20% lower than in the control group. The comparative effects of TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 1,3,7,8-TCDD, and 1,2,4,7,8-PeCDD on the down regulation of uterine and hepatic ER levels were found to be structure-dependent. An excellent correlation was found between the binding affinities of these congeners for the Ah receptor and their antiestrogenic activity. The most active congeners exhibited high affinities for the Ah receptor (TCDD and 1,2,3,7,8-PeCDD) whereas less active compounds (1,3,7,8-TCDD and 1,2,4,7,8-PeCDD) exhibited lower affinities for the Ah receptor. These data support the role of Ah receptor in mediating the down regulation process that may be responsible for TCDD-induced reproductive toxicity.

In earlier studies, Moore et al. (1985) reported that TCDD causes pronounced androgenic deficiency in male rats. Recently, Moore and Peterson (1988) examined the extent to which enhanced catabolism and/or excretion could account for the androgenic deficiency in TCDD-treated rats. Male Sprague-Dawley rats were castrated, implanted subcutaneously with testosterone containing
capsules, and dosed with TCDD (15 or 100 \( \mu g/kg \)) or vehicle (corn oil-acetone 19:1). Seven days later substantial increases in food intake and body weights were seen in TCDD-treated rats and their weight-matched pair fed controls. Plasma testosterone and dihydrotestosterone concentrations or seminal vesicle and ventral prostate weights were also similar in both groups. These results clearly demonstrate that TCDD does not produce an androgenic deficiency when rats are exposed to a constant physiological dose of testosterone. Therefore, the proximate cause of the androgenic deficiency must be a decrease in testicular testosterone secretion secondary to testicular, pituitary, and/or hypothalamic dysfunction. (For further details refer to Chapter V.)

TCDD is known to produce atrophy, morphological changes, impaired spermatogenesis, and epididymal lesions in the testes of experimental animals. Al-Bayati and coworkers (1988) investigated the mechanism of TCDD-induced toxic effects in rat testes. Male Sprague-Dawley rats were administered 40 \( \mu g \) TCDD/kg/day in 10% acetone (in corn oil) for 3 days. Control animals received the vehicle. Animals were sacrificed 1, 3, 6, 9, and 12 days following treatment. Nine days after TCDD treatment significant decreases in body and testes weights occurred. However, the testes weight as a percentage of body weight was significantly higher than control animals. An increase in lipid peroxidation (contents of thiobarbituric acid reactive substances) occurred as the testicular weights decreased. Significant alterations in iron and copper content distribution, and an increase in protein kinase C activity with decreases in superoxide dismutase and glutathione peroxidase activities, were also noted. The authors suggested that TCDD caused testicular atrophy that may be associated with enhanced lipid mobilization and peroxidation in conjunction with increased formation or decreased removal of reactive oxygen species and the induction of protein kinase C.
Hassoun and Arif (1988) investigated the role of ornithine decarboxylase (ODC) activity in the teratogenic action of the TCDD congener 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) in NMRI mice. D,L-α-difluoromethyl ornithine (DFMO), an inhibitor of ODC activity when administered in three doses each of 200 mg/kg on days 11 to 12 and 12 to 13 of gestation, significantly decreased the rate of cleft palate formation induced by TCAOB administered at 4 mg/kg body weight on days 11-12 of gestation. However, the incidence of fetal deaths induced by TCAOB was unaffected by the DFMO treatment. Preliminary evidence shows that the mechanism of induction of cleft palate by TCDD and TCAOB is by failure of programmed palatal epithelial cell death. These compounds possibly induce enzymes like ODC, which help the proliferation of epithelial cells leading to cleft palate. In the present study, DFMO inhibits ODC activity, thus resulting in decreased rate of cleft palate without affecting the rate of fetal death. This observation shows that the mechanisms of TCAOB-induced fetal death and cleft palate are unrelated. This is confirmed by the authors' finding in other studies in which benzo(a)pyrene increased the rate of fetal deaths induced by TCDD without affecting the rate of cleft palate formation.

3. Conclusions

The two new studies that assessed the proposed association between exposure of Vietnam veterans to Agent Orange and the occurrence of birth defects in children failed to establish a positive association. The epidemiological investigations conducted in New Brunswick, Canada, did not reveal any association between exposure to phenoxy herbicides and adverse reproductive outcomes. No new studies on the effects of 2,4-D and 2,4,5-T in humans have been published in 1988. The available studies involving exposure to TCDD do not provide convincing evidence that TCDD produces adverse reproductive effects in humans.
Prenatal exposure to a mixture of 2,4-D and 2,4,5-T has been reported to produce behavioral and neurochemical alterations in rat neonates. Studies in experimental animals were designed to elucidate the mechanism of action of TCDD and its congeners in causing birth defects or impaired reproductive performance. Results of these studies are of limited relevance in assessing the reproductive consequences of exposure of human males to phenoxy herbicides.
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E. Effects on the Immune System

No studies have provided conclusive evidence that exposure to phenoxy herbicides causes alterations in immune function in humans or experimental animals. However, until recently the evaluation of immune function or status was not common in the design of epidemiologic studies. Numerous studies have shown that chlorinated dioxins alter immune function in experimental animals. The results of the few epidemiologic studies in which such parameters have been measured in humans exposed to dioxins have been inconclusive.

1. Studies in Humans

As part of the medical and physical examination component of the VES (USCDC 1989), indicators of immune status and function were assessed (see Section A above for a detailed description of the design and methods of this study). Blood samples were analyzed for the following indicators of immune status: total lymphocytes, total B-cells and T-cells, T-cell subset populations, the ratio of T4 to T8 cells, and the concentrations of IgG, IgM, and IgA. In addition, all subjects in the medical examination component underwent a skin test for delayed hypersensitivity using seven recall antigens. There were no differences between Vietnam and non-Vietnam veterans in any of the parameters that were measured, nor were there significantly more Vietnam veterans outside of the normal ranges for these parameters than non-Vietnam veterans. In the skin test, 3.5% of the Vietnam veterans and 3.9% of the non-Vietnam veterans were anergic.

Studies of immune parameters and function among residents of dioxin-contaminated areas in Missouri have been discussed in the two preceding issues of this review (see Clement 1987 and 1988). Originally Hoffman et al. (1986) and Knutsen et al. (1987) reported that residents with a high probability of dioxin exposure had more anergy (as assessed by a skin test to seven recall antigens) than did residents with a lower probability of
dioxin exposure. Questions regarding the reading of the skin tests led to a retesting of the subjects that were anergic or relatively anergic a year later. Evans et al. (1987) reported at a scientific meeting that the repeated study showed no differences between the two groups in responses to the skin test. A full article describing the results of this follow-up study was published in 1988 (Evans et al. 1988). While there were no differences between the two groups with respect to the results of the skin tests, there were differences in T4 counts and ratios of T4/T8. Two exposed individuals had abnormally low T4 counts, and four exposed individuals demonstrated abnormally low T4/T8 ratios. These authors attributed the relatively high degree of anergy that was observed in the original study to methodological problems. However, a paper published by other authors of the original study reported the conflicting results of the follow-up study but suggested that the later findings could be explained by recovery of immune function by the anergic individuals in the exposed group in the period between the two tests (Stehr-Green et al. 1988). It seems unlikely that such recovery would take place in 1 year in light of the fact that 4 or 5 years had elapsed between dioxin exposure and the first set of skin tests. Furthermore, there was no change in the T4 and T4/T8 parameters measured in the exposed group during the same period.

In a related investigation, Andrews et al. (1988) described the results of immunological tests among individuals from dioxin-contaminated areas of Missouri whose adipose tissue had been sampled and analyzed for dioxin content. A total of 40 people were tested. Sixteen of these were classified as having low body burdens (less than 20 ppt), 12 had moderate body burdens (20–60 ppt), and 12 had concentrations greater than 60 ppt. None of the 40 participants was anergic or relatively anergic, and there were no differences among the groups in their responses to a delayed hypersensitivity skin test. Increased serum globulins and decreased albumin/globulin ratios were associated with dioxin
body burdens. Seven of the 12 individuals in the high body burden group had T4/T8 ratios of less than 1.2 compared to only two in the other two groups combined. However, the four individuals with the lowest T4/T8 ratios had tissue levels of 10, 33, 165, and 294 ppt), suggesting no real correlation between body burden and T4/T8 ratios. Furthermore, there was no clinical evidence of impaired immune function in any of these individuals, and the significance of decreased numbers of T4 cells or T4/T8 ratios has not been determined. Because body burdens of TCDD are probably more accurate indicators of past dioxin exposure than place of residence or recall, this study is more reliable than the earlier studies in assessing the relationship between dioxin exposure and measures of immune status in Missouri residents. Unfortunately, it is available only as an abstract at the present time.

Jennings et al. (1988) conducted an immunologic investigation among 18 workers who were exposed to dioxin as a result of an industrial accident at the Coalite Oils and Chemicals Ltd. plant in England in 1968. Earlier studies of these workers have been reviewed in previous volumes of this review (see May 1982, 1983a,b in Clement 1984 and Martin 1984 in Clement 1985). Blood samples were taken from the 18 exposed workers and from 15 control subjects from the same factory whose jobs precluded extensive exposure to dioxin. The control subjects were matched to the exposed subjects for age, sex, percentage of ideal body weight, social class, alcohol consumption, and smoking behavior. The blood parameters assessed were serum concentrations of IgG, IgA, IgM, IgD, concentrations of antinuclear antibodies and immune complexes, the total number of lymphocytes, lymphocyte subpopulations, and the ratio of helper to suppressor cells (T4/T8). They also evaluated the lymphocyte proliferative response to phytohemagglutinin A (PHA). The only differences between the two groups were the presence of significantly more antinuclear antibodies, immune complexes, and
natural killer (NK) cells in the blood of the dioxin-exposed workers. The mean T4/T8 ratio was lower among dioxin-exposed workers than among the controls, but this difference was not statistically significant. There was no difference between the groups in the one measure of immune function, i.e., the lymphocyte proliferative response to PHA.

This study is similar to the studies of dioxin-exposed people in Missouri in its suggestion that there might be subtle alterations in measures of immune status in humans exposed to dioxins but the significance, if any, of these changes is unclear. The function of NK cells and the consequences of increased concentrations of them are not fully understood.

2. Animal Studies

The effects of phenoxy herbicides on immune function has not been systematically studied in experimental animals. A large volume of literature has indicated, on the other hand, that chlorinated dioxins and furans are potent suppressors of immune function in many different animal species. Current research in experimental animals has gone beyond the identification of these effects to investigate mechanisms of action. At present this line of investigation is incomplete, and the implications for assessing potential health consequences in humans exposed to these compounds are not clear.

Davis and Safe (1988) investigated structure-activity relationships for the suppression of the splenic plaque-forming cell response to sheep red blood cells in mice by a series of chlorinated dioxins and furans. The structural requirements for activity and antagonism of the suppressive effect by certain compounds supported earlier evidence that this effect is mediated by binding of the dioxin to a specific receptor in spleen cells. The authors also concluded that TCDD modulates some early stage of B-cell differentiation. This conclusion is consistent with that of Luster et al. (1988) who compared the effects of dexamethasone and TCDD on the maturation of B-cells in vitro.
TCDD interfered only with the expression of plasma cell marker suggesting action at a very early stage of cellular programming. Tomar and Kerkvliet (1988) provided evidence that suppression of the sheep red blood cell response by TCDD may also be due, in part, to a defect in T-helper cells. Lundberg et al. (1988) found that TCDD may interfere with T-cell differentiation by altering the interaction between immature thymocytes and the thymic reticulum in young mice. Nikolaidis et al. (1988a,b) investigated the effects of TCDD on the development of lymphoid cells in the Bursa of Fabricius of chicken embryos and found similar results to those observed in the thymus of mice.

3. Summary and Conclusions

Studies of humans who may have been exposed to phenoxy herbicides and/or dioxins failed to indicate any effects of these exposures on immune function. There were no differences in any measure of immune status between Vietnam and non-Vietnam veterans in the VES, but this study was not designed to examine effects that might be related to herbicide exposure. Studies of blood parameters that may be relevant to immune status in individuals who were exposed to dioxins in the past reveal some differences from unexposed controls groups. Whether these alterations are caused by the dioxin exposure and whether they have any functional significance remains to be determined. No studies of the effects of phenoxy herbicides on immune function in experimental animals were published during 1988. Studies of the effect of TCDD and related dioxins and furans on the immune systems of experimental animals were mechanistic in nature and indicate that the action of these compounds are complex and not fully understood.


F. Neurobehavioral Effects

1. Studies of Humans

Several studies were published in 1988 that investigated the neurobehavioral toxicity of chlorinated dibenzo-p-dioxins in humans. Three studies reported on findings in Vietnam veterans and two studies described the neurobehavioral effects resulting from point-source exposure to 2,3,7,8-TCDD. The results of these four studies are discussed below.

As part of their ongoing VES, the CDC (USCDC 1988a, 1989) reported on the psychosocial characteristics of Vietnam veterans. This study has been described in detail in Section A above. It is important to note that the VES is intended to evaluate health effects associated with the general Vietnam military experience, and no attempt was made to assess adverse effects related specifically to Agent Orange exposure.

A random subsample of 2,490 Vietnam veterans and 1,972 non-Vietnam veterans underwent a comprehensive health examination that included a psychological evaluation. The veterans' psychological health status was evaluated with the Diagnostic Interview Schedule (DIS) and the Minnesota Multiphasic Personality Inventory (MMPI). The DIS is a standardized questionnaire that assesses the occurrence of certain psychiatric conditions as defined by the DSM-III criteria of the American Psychiatric Association. Conditions of interest in this study included generalized anxiety, depression, alcohol abuse or dependence, drug abuse or dependence, and post-traumatic stress disorder (PTSD). The MMPI is a self-administered questionnaire designed to make a quantitative assessment of personality, emotional status, and level of psychopathology. If a veteran satisfied full DIS criteria for generalized anxiety, depression, or substance abuse in the past month and if he had elevated scores on at least two of eight clinical MMPI scales, he was considered to have poor psychological status.
The CDC found that, in general, "Vietnam veterans seem to be functioning socially and economically in a manner similar to Army veterans who did not serve in Vietnam" (USCDC 1988a). However, the results of this study did reveal that more Vietnam veterans are experiencing psychological problems than non-Vietnam veterans. These problems include depression (4.5% of Vietnam veterans vs. 2.3% of non-Vietnam veterans), anxiety (4.9% vs. 3.2%), and alcohol abuse or dependence (13.7% vs. 9.2%).

Interestingly, despite the increased incidence of these psychological problems, fewer than 1% of either cohort were found to meet the DIS criteria for drug abuse or dependence. Approximately 15% of the Vietnam veterans met DIS criteria for PTSD at some point after their combat experience, and 2% experienced PTSD in the month prior to examination. Furthermore, those veterans who experienced PTSD were also more likely to meet DIS criteria for other psychiatric conditions of interest. The authors concluded that the higher prevalence of psychological problems seen in Vietnam veterans was not due to organic defects since few differences were observed between Vietnam and non-Vietnam veterans with regard to neuropsychological performance, neurological findings, or other objective measures of current physical health. In conclusion, the results of this study indicate that the Vietnam experience resulted in adverse psychological effects in some veterans, but the design of the study does not permit an evaluation of the role of Agent Orange exposure in producing these effects.

In addition to determining the psychological characteristics of Vietnam era veterans, neurological examinations and tests were conducted as part of the medical examination phase of the study (USCDC 1989). Nerve conduction velocities and the amplitudes of upper and lower extremity sensory and motor nerves were determined. Vibratory and thermal sensation were evaluated. Auditory acuity was measured, and veterans were questioned regarding current and past symptoms of neurological impairment.
Very few veterans in either the Vietnam or non-Vietnam cohorts (1.0% and 0.8%, respectively) had signs and symptoms consistent with peripheral neuropathy. Although Vietnam veterans were more likely to have symptoms and/or signs of neurological impairment, none of these differences was statistically significant. High-frequency hearing loss was more prevalent among Vietnam veterans (9.4%) than among non-Vietnam veterans (6.2%). The difference was accounted for by veterans who had tactical military occupational specialties, i.e., were more likely to have been in combat. These results do not support an association between service in Vietnam and peripheral neuropathy. The study was not designed to evaluate associations between any of these effects and herbicide exposure.

A number of the questions in the self-administered questionnaire used to gather data for the American Legion Study were designed to assess psychological and social status among Vietnam-era veterans (Stellman et al. 1988a,b). These included questions regarding marital well-being, satisfaction with parenting, sexual satisfaction, happiness, life satisfaction, and intensity, duration, and changes in smoking, drinking, and drug use. In addition, questions were derived from the Psychiatric Epidemiology Research Interview to assess psychological well-being.

The results of the questionnaire indicated that a large number of adverse social and psychological outcomes were significantly associated with combat exposure in Vietnam. Men who reported high exposure to combat were more likely to be divorced, were less happy and satisfied with their lives, experienced more sexual dysfunction, and had more problems in their marital and parental relationships than did veterans with little or no combat exposure. Furthermore, these individuals engaged in behaviors that were more likely to result in current and future health impairment such as alcohol, tobacco, and drug use. Vietnam veterans had worse scores than non-Vietnam veterans.
for depression, anxiety, irritation, and feelings of helplessness, and these scores increased with increasing combat exposure. On the other hand, no association was found between any measure of psychological and social well-being and exposure to Agent Orange as evaluated in this study. There was some apparent interaction between herbicide exposure and psychological impairment among Vietnam veterans with the highest level of combat exposure; the authors speculated that this might indicate a herbicide effect in individuals who were highly stressed. The authors concluded that further study, with larger populations, was necessary to control for the confounding effects of combat and determine the possible effects of herbicides more accurately.

Levy (1988) attempted to assess whether exposure to Agent Orange contributed to the occurrence of PTSD or other psychological deficits in Vietnam veterans and whether exposure exacerbated the incidence of PTSD. The subject population was drawn from veterans who received bonus checks from the Commonwealth of Massachusetts for serving in Vietnam. Six exposed veterans were selected based on self-reported chloracne, an indicator of TCDD exposure. Matched control subjects (n = 25) who had no past or present evidence of chloracne were selected from the same list. A neuropsychological battery, designed to identify nonfocal effects of brain dysfunction, consisting of the WAIS Vocabulary Subtest, Rey Auditory Verbal Test (5-trial total, list A Intermediate Recall, list A Recognition score, list A Delayed Recall, list B), the Symbol Digit Modalities Test in written and oral versions, and the Word Fluency Test, was administered to the subjects. Vietnam veterans were also questioned to determine if they experienced PTSD and its associated features (depression, anxiety, and aggressiveness) in the past 6 months.

Levy (1988) found that the exposed veterans scored significantly lower than the control group on six of the nine neuropsychological tests for brain dysfunction, indicating the
presence of organic psychological deficits. The veterans exposed to Agent Orange also had significantly higher scores for measures of PTSD. Furthermore, all of the exposed subjects met the diagnostic criteria for PTSD and the three associated features, whereas only 20% of the control subjects had PTSD and 8% had the three associated features. Based on these results, Agent Orange exposure appears to exacerbate the incidence of PTSD in Vietnam veterans.

Levy (1988) concluded that PTSD alone cannot account for the apparent neuropsychological deficits seen in the exposed veterans. Furthermore, he concluded from combat reports that the level of combat experience cannot account for the higher incidence of PTSD in exposed veterans. Therefore, exposure to Agent Orange may be responsible for the organic neuropsychological deficits as well as the greater incidence and severity of PTSD. Levy acknowledged that his exposed sample population is very small and that his method of selection may actually underestimate the number of exposed Vietnam veterans for several reasons.

This study can best be described as a case report and cannot be considered a controlled epidemiology study. There are several problems in the study that limit its usefulness. The occurrence of chloracne was used to select exposed veterans, and no indication was given of how the occurrence of chloracne was established. Apparently, no provisions were made to account for past occurrences of chloracne that resolved, thus biasing the selection of participants. Furthermore, the absence of chloracne does not preclude exposure to Agent Orange, so control subjects could also have been exposed. Finally, Levy did not attempt to account for important confounding variables such as combat experience (see discussion on Stellman et al. 1988a,b), smoking, alcohol and drug use, and civilian employment. Therefore, it must be concluded that this study provides no reliable evidence for a cause-effect relationship.
The neurological sequelae of TCDD exposure can also be studied following point-source exposure. Klawans et al. (1987) evaluated the neurological effects of TCDD in 47 railroad workers following exposure to PCBs including dioxin (present at a level of 45–46 ppb) in early 1979 while cleaning up a chemical spill from a damaged tank car. Two workers subsequently committed suicide. The initial complaints in the remaining 45 workers following exposure included fatigue (41 of 45), muscle ache (23 of 45), and distal paraesthesia (42 of 45). Recovery from the fatigue and muscle cramping was usually seen after about 2 years, but the paraesthesia tended to persist. At this time, depression and alteration of concentration became prominent features in the exposed workers. The workers underwent detailed neurological examinations including nerve conduction velocities and evoked potential studies again in 1985. Peripheral neuropathies were still evident in 43 of the 45 workers. Postural and terminal intention tremor was seen in 35 of the 45 subjects, and action dystonias of the hands were seen in 24 of the 45 workers. This study provides an in-depth chronological accounting of the neurological sequelae associated with exposure to chemicals including TCDD exposure when the exposure level and duration is known and the entire exposed population can be evaluated. However, the absence of controls and the concurrent exposure to other potentially neurotoxic chemicals limit its usefulness.

In July of 1976 an explosion occurred at a chemical factory manufacturing 2,4,5-T in Meda, Italy. The explosion released a cloud of toxic debris that resulted in contamination of the soil with 2,3,7,8-TCDD in the towns of Seveso, Meda, Cesano Maderno, and Desio. Barbieri et al. (1988) studied the peripheral neuropathologic effects in a cohort of 152 exposed individuals with chloracne resulting from dermal and inhalation exposure to, as well as ingestion of, 2,3,7,8-TCDD after a period of 6 years. The controls consisted of 123 age- and sex-matched individuals living in nearby towns. A detailed medical history including use
of neurotoxic drugs, presence of confounding habits, and data on TCDD exposure (number of days at home after the explosion and before evacuation, place of residence, ingestion of contaminated food) was obtained from each subject. Each subject then underwent examination by an internist and neurologist. An evaluation of strength, deep tendon reflexes, and past and current symptoms of peripheral nervous system involvement was part of the neurological examination. Electrophysiological studies were also conducted to measure motor conduction velocities, and action potential latencies and amplitudes.

No peripheral neuropathies were found in any of the subjects. However, the exposed cohort had significantly more subjects that presented with at least two bilateral clinical signs and at least one electrophysiological abnormality. The significance of these findings in the absence of peripheral neuropathies is not known.

Assennato et al. (1988) described very similar results in an abstract. It is not clear whether this is the same or a different study from that of Barbieri et al. (1988), but the two reports have no authors in common. Assennato et al. reported that nerve conduction velocities in 193 individuals who developed chloracne as a result of the accident in Seveso, Italy, were not different from those in control subjects selected from nearby communities. This abstract lacked sufficient detail to allow critical evaluation of the authors' conclusions.

2. Studies in Experimental Animals

Three studies were published this year by Schulze's laboratory that investigated the neurobehavioral toxicity of 2,4-D and the possible mechanisms of action for these effects (Schulze 1988, Schulze and Dougherty 1988a,b). Various neurobehavioral parameters were assessed in rats given 150-250 mg/kg/day 2,4-D subcutaneously. These included schedule-controlled lever pressing, photocell locomotor activity, landing foot splay (a measurement of motor coordination), rota
rod, and grip strength. The results obtained in these studies can be summarized as follows:

1. Administration of 2,4-D n-butyl ester (150 mg/kg/day) produced significant depression of schedule-controlled lever pressing and photocell locomotor activity, and a significant increase in landing foot splay. Peak effects occurred by the third injection and tolerance developed by the tenth injection. The behavioral effects were rapidly reversible within 24-48 hours after cessation of treatment.

2. These effects were observed only after administration of the n-butyl ester. Equimolar doses of 2,4-D acid and 2,4-D mixed butyl esters were without effect.

3. n-Butanol, a metabolite of 2,4-D n-butyl ester, also depresses locomotor activity and increases landing foot splay. However, there was no cross-tolerance between the two chemicals for these effects indicating that they act by different pharmacological mechanisms.

4. Since brain 2,4-D levels remained elevated when tolerance was observed, a biochemical basis for the tolerance to 2,4-D, behavioral effects had to be discounted. The authors therefore concluded that repeated administration of 2,4-D produced an altered response in the target cells suggestive of functional/cellular tolerance.

Taken together, these results indicate that very high doses (1/3 to 1/2 of the LD$_{50}$) of a specific 2,4-D formulation induces neurobehavioral toxicity in animals. Tolerance to these effects develops most likely as a result of an altered cellular/functional response. The relevance of these effects to humans is not known because of the high doses required to elicit these responses and the lack of convincing neurologic effects noted in humans following exposure to phenoxy herbicides (see also Volumes X and XI of this review). However, the observation that some sort of cellular/functional tolerance develops to the neurotoxic effects observed has implications with regard to health effects associated with chronic exposure in humans.

Prenatal exposure to a combination of 2,4-D and 2,4,5-T at nonteratogenic levels has been demonstrated to induce specific
changes in brain neurotransmitter levels (Mohammed and St. Omer 1988). These investigators found that daily gavage administration of 50 or 125 mg/kg of a 1:1 mixture of 2,4-D and 2,4,5-T to pregnant rats on gestation days 6–15 resulted in regional reductions of glutamate but not GABA concentrations in 1-day-old pups, but not in 15- or 22-day-old pups. These results indicate that 2,4-D and 2,4,5-T can potentially adversely affect neurological development in the fetus, but the relevance of these findings to Vietnam veterans exposed to Agent Orange is limited.

3. Conclusions

The studies published this year indicate that Vietnam veterans exhibit a prevalence of neurobehavioral disorders as compared to non-Vietnam veterans. No conclusive evidence was found to indicate that these disorders may be due to Agent Orange exposure. One study that attempted to assess the role of Agent Orange in these effects was limited by a small sample size and biased subject selection, and another well-controlled study found that there was no association between psychological and social well-being and exposure to Agent Orange. Point-source exposure to TCDD has been associated with adverse neurological effects in humans. Studies in animals indicate that 2,4-D is neurotoxic at high doses, but the relevance of these findings to humans is not known.


Schulze, G.E. 1988. 2,4-D-n-butyl ester (2,4-D ester) induced ataxia in rats: Role for n-butanol formation. Neurotoxicol. Teratol. 10:81-84


Schulze, G.E., and Dougherty, J.A. 1988b. Neurobehavioral toxicity and tolerance to the herbicide 2,4-dichloro-phenoxyacetic acid-n-butyl ester (2,4-D ester). Fund. Appl. Toxicol. 10:413-424


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G. Other Toxic Effects

1. Acute Phenoxy Herbicide Intoxication in Humans

The acute toxic effects of phenoxy herbicides in humans are not well understood because cases of severe overexposure are relatively rare. A total of three cases of intoxication through ingestion of relatively large quantities of commercial phenoxy herbicide formulations were described in two separate publications in 1988 (Kancir et al. 1988, Meulenbelt et al. 1988). In two cases, adult men ingested unknown quantities of an herbicidal formulation in which 2-methyl-4-chlorophenoxy acid (HCPA) was the active ingredient. In the third case, an adult woman ingested an unknown quantity of a formulation containing 9.1% 2,4-D and 22.7% MCPA. The clinical aspects of all three cases were remarkably similar. All three patients became comatose and experienced respiratory failure, presumably as a result of central nervous system depression. Subsequently all three patients experienced acute renal failure, which was probably secondary to rhabdomyolysis. All three patients also had hypotensive crises and severe disruptions of serum electrolyte levels. One group of authors noted a severe drop in serum calcium concentration (Kancir et al. 1988). After aggressive therapeutic intervention, one of the three patients died from complications related to the acute intoxication. The other two recovered fully.

The similarities among these three cases are remarkable, especially in view of the independence of the two separate reports. The clinical courses of intoxication in these three cases were consistent with the results of studies in experimental animals that indicate that the central nervous system and the musculoskeletal system are primary targets for the acute toxic effects of phenoxy herbicides. In humans, the depressive effects on the central nervous system are manifested as coma and respiratory failure while the muscular effects are manifested by...
rhabdomyolysis leading to kidney failure and severe electrolyte imbalances. Aggressive therapy can result in full recovery.

2. Hepatic Effects

a. Studies of Humans. Assennato et al. (1986) reported the results of a cross-sectional study of biochemical indicators of liver function in a cohort of workers from the ICMESA plant in Seveso, Italy. The study was carried out in 1981. The study cohort consisted of 196 individuals who were likely to have been exposed to chlorinated dioxins at the ICMESA plant before, during, and after the 1976 accident. Blood samples were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transpeptidase (GGTP), cholesterol, and triglycerides. Urine samples were analyzed for porphyrins and aminolevulinic acid. Values of the parameters for the cohort were compared to those determined in two control groups of workers from plants where exposure to hepatotoxic substances was unlikely. Information on the size and methods for selecting the control groups was not included in the paper.

No information on dioxin exposure or length of employment was provided in this paper. None of the workers had chloracne. The only significant biochemical differences between the ICMESA group and the comparison groups were elevated levels of urinary porphyrins and serum alkaline phosphatase in heavy drinkers among the ICMESA workers compared to heavy drinkers in the comparison groups. The authors concluded that there was an interaction between dioxin exposure and alcohol intake in altering porphyrin metabolism. The report of this study is deficient regarding experimental methods. The results must be interpreted with caution.

A very brief abstract by Assennato et al. (1988) summarized the results of three follow-up surveys among 193 subjects who developed chloracne as a result of the accident at the ICMESA plant. It is presumed that these are the same 193 subjects who
were observed by Caputo et al. (1988). Assennato et al. reported no significant differences between the chloracne subjects and an age- and sex-matched comparison group in biochemical indicators of liver function or in nerve conduction velocities. Furthermore, they reported that only one case of clinically evident chloracne remained in 1985. The lack of detail in this abstract precludes independent evaluation of the validity of the authors' conclusions.

Nagayama et al. (1987) examined the basal activity and inducibility of ary1 hydrocarbon hydroxylase (AHH) activity in lymphocytes from people who developed cutaneous lesions as a result of consuming cooking oil contaminated with PCBs and chlorinated dibenzofurans in the Yusho incident in 1969. The results of this study indicated that both basal AHH activity and the inducibility of this enzyme were correlated with both the severity of skin lesions and PCBs in the blood in the years shortly after the exposure (1969-1976). However, neither enzyme activity nor inducibility appeared to be correlated with more recent measures (1981-1984) of the severity of skin lesions or PCB levels. The authors suggested that these results indicate that current AHH activity and inducibility reflect genetic differences and are not compound related. They suggest that individuals who were genetically predisposed to higher AHH activity were more sensitive to the dermatologic effects and accumulated more halogenated aromatics than individuals with lower AHH activity. This is consistent with interindividual variability in the properties of the Ah receptor and resulting variability in sensitivity to many of the adverse effects of dioxins.

b. Studies in Experimental Animals. The liver is a target organ of TCDD toxicity in experimental animals. The hepatotoxic effects of TCDD in C57BL/6J male mice were evaluated by Brooks et al. (1988). They administered a single intraperitoneal injection of 0, 25, 37.5, 75, or 15 µg TCDD/kg body weight to the mice, and
at least three mice per dose group were sacrificed at 1, 7, and 30 days post-injection. Liver samples were taken for histopathologic evaluation.

No evidence of TCDD-induced hepatotoxicity was observed in liver samples from treated animals 1 day after dosing. By 7 days, all livers from animals treated with 75 and 150 μg/kg exhibited minor inflammatory changes with foci of cellular coagulative necrosis. These changes were also evident in two-thirds of the animals given 37.5 μg/kg and one-third of those given 25 μg/kg. By 30 days post-administration, hepatocellular damage that was graded in severity and dose-dependent was evident in the three highest dose groups. These lesions consisted of hepatocellular necrosis and focal architectural collapse. No cirrhosis was seen. These results indicate that single exposures to TCDD can induce severe liver damage in rats that is both dose- and time-dependent, and the full effect is not manifested until 30 days post-exposure.

Phenoxy herbicides have been shown to induce several hepatic enzyme systems in experimental animals. Bacher and Gibson (1988) investigated the effects of several phenoxy herbicides (including 2,4-D and 2,4,5-T) on the cytochrome P-450 mixed function oxidase system in rat liver, particularly the hypolipidemic-induced cytochrome P-450 IVA1 isoenzyme. Male Wistar rats were administered 50, 100, or 200 mg/kg of the herbicides by gavage once a day for 3 days. The animals were sacrificed 24 hours after the last injection, and the livers were removed for enzyme assays. The liver-to-body weight ratio was increased by 2,4,5-T but not 2,4-D, whereas total cytochrome P-450 content, as well as specific P-450 IVA1 content, were significantly increased in a dose-dependent manner by both 2,4-D and 2,4,5-T. The specific induction of P-450 isoenzymes by 2,4-D and 2,4,5-T was further demonstrated by their ability to induce significantly the activity of ethoxyresorufin-O-deethylase, a marker substrate for P-450 IA1. Benzphetamine N-demethylation, a marker of cytochrome
P-450 II B1 activity, was unaffected by these herbicides. Thus, 2,4-D and 2,4,5-T can function as "mixed inducers" of various cytochrome P-450 isoenzymes.

3. Cardiovascular Effects
   
a. Studies of Humans. In a brief abstract, Fabig (1988) described a study in which three groups of individuals exposed to chlorinated dibenzo-p-dioxins or dibenzofurans underwent a single photon emission computed tomography of the cerebrum. This technique is useful for measuring cerebral blood flow. Fabig reported that there was a reduction in prefrontal cerebral blood flow compared to a group of people not exposed to these compounds. The lack of experimental detail in this brief abstract precludes critical evaluation of this report. However, the reported findings are interesting especially in view of the finding of a possible increased risk of stroke among employers of Dow Chemical Co. with a history of chloracne (Bond et al. 1987).

   b. Studies in Experimental Animals. It has previously been demonstrated indirectly that acute exposure to TCDD can adversely affect the heart in experimental animals. However, functional changes and the mechanism behind the possible cardiotoxicity of TCDD have not been studied. By examining biochemical, functional, and morphological changes in the hearts of female rats, Hermansky et al. (1988) attempted to determine whether and how TCDD affects the intact heart. The rats were administered 40 µg/kg TCDD by gavage for 3 consecutive days, and various parameters were measured 3, 6, and 9 days after treatment.

   Blood pressure and heart rates were significantly decreased in treated animals 6 days after treatment. Furthermore, TCDD treatment attenuated the tachycardia normally seen with isoproterenol (a β-receptor agonist) in a dose-dependent manner. Despite these apparent functional changes, no morphological changes were observed in the hearts of the treated animals at necropsy. Furthermore, TCDD had no significant effect on cardiac lipid peroxidation or glutathione peroxidase and catalase.
activities, which, together with the lack of morphological changes, indicates that TCDD did not cause direct injury to the heart. However, serum thyroxin levels were decreased by 66% in TCDD-treated animals, as compared with control animals. Other investigators have linked decreases in serum thyroid hormones to changes in myocardial β-receptors (Pilcher and Langley 1986). This led Hermansky et al. (1988) to speculate that the functional changes observed in the present experiment were a result of myocardial β-receptor down regulation secondary to the TCDD-induced hypothyroid state and not a direct toxic action on the heart. Thus, as concluded in previous volumes, the significance of these findings to human health is limited because the heart does not appear to be a major target organ of TCDD toxicity. However, the effects on thyroid function observed may be a significant health concern.

Canga et al. (1988) examined the effect of a single intraperitoneal injection of TCDD on measures of cardiac function in guinea pigs. A single dose of 10 μg/kg caused a significant decrease in β-adrenergic responsiveness as manifested by a decrease in the positive effect of isoproterenol. Other findings suggested that TCDD treatment caused an increase in intracellular calcium ion concentration in papillary muscle. The authors concluded that the heart may be a major target organ for the toxic effects of TCDD.

4. Chloracne

In July 1976 an explosion occurred at a chemical factory manufacturing 2,4,5-T in Meda, Italy. The explosion released a cloud of toxic debris that resulted in contamination of the soil with 2,3,7,8-TCDD in the towns of Seveso, Meda, Cesano Maderno, and Desio. Caputo et al. (1988) has described the results of a 10-year follow-up study of dermatologic effects in exposed victims.

The dermatologic manifestations observed in exposed people took the form of early irritative lesions and late acneic
(chloracne) lesions. The early irritative lesions seen in 447 of 1,660 people examined were characterized by erythema and edema of exposed areas, vesiculobullous and necrotic lesions, and papulonodular lesions. These lesions were observed within hours of the event, and other toxic substances released could have contributed to their occurrence. All resolved within 1 month and left no sequelae, with the exception of mild hyperpigmentation, which may have been the result of phototoxicity.

Chloracne was observed in 44 of the subjects (mostly children aged 2-10 years or adolescents) approximately 30-60 days after the incident. Twenty-eight of the affected subjects had also presented with the early lesions. The lesions were characterized by comedo-like and cystic lesions predominantly in the malar region but not often in the centrofacial region. Severe lesions were observed in eight children, all of whom had suffered from early lesions. An additional 149 subjects with mild to minimal chloracne were detected in screenings conducted in school-age children between 1977 and 1979. Recovery was observed in most patients within 2 years, and only residual atrophic lesions remained. No systemic toxicity was observed in any of the affected individuals.

In summary, these results indicate that chloracne is a sensitive and reliable indicator of TCDD exposure that affects mainly children and adolescents. The condition was most severe in those who manifested early irritative lesions, persisted for approximately 2 years, had no systemic involvement, and left only atrophic scars.

5. Renal Effects

Lukowicz-Ratajcak and Krechniak (1988) investigated the effects of subchronic 2,4-D treatment on renal function in rats. Male Wistar rats were administered 2,4-D (100 and 150 mg/kg) by intraperitoneal injection every other day for 12 weeks, and blood and urine were collected at various intervals throughout the study and analyzed for urea, Na⁺, K⁺, H⁺, α-ketoacids,
α-aminonitrogen, and protein. In addition, glomerular filtration and blood acid base balance were measured at 3 and 8 weeks. Significant changes observed included a dose- and time-dependent increase in urinary output, an increase in sodium excretion, a decrease in glomerular filtration rate, and an increase in blood urea and H⁺ excretion in the high-dose animals only. Acid-base balance was unaffected, and no protein was excreted. No histopathologic examinations were conducted. Based on these results, the authors concluded that subchronic 2,4-D induced no damage to the proximal tubule and that the Loop of Henle was the probable site of action for 2,4-D renal effects. Though the route of administration was inappropriate for extrapolation to human health and the doses studied were very high, these results suggest that 2,4-D has the potential to adversely affect the kidney.


H. Metabolism and Distribution Studies

1. 2,4-Dichlorophenoxyacetic Acid

Schulze and Dougherty (1988b) studied the metabolism of 2,4-dichlorophenoxyacetic acid-n-butyl-ester (2,4-D-ester) in rats and monitored its distribution in brain tissue. Rats were administered a daily dose of 150 mg/kg of 2,4-D-ester subcutaneously for 14 days. No parent 2,4-D-ester was detectable in blood or brain tissues 4 hours after injection on any dosing day. Blood concentrations of the metabolite, 2,4-D-acid, varied from 350 to 475 μg/g over the dosing period, but peak levels of 600 μg/g occurred on the 10th dosing day. In contrast to blood levels, brain 2,4-D-acid levels increased over time until maximum levels of 143 μg/g were reached on day 10. These results indicate that 2,4-D-acid accumulated in the brain. Brain levels of 2,4-D-acid remained elevated from days 10-12 but declined to 90 μg/g by day 14. Blood-brain ratios of 2,4-D concentrations also declined with time. In this study, Schulze and Dougherty (1988b) conducted several behavioral tests. Analysis of the behavioral data suggested that the metabolism of 2,4-D-ester to an active product was important to the expression of neurobehavioral toxicity. Moreover, the behavioral and metabolic data indicated that tolerance developed. However, metabolic data indicated that the mechanism of tolerance development was functional, while behavioral data indicated a dispositional mechanism. Although it was apparent that 2,4-D-acid accumulated in the brain, it remains unresolved whether brain levels of 2,4-D-acid are appropriate indicators in determining the mechanism of tolerance.

Kim et al. (1988) directly examined the regional distribution of 2,4-D in the maternal and fetal brain to assess the mechanisms responsible for 2,4-D accumulation in the brain. Pregnant mice received intraperitoneal injections of [14C]-2,4-D (1.8 μM/kg) on day 17 of gestation. One group received a pretreatment of unlabeled 2,4-D at doses of 80 mg/kg/day for
2 days prior to administration of the radiolabeled compound. Autoradiography showed that the brain $[^{14}\text{C}]-2,4\text{-D}$ content in control and pretreated animals was below that of most tissues, but pretreatment resulted in a greater accumulation in the brain. Treatment of the pregnant mice with 0.9 $\mu\text{M}[^{14}\text{C}]-2,4\text{-D/kg}$ on day 17 of gestation resulted in a 2,4-D concentration in maternal brain that was 4% of its plasma concentration within 1 hour after administration; the fetal brain 2,4-D concentration was approximately 8% of the maternal plasma concentration. Maternal and fetal contents did not significantly change over the 6 hours following administration. Detailed analysis of the maternal brain showed that the brain stem, cerebellum, and frontal cortex had similar 2,4-D contents that were significantly higher than 2,4-D concentrations in the hypothalamus and caudate nucleus. However, pretreatment with 40 mg/kg/day for 2 consecutive days significantly increased the regional distribution in the maternal brain five- to nine-fold. Greater increases were seen following pretreatment with 80 mg/kg/day. Fetal brain concentration increased three- and six-fold following pretreatments of 40 and 80 mg/kg/day, respectively.

The pattern of $[^{14}\text{C}]-2,4\text{-D}$ distribution within the brain of the rabbit was similar to that seen in the mouse. An increased accumulation of $[^{14}\text{C}]-2,4\text{-D}$ in the brain following pretreatment with unlabeled 2,4-D was also observed (Kim et al. 1988). Brain levels of 2,4-D were 3-5% of plasma values. In addition, the study in rabbits showed that the $[^{14}\text{C}]-2,4\text{-D}$ concentration in the cerebrospinal fluid was 10% that of brain tissue. Pretreatment with 160 mg/kg/day of 2,4-D increased the cerebrospinal fluid concentration in absolute terms and in relation to the brain tissue to reach 50% of the brain content. Choroid plexus levels were 6-9 times that of brain tissue but fell to 1.5 times that of brain tissue following pretreatment with 160 mg 2,4-D/kg. The choroid plexus is thought to mediate the elimination of solutes from the brain. Pretreatment of rabbits with varied doses of
2,4-D resulted in dose-dependent increases in brain content. The dose-dependent effects of 2,4-D pretreatments can be explained by the competitive inhibition of elimination of $^{[14}C\text{-2,4-D}}$ from the brain by active transport on the organic anion system of the choroid plexus. Saturation of the excretory process by unlabeled 2,4-D would cause $^{[14}C\text{-2,4-D}}$ to accumulate in the cerebrospinal fluid and the brain. This was verified by an in vitro experiment where choroid plexus tissue was incubated with 10, 100, and 1,000 μM solutions of unlabeled 2,4-D. The 10 μM 2,4-D solution was sufficient to inhibit transport of $^{[14}C\text{-2,4-D}}$ by 45%. Competitive inhibition of the organic anion transport mechanism by organic anions like 2,4-D may exacerbate the neurotoxic effects of the chemical by slowing its elimination from the brain and the cerebrospinal fluid.

Moreover, 2,4-D had no effect on the permeability of the blood-brain barrier. The penetration of the organic solute $^{[14}C\text{-2-deoxyglucose}}$ was unchanged by pretreatment with 160 mg/kg of 2,4-D (Kim et al. 1988).

2. Octachlorodibenzo-p-Dioxin

Birnbaum and Couture (1988) measured the effect of dose of octachlorodibenzo-p-dioxin (OCDD) on its absorption, tissue distribution, routes of elimination, and metabolism. They also examined the effect of repeated exposure on tissue levels and elimination. Following intravenous administration of 50 μg OCDD/kg to rats, the liver, skin, and adipose were the only tissues that contained more than 1% of the administered dose at any time up to 56 days after administration. Fecal excretion was the major route of elimination. Elimination via the feces was the result of biliary excretion. Thin-layer chromatography analysis of tissue extracts and bile indicated that only parent compound was excreted. OCDD was not metabolized by the rat. Rats treated orally with 50, 500, and 5,000 μg $^{[14}C\text{OCDD}}$ excreted more than 85% of the dose in the feces 3 days after administration; only 14% was excreted in the feces 3 days after administration.
intravenous administration. These results indicate that OCDD is poorly absorbed. As seen following intravenous administration, the liver contained the largest amount of OCDD regardless of oral dose. However, intravenous administration of a comparable concentration resulted in nine times as much of the dose in the liver and adipose 3 days after treatment relative to oral exposure. Lower proportions of the dose were present in the liver as dose increased. This nonlinear relationship suggested that the oral absorption of OCDD may be saturated. Also, less OCDD was present in the blood after oral exposure when compared with intravenous exposure. OCDD rapidly partitioned out of the blood into the liver, adipose, and skin, where it has a greater affinity. The low gastrointestinal absorption of OCDD is probably a function of its extreme insolubility. As concentrations of OCDD increased, the dosage form became a suspension, a form that is not easily absorbed.

Groups of rats were exposed to daily oral doses of 50 $\mu$g OCDD/kg for 1-10 days. OCDD accumulated in the liver where 3%-4% of the dose was present regardless of the number of doses. Similar trends were seen in the adipose. This suggested that the nonlinearity of absorption was due to the saturation of the absorption process and not the saturation of the depots. The insolubility of OCDD could contribute to this limited absorption. Elimination half-lives for the liver and adipose were approximated at 130 days and 7 years, respectively. The slow rate in the adipose is a reflection of the increased depot size as the animal grows. The whole body elimination half-life was 173 days (Birnbaum and Couture 1988).

In the companion study, Couture et al. (1988) evaluated whether subchronic exposure to low levels of OCDD would result in continuously increasing body burdens. They also assessed the significance of any concomitantly observed toxicities. Rats were treated with 50 $\mu$g/kg [14C]OCDD by gavage for 10, 20, 40, or 65 times (once daily, 5 days per week) and sacrificed 3 days post-
They confirmed their previous results that the percentage of total dose that localized in the liver remained constant. Treatment with 65 doses of OCDD elevated 7-ethoxyresorufin-O-deethylase activity (EROD) to 40 times that of controls. Cytochromes P-450c and P450d were also induced, which was in agreement with increased EROD activity, since EROD activity is associated with cytochrome-P450c. Also, total cytochrome-P450 levels doubled, and there was a 2 nm shift in the Soret absorption maximum. The selective induction of cytochrome-P450c in combination with the shift in the Soret absorption maximum was characteristic of a 3-methylcholanthrene-type induction. OCDD resulted in the manifestation of cytoplasmic fatty vacuolization in the liver, which was centrilobular to midzonal in distribution. The authors reported that subchronic exposure to OCDD caused effects similar to those observed following exposure to low levels of TCDD but was only 0.01-0.001 times as potent. The persistent accumulation of OCDD over time may eventually result in a toxic response, thus rendering OCDD a potent health hazard.

3. Pentachlorodibenzoofurans

Brewster and Birnbaum (1988) evaluated the distribution and excretion of 1,2,3,7,8-pentachlorodibenzoofuran (1PeCDF). Only 6% of an intravenous dose (0.1 μM/kg) of [3H]1PeCDF remained in the blood of the rat 15 minutes after injection. 1PeCDF primarily distributed to the liver and muscle and to a lesser degree to adipose and skin. After 3 days, levels in adipose and liver were similar. The decline in tissue activity was exponential. Apparently, the rapid elimination from the blood reflected its rapid distribution to tissues. Loss of radioactivity from all tissues except adipose followed a two-component exponential decay; adipose followed a one-component exponential decay. Persistence in adipose may be a cause for concern since bioaccumulation could occur upon repeated exposure. Daily elimination of 1PeCDF activity was primarily via the feces and
followed a two-component exponential decay. Less than 0.5% of the dose per day was detected in the urine. High-pressure liquid chromatography analysis of the urine revealed at least two metabolites that constituted greater than 90% of the urine's activity. Since the major excretory route was via the feces, the authors examined the biliary excretion to see if the activity in the feces originated in the bile. The amount of activity in the bile suggested that it entered the intestine and was excreted without recirculation. HPLC analysis of the bile revealed a single metabolite similar to the more polar metabolite in the urine. No parent compound was ever detected in the bile. Metabolism seems to be a requirement for the elimination of 1PeCDF.

Brewster et al. (1988a) studied the disposition of 2,3,4,7,8-pentachloridibenzofuran (4PeCDF) in rhesus monkeys. Following oral administration of \[^{14}C\]4PeCDF, approximately 70% of the dose remained in the stomach. Less than 0.1% of 4PeCDF activity was detected in the blood; none was detected in the bile. Six hours after administration, 4PeCDF activity was primarily distributed to muscle and skin. Monkeys were also intravenously injected with 34 \(\mu g\) \[^{14}C\]4PeCDF. Within 20 minutes, 4PeCDF was eliminated from the blood and distributed to the liver, skin, adipose, and muscle tissues. Elimination from the liver, skin, muscle, and adipose tissues followed one-compartment models with half-lives of 52.1, 37.6, 31.6, and 7.4 days, respectively. The relatively rapid elimination rate from adipose was attributable to the loss of fat stores after 4PeCDF administration. Excretion of 4PeCDF activity was primarily by the feces with a minimum whole body half-life of approximately 38 days. However, the half-life value is only a minimum estimate because of the inherent toxicity of this chemical in the monkey. The majority of the excreted radioactivity was due to polar metabolites. The authors speculated that, as the monkeys became toxic, 4PeCDF was translocated from tissues, thereby resulting in enhanced excretion and/or toxicity.
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PCDDs that are substituted in the 2,3,7,8-positions of the dioxin structure tend to accumulate in lipid-rich tissues of humans and can still be measured in these tissues 20 years after exposure. Analytical techniques have advanced to the point where PCDDs can be detected in human adipose tissue, blood serum, and milk at the pg/g (ppt) level. Quantification of PCDDs in human tissue affords investigators the opportunity to characterize past exposures to PCDDs either in the diet or in the environment without relying solely on interviews, employment records, or military records.

A. Studies of Vietnam Veterans

Recent investigations have attempted to assess objectively the exposure of Vietnam veterans to 2,3,7,8-TCDD (present as a contaminant in 2,4,5-T, a component of Agent Orange) by measuring this chemical in the adipose tissue and/or blood. The results of these investigations are presented below.

In Volume XI of this review, preliminary results of a study conducted by the USCDC were discussed (Anonymous 1987 in Clement 1988, Karon et al. 1987 in Clement 1988, Marshall et al. 1987 in Clement 1988). An update of this study is presented here. Blood serum TCDD levels were measured in a cohort of Vietnam veterans in an attempt to determine if military records could be used to identify U.S. Army veterans exposed to TCDD-contaminated herbicides (USCDC 1988d). Vietnam veterans who served in the heavily sprayed III Corps military region near Saigon during the period 1967-1968 were identified, and five exposure scores or indices were computed from military records. Three indices of exposure were calculated assuming that TCDD undergoes rapid degradation on vegetation. Two other methods of scoring exposure were also chosen to reflect a longer environmental persistence of TCDD. A group of 979 Vietnam veterans were invited to
participate; men with highest exposure scores were deliberately over-sampled. Of the 979 invited participants, 68% (665) completed telephone interviews and medical examinations and gave blood samples for residue analysis. A stratified random sample of 200 non-Vietnam veterans of the same era was invited as a comparison group, and 52% (103) participated fully. Each participant underwent a detailed interview regarding military and civilian exposure to herbicides. While 25% of Vietnam veterans reported direct exposure (present during spraying or handled spraying equipment) and 71% reported indirect exposure (walked through defoliated areas), 6% of non-Vietnam veterans reported such exposures.

The update of this study provides the results of TCDD analyses that met laboratory quality control criteria. These analyses were conducted on blood samples from 646 Vietnam veterans and 97 non-Vietnam veterans, or 66% and 48%, respectively, of those eligible for the study. There was no significant difference between Vietnam and non-Vietnam veterans with respect to serum TCDD levels. Both groups were found to have mean serum TCDD levels of approximately 4 ppt. Only two Vietnam veterans had clearly elevated TCDD levels (more than 20 ppt). Furthermore, there was no correlation between serum TCDD level and any of the five exposure scores based on military records or self-reports of exposure. The more complete results reported in this update corroborate the conclusions drawn previously; few of the participants in this study had unusually heavy exposure to TCDD, and military records cannot be used to categorize potential exposures of veterans to TCDD.

The Air Force conducted a nonconcurrent prospective study, the Air Force Health Study, of Ranch Hand personnel (Anonymous 1988a, Wolfe et al. 1988). This study was performed in conjunction with the ongoing Air Force Health Study of Ranch Hand personnel (see Lathrop et al. 1984 in Clement 1985, Lathrop et al. 1987 in Clement 1988, Wolfe et al. 1986 in Clement 1987).
The phase of the study reported here measured serum TCDD levels in 150 Ranch Hand veterans all of whom were either herbicide loaders or herbicide specialists in Vietnam and 50 matched controls. The matched controls consisted of Air Force veterans who served in air cargo units stationed in Southeast Asian locations other than Vietnam during the same period of the Ranch Hand unit. TCDD analyses that met laboratory quality control criteria were obtained from 147 Ranch Hand veterans and 49 controls. The mean serum TCDD level in the Ranch Hand personnel was 49 ppt (median 26 ppt), and 62% had levels above 20 ppt, which are considered to be elevated. The mean serum TCDD level in the controls was 5 ppt (median 5 ppt), and only one person had a level above 20 ppt. These results indicate that relatively heavy exposure to TCDD in those veterans who were either herbicide loaders or herbicide specialists can be detected 20 years later in the blood.

Similar results were obtained by Kahn et al. (1988) in a pilot study that measured TCDD levels in adipose tissue and blood of Vietnam veterans heavily exposed to Agent Orange. This pilot study is part of the Pointman Project sponsored by the State of New Jersey, which is designed to compare exposure of Vietnam veterans to Agent Orange with their 2,3,7,8-TCDD body burdens and state of health. The objectives of the pilot study were to determine, by isomer-specific analysis of all tetrachlorinated to octachlorinated dibenzodioxins and dibenzofurans, if a relationship exists between exposure and 2,3,7,8-TCDD tissue levels 15-20 years after heavy exposure in Vietnam veterans, and whether a correlation exists between blood and adipose levels.

The study population consisted of 27 men, all of whom were identified by questionnaires; their military exposure status was confirmed by U.S. Army/Department of Defense Joint Environmental Support Group. Ten of the subjects were heavily exposed Vietnam veterans; nine of those ten were herbicide handlers. Five Ranch Hand veterans were included in this group. The remaining four
classified as herbicide handlers were an Air Force freight handler who handled drums of defoliants, two Army chemical corps specialists, and one Army helicopter crew chief who filled defoliant tanks and flew spray missions. Another 10 Vietnam veterans with little or no history of exposure served as controls, and an additional seven veterans who served at the same time as the Vietnam war but who were not stationed in Southeast Asia were Vietnam-era control subjects. The controls were matched to the exposed subjects based on sex, age at time of entry into the study ±36 months, dates of military service ±36 months, race/ethnicity, and rank. Subcutaneous abdominal adipose tissue samples and blood samples were analyzed for PCDDs and PCDFs by high-resolution gas chromatography/mass spectrometry.

The levels of 2,3,7,8-TCDD in the adipose tissue and blood of the exposed men were significantly higher than either control group. Exposed men were found to have a mean 2,3,7,8-TCDD level in adipose tissue of 41.7 ppt (median 15.4 ppt) and a mean 2,3,7,8-TCDD level in blood of 46.3 ppt (lipid basis) (median 25.1 ppt). The mean adipose 2,3,7,8-TCDD level in pooled control subjects was 4.3 ppt (median 4.5 ppt), and in blood the mean 2,3,7,8-TCDD level was 5.7 ppt (lipid basis) (median 4.6 ppt). Furthermore, 2,3,7,8-TCDD levels in adipose tissue of exposed men exceeded their matched control values in 9 of the 10 pairs. The same was true for blood levels in six of the nine pairs suitable for comparison. The 2,3,7,8-TCDD congener was also the only one found to be elevated in the fat of the exposed men. Other congeners were present at higher levels in the blood of exposed men, but the differences were not as striking as they were for 2,3,7,8-TCDD. The authors attributed the higher levels of other congeners found in the blood of the exposed men partially to recovery problems with the 13-C-labeled surrogates.

Based on these results, Kahn et al. (1988) drew the following conclusions:
1. Although exposure cannot be ruled out by a low blood or fat level of \(2,3,7,8\text{-TCDD}\), it is easy to identify cases of heavy exposure by measuring fat or blood levels even 15-20 years after exposure.

2. Adipose tissue and blood levels were highly correlated when the blood levels were expressed on a fat content basis, which suggests that blood levels may present a reliable, easier, and less invasive means of detecting \(2,3,7,8\text{-TCDD}\) exposure.

3. The selective elevation of \(2,3,7,8\text{-TCDD}\) levels in exposed veterans over other congeners (none of which were present in Agent Orange but are present in the industrialized world) indicates that this is a sensitive indicator of Agent Orange exposure.

4. There is good quantitative agreement between the results obtained in blood in the present study and the CDC (USCDC 1988d) study (49 ppt vs. 46.3 ppt), lending credence to the methods used.

Schecter et al. (1987a, 1988a) reported the results of an investigation carried out as part of a Commonwealth of Massachusetts study on Vietnam veterans. They sampled the adipose tissue of 10 Vietnam veterans for \(2,3,7,8\text{-TCDD}\) levels. Six of these men were Ranch Hand veterans, and the remaining four believed they were exposed to Agent Orange as helicopter sprayers or herbicide handlers. No indication as to how the men were selected was provided in the paper. The Ranch Hand personnel had TCDD adipose levels of 7, 7, 43, and 55 ppt with two as "unconfirmed positives." The unconfirmed positives satisfied two of the three analytical criteria for a positive sample (i.e., accurate mass of the m/z 320 and m/z 322 molecular ions and retention time of the eluted material). Further work is being conducted on these samples. The other four men exhibited levels of 3—9 ppt, which are not considered to be elevated. These results indicate that it cannot be assumed that all Vietnam veterans, even those claiming to be "heavily exposed," were uniformly heavily exposed to Agent Orange, and that heavily exposed Vietnam veterans can still be identified by elevated levels of \(2,3,7,8\text{-TCDD}\) in the fat 15-20 years after exposure.
This study is limited by the small sample size, lack of controls, and lack of detail on subject selection. Nevertheless, the results are consistent with those found by others (e.g., Kahn et al. 1988).

Taken together, the results of these studies indicate that both blood and adipose tissue levels of \(2,3,7,8\)-TCDD provide a sensitive indicator of relatively heavy exposure to Agent Orange, even 15-20 years after exposure. It is likely that the cohort of Vietnam veterans that would meet the criteria for "relatively heavily exposed" to Agent Orange is composed of the Ranch Hand personnel. The cohort numbers 1,267 men and represents only a small fraction of all Vietnam veterans. As Schecter et al. (1987a, 1988a) demonstrated, not all Ranch Hand veterans were uniformly heavily exposed to Agent Orange, so a more accurate estimate would be that the number of "relatively heavily exposed" Vietnam veterans is under 1,000. These methods apparently cannot be used to detect or distinguish low to moderate exposure, since the levels in Vietnam veterans in general did not vary from control values. Furthermore, blood TCDD levels may serve as an easy, reliable, less expensive, and less invasive means to detect Agent Orange exposure. This becomes particularly important when the expense and difficulty of sampling all Vietnam veterans is considered. Proponents of this method argue that blood TCDD levels may be a better index of potential health hazard since blood circulates to vital organs and TCDD in fat is sequestered (Kahn et al. 1988). Others argue that although fat sampling is more difficult, it is still preferred over blood because it is less susceptible to diet variations and it contains a higher content of lipid with which to hold TCDD and is thus a more reliable indicator of TCDD body burden.

B. Studies of other Exposed Populations,

Several studies have been published that investigated the levels of PCDDs and PCDFs in human milk, liver, and adipose from
other exposed populations. These studies can be separated into two categories: general population or occupational studies and studies of populations exposed to a specific point source. The annotated bibliography that accompanies this review contains approximately two dozen citations that describe the levels of PCDDs and PCDFs in tissues from general industrial or nonindustrial populations across Europe, Asia, and North America. Most of these studies were reported in abstract form only and analytical results were often not reported. They will be summarized briefly here. Beck et al. (1988, abstract only) attempted to correlate previous exposures to the fat content of PCDDs and reported that the maximum PCDD levels in the fat of occupationally exposed German chemical workers were 2,252 ppt (2,3,7,8-TCDD), 9,613 ppt (HxCDD), and 4,120 ppt (HpCDD). This level of TCDD is far higher than those reported for relatively heavily exposed Vietnam veterans. In a study reported by Fingerhut et al. (1988, abstract only) the 2,3,7,8-TCDD levels in the serum of chemical workers involved in the production of 2,4,5-trichlorophenol and 2,4,5-T from 1951-1973 were compared to referent controls. The levels found in the exposed workers were reported to "greatly exceed background levels." Whole blood and adipose tissue samples were taken from two subjects with a 13-year occupational exposure to PCDDs and PCDFs at an indoor air concentration of 0.2 pg tox. equivalent/m³ (Päpke et al. 1988, abstract only). Levels were reported to be within the range of other unexposed subjects, leading the authors to conclude that no correlation exists between tissue levels and low-level exposure.

Other recent publications reported the levels of PCDDs and PCDFs in human milk, liver, and/or adipose from three highly exposed populations: South Vietnamese women, residents of Missouri, Japanese Yusho and Chinese Yucheng victims, and residents of Seveso, Italy. The results of these studies are described below.
In an ongoing study discussed in previous volumes of this review (Schecter et al. 1986a in Clement 1987, Schecter et al. 1987c,d in Clement 1988), Schecter et al. (1987c) determined the levels of PCDDs and PCDFs in human milk samples collected from South Vietnamese women in 1973 and 1985 believed to have had heavy exposure to Agent Orange. They compared these levels with those from samples taken from different women in 1970 (during or immediately after Agent Orange was sprayed) and current North American and North Vietnamese samples. The sample size was quite small (18 in 1970, 9 in 1973, and 19 in 1985), the mothers were not all from the same geographical location, and the sampling cannot be considered representative of the larger population. All of these factors tend to limit the usefulness of these results. Nevertheless, certain trends can be identified in the data. TCDD levels decreased in human milk from South Vietnamese women from 1970 to 1985: average levels from positive samples (one-half to two-thirds of the total samples) were 484 ppt in 1970, 121 ppt in 1973, and 12 ppt in 1985. The 1985 levels were slightly higher than those seen in North American human milk (5 ppt) and markedly higher than levels found in samples from North Vietnam where Agent Orange was not sprayed. The authors concluded that the elevated TCDD levels seen in South Vietnamese women's milk resulted from exposure to Agent Orange 15 years previously. Thus, human milk provides a sensitive source to measure exposure to dioxin, even though it can only be studied in a limited portion of the general population.

Another ongoing study discussed in previous volumes of this review is the Missouri Dioxin study (Patterson et al. 1986a,b, in Clement 1987, Patterson et al. 1987a in Clement 1988). Soil samples from Missouri have been found to be contaminated with 2,3,7,8-TCDD at levels higher than 1 ppb. In previous studies (Patterson et al. 1986a,b) exposed populations were defined as (a) persons exposed for 2 or more years to areas with 2,3,7,8-TCDD levels in soil between 20 and 100 ppb, or (b) persons
exposed for at least 6 months to areas with soil levels of more than 100 ppb of TCDD. The levels of 2,3,7,8-TCDD in the adipose tissues of these exposed people were then measured. Levels of PCDDs and PCDFs in adipose tissue were recently evaluated in seven selected residents with the highest exposure levels (determined previously as described above) and compared with seven randomly selected controls (Needham et al. 1987). The mean value of 2,3,7,8-TCDD in adipose tissue (expressed on a lipid basis) in the exposed cases was about 345 ppt and in the controls was about 10 ppt. However, the exposed cases did not differ from controls with regard to the levels of other congeners present in the adipose tissue. These results indicate that background exposure to PCDDs and PCDFs in general was similar in the two study groups, but that the exposed cases had experienced exposure to a 2,3,7,8-TCDD-specific source.

In July 1976 an explosion occurred at a chemical factory manufacturing 2,4,5-T in Meda, Italy. The explosion released a cloud of toxic debris that resulted in contamination of the soil with 2,3,7,8-TCDD in the towns of Seveso, Meda, Cesano Maderno, and Desio. The contaminated area was divided into three zones (A, B, and S) depending on the level of TCDD found in the soil. Zone A was the most highly contaminated and Zone S was a control zone. The results of serum sample analyses for TCDD from nine Zone A residents and five Zone S residents have recently been reported (Anonymous 1988b). The levels in the Zone A residents were the highest ever reported and ranged from 828 to 27,821 ppt (on a lipid basis). The three highest levels were obtained from children who developed chloracne. The levels in two other chloracne cases were similar to the four other Zone A residents who did not have chloracne. TCDD could not be detected in four of the five control samples. Thus, there is some overlap with regard to tissue TCDD levels in children who have chloracne and those who do not, and consequently there is no obvious tissue threshold level of TCDD for the manifestation of chloracne.
These results also suggest that there is significant variation in the sensitivity to the toxic effects of TCDD. Based on the extremely high body levels of TCDD seen following the Seveso incident, this population is more likely to show long-term adverse health effects than either the Vietnam veterans or the Missouri residents. This incident therefore provides a valuable research tool with which to study the health effects of acute exposure to high levels of TCDD, to correlate tissue levels with health effects, and to study the pharmacokinetics of TCDD in humans.

Two incidents of extensive population poisoning with PCDFs via ingestion of contaminated rice oil occurred: one incident took place in Japan in 1968 (Yusho) and the other took place in Taiwan in 1979 (Yucheng). Ryan et al. (1987) reported the results of human tissue sample analyses of PCDD and PCDF levels from Chinese, Japanese, and two Japanese Yusho victims. The lowest levels were found in adipose tissue taken from Chinese subjects (less than 30 pg/g). Levels in Japanese tissues were similar to those observed in other industrialized nations. The tissue samples from a Yusho victim contained PCDFs at levels that were 50 times the levels seen in the Japanese subjects, indicating that relatively high level exposures can be detected in tissues 9 years after exposure.

Kuroki et al. (1987) also found that the PCDF content in human tissues is greatly elevated up to 9 years following relatively heavy exposure. They reported that PCDF levels in the livers of Yusho victims (number not specified) were 11–69 ng/g 1–9 years after exposure, 9–22 ng/g in adipose tissue 1 year after exposure, and 0.8 ng/g in lung tissue 9 years after exposure. In comparison, the level of PCDFs in the adipose tissue of a control Japanese subject was 41–79 pg/g.
C. Pharmacokinetic Parameters

The studies presented above indicated that analyses of adipose tissue, blood, and breast milk may be useful indicators of exposure to PCDDs/PCDFs. However, measurements of body residues of these compounds provide no more than a semiquantitative measure of relative exposure in the absence of an understanding of the pharmacokinetic parameters required to relate tissue residues to cumulative dose.

The half-life of TCDD in humans was estimated as approximately 6-10 years (Anonymous 1987 in Clement 1988). This estimate is based on assumption of first-order kinetics and measurements in paired blood sera drawn in 1982 and 1987 from Ranch Hand personnel.

A steady increase in adipose tissue concentrations of PCDDs with age was shown in research presented in the previous volume of this review and by Stanley (1986a,b in Clement 1988). It is apparent that PCDD residues probably do not reach equilibrium in human tissues and that individuals may slowly accumulate PCDDs throughout their lifetimes. By contrast, a negative correlation between human milk residues of PCDDs and PCDFs and period of lactation or number of children nursed was reported by Fürst et al. (1987), with levels of PCDDs/PCDFs decreasing as a result of nursing.

Pharmacokinetic considerations may account for some of the apparently discrepant tissue level data seen in heavily exposed Vietnam veterans and Seveso children with chloracne. It is possible that individuals vary considerably with regard to the elimination of TCDD, so that comparable levels of exposure may not result in the same tissue levels since some individuals may metabolize and/or excrete TCDD at a much greater rate.

The conclusion expressed in the previous volume of this review remains appropriate. Until the pharmacokinetic parameters are more accurately defined, tissue residues of PCDDs/PCDFs are probably more appropriate as indicators of relative exposure than
as measurements of cumulative **dose**. It is also likely that mean or median tissue levels determined from a representative sample of a given population are more indicative of exposure than are individual levels, because of large inter-individual variability in **pharmacokinetic** parameters.

D. **Conclusions**

Based on the studies discussed above, both blood and adipose tissue levels of **2,3,7,8-TCDD** provide a sensitive indicator of relatively heavy exposure to Agent Orange, even **15—20** years after exposure. **Thus**, these methods are valid only for a small percentage of Vietnam veterans who were heavily exposed. They cannot be used to detect or distinguish low to moderate exposure, since the levels in Vietnam veterans in general did not vary from control values. Studies of TCDD levels in the tissues of other exposed populations are consistent with the findings in Vietnam veterans in that only relatively heavy exposure can be reliably detected. These studies also indicate that there is considerable population variation with regard to the susceptibility to **TCDD**-induced toxicity and possibly the rate of metabolism and/or elimination of TCDD.
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REVIEW OF LITERATURE ON HERBICIDES, INCLUDING PHENOXY HERBICIDES AND ASSOCIATED DIOXINS

Volume XIV: Annotated Bibliography of Literature on Health Effects Published in 1988

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BIBLIOGRAPHY

This volume is a bibliography of published and unpublished literature relevant to the human health effects of 2,4-D, 2,4,5-T, PCDD, cacodylic acid, and picloram that became available during 1988. Some of the literature cited was published in 1987 or earlier but were not available until 1988. Similarly, some items which will carry a publication date of 1988 were not available during the year. The citations are arranged alphabetically by author. Each citation is followed by a series of keywords. These keywords describe the information contained in the paper including the health effects(s) or type of study, the route of administration/exposure, the chemical, the species, and the type of report.

Following the key words is a line that indicates those pages of the critical analysis (Volume XIII) on which that document is discussed. Because many resources, e.g., review articles, new reports, and commentaries, are not cited in the critical review, short narrative statements describing the contents of those documents are included in the bibliography.

Keywords: Absorption, distribution, metabolism, and excretion; Unspecified route of exposure; Dioxins; Other contaminating compounds; Monkey; Rat; Abstract

The absorption and distribution of total PCDD/Fs after a single oral dose of 23,200 ng/kg b.w. was studied in rats and monkeys. Absorption after seven days was greater than 90%. Analytical results were not provided.


Keywords: Genetic toxicity; Injection; 2,4-D; Rat

See page 55.


Keywords: Dioxins; Human; Review article

The potential adverse health effects of chlorinated hydrocarbons (including 2,3,7,8-TCDD) that are present in emissions from municipal solid waste incinerators were reviewed in this article. Data on environmental concentrations and relative biologic activities of PCDDs/PCDFs were discussed in some detail (81 references).


Keywords: Cancer; Chloracne; Hepatotoxicity; Cardiovascular toxicity; Neurobehavioral effects; Reproductive toxicity; Immunotoxicity; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human;

This is a report of the update of the morbidity study conducted by the Air Force among personnel assigned to
Operation Ranch Hand in Vietnam. This report is similar to that of Lathrop et al. 1987, which was reviewed in Volume XI of this review (Clement 1988).


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat

The authors investigated the effects of acutely toxic doses of TCDD on concentrations of calcium (Ca²⁺) in the livers of rats. TCDD caused a doubling of calcium concentration in whole liver homogenates five days after treatment. Greatest increases were observed in the mitochondria and the microsomes. The significance of these findings is unclear.


Keywords: Acute toxicity; Mechanism of action; Reproductive toxicity; Oral; Dioxins; Rat

See page 78.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat

The authors compared the effects of TCDD and carbon tetrachloride (CCl₄) on lipid peroxidation in rat liver. The effects of TCDD were different from those of CCl₄. The authors concluded that the effects of TCDD on lipid peroxidation were so minor that they could not account for the acute toxic effects of this compound.

The authors studied the interaction between dietary iron and large single doses of TCDD on lipid peroxidation in rat liver. An iron deficient diet was protective against the induction of lipid peroxidation but not against body weight loss induced by TCDD.

Anderson, H.E., Hanrahan, L.P., Jensen, M., Laurin, D., Yick, W-Y., and Wiegman, P. 1986. Wisconsin Vietnam veteran mortality study - Proportionate mortality ratio results - Standardized mortality ratio results (Final report). State of Wisconsin Department of Health and Social Services, Division of Health and Social Services, Division of Health, Section of Environmental and Chronic Disease Epidemiology, Madison, WI. March 1986, 64 pages

Keywords: Cancer; Other toxic effects; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 13, 24, 26 and 45.


Keywords: Chloracne; Epidemiological study; Immunotoxicity; Environmental exposure; Occupational exposure; Dioxins; Human; Abstract

See page 85.

Anonymous. 1988a. Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in Air Force Health Study participants - Preliminary report. MMWR. 37:309-311

Keywords: Tissue levels; Occupational exposure; Phenoxy herbicide formulations; Human

See page 121.

Anonymous. 1988b. Preliminary report: 2,3,7,8-Tetrachlorodibenzo-p-dioxin exposure to humans - Seveso, Italy. MMWR. 37:733-736

This abstract summarizes a study that compared the properties of cytochrome P-450s induced by TCDD in rats and hamsters. In rats TCDD induced a single protein. In hamsters TCDD induced two cytochrome P-450s immunologically similar to that in rats but differing in enzymatic properties.


Biochemical tests, skin examination, and electrophysiological measurements were taken on subjects (and comparison controls) who developed chloracne following exposure to TCDD at Seveso. Hepatic function and nerve conduction were unaffected and chloracne was reversible.
See pages 98, 104 and 105.


Keywords: Enzyme induction or inhibition; Alterations in sex hormones; Mechanism of action; Injection; Dioxins; Rat

The authors compared the antiestrogenic and microsomal mixed function oxidase inducing activities of 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) and TCDD in female rats. MCDF had significant antiestrogenic activity at doses below those that induced MFO activity, suggesting that the hormonal effects are not secondary to increased estradiol metabolism.

Astroff, B. and Safe, S. 1988b. 6-Methyl-1,3,8-trichlorodibenzo-furan (MCDF) and related analogs as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) antagonists: Structure-activity relationships. Toxicologist. 8:107

Keywords: Enzyme induction or inhibition; Mechanism of action; Other toxic effects; Unspecified route of exposure; Dioxins; Rat; Mouse; Abstract

This abstract summarizes information that was published in full in Astroff et al. 1988.


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes studies that were described in full in Astroff and Safe 1988a.


Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; Injection; Dioxins; Rat
A structural analog of TCDD, 6-methyl-1,3,8-trichlorodibenzo-furan antagonizes the induction of cytochrome P-450 by TCDD in rat liver but is not a potent inducer itself. The antagonist binds to the Ah receptor but does not cause gene transcription.


Keywords: Acute toxicity; Lethality; Mechanism of action; Other toxic effects; Injection; Dioxins; Other species

The effects of intraperitoneal injection of TCDD and EGF on developmental parameters were studied in neonatal mink. High doses of both TCDD and EGF caused both decreased weight gain and mortality. However, there were important differences in the nature of other effects caused by these compounds.


Keywords: Epidemiological study; Cancer; Occupational exposure; Phenoxy herbicide formulations; Human; Review article

The author provides a comprehensive review of the epidemiology of cancer among agricultural workers with emphasis on the possible etiologic role of pesticides including phenoxy herbicides (88 references).


Keywords: Acute toxicity; Enzyme induction or inhibition; Oral; 2,4-D; 2,4,5-T; Rat

See page 106.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat; Abstract
This abstract summarizes a study of the mechanism by which TCDD depletes stores of vitamin A in the liver. The authors found that TCDD induces the glucuronidation of vitamin A and its metabolites in the liver of rats, thus enhancing the excretion of vitamin A in the liver.

Bannister, R. and Safe, S. 1987. The effects of receptor antagonists on the AHH induction activity of 2,3,7,8-TCDD in C57BL/6 and DBA/2 mice: 1,3,6,8-Tetrachlorodibenzoofuran. Chemosphere. 16:1739-1742

Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; Injection; Dioxins; Mouse

The authors found that 1,3,6,8-tetrachlorodibenzoofuran antagonized the induction of AHH activity by TCDD in both responsive and non-responsive mouse strains but that it was a much less effective antagonist in the responsive strain.


Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; Injection; Dioxins; Rat

Treatment of Ah responsive mice with 2,2',4,4',5,5'-hexachlorobiphenyl increased the concentration of Ah receptor in the liver and enhanced the induction of microsomal enzyme activity by subsequent treatment with TCDD. The mechanism of this interaction is being investigated.


Keywords: Enzyme induction or inhibition; Immunotoxicity; Mechanism of action; Birth defects; Unspecified route of exposure; Dioxins; Mouse; Abstract

This abstract summarizes studies that are described in full in Astroff et al. 1988b.

Keywords: Epidemiological study; Neurobehavioral effects; Environmental exposure; Dioxins; Other contaminating compounds; Human

See pages 97 and 98.


Keywords: Tissue levels; Occupational exposure; Dioxins; Other contaminating compounds; Human; Abstract

See page 126.


Keywords: Chloracne; Cancer; Mechanism of action; In vitro; Dioxins; Human; Abstract

This abstract summarizes studies of the effect of 2,3,7,8-TCDF on primary cultures of human epidermal keratinocytes. TCDF treatment caused a marked reduction in DNA synthesis and morphological alterations characteristic of enhanced differentiation.


Keywords: Porphyria cutanea tarda; Dioxins; Human; Review article

The author reviewed the pathology of the skin that is associated with various porphyrias in humans including porphyria cutanea tarda (PCT) (29 references).


Keywords: Birth defects; Mechanism of action; Unspecified route of exposure; Dioxins; Mouse; Abstract

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This abstract summarizes studies of the antagonism of the induction of birth defects (cleft palate) in mice by various polychlorinated biphenyl compounds.


Keywords: Absorption, distribution, metabolism, and excretion; Injection; Oral; Dioxins; Rat

See page 116.


Keywords: Birth defects; Mechanism of action; Oral; Dioxins; Mouse; Abstract

The interaction between the known teratogens TCDD and vitamin A in inducing birth defects in mice was studied. The combination resulted in an enhanced incidence of cleft palate (an effect common to both chemicals) over what would be expected if additivity is assumed. However, co-administration of TCDD and vitamin A had no synergistic or antagonistic effect on other teratogenic end points that were unique to each chemical.

Bombick, D.W. and Matsumura, F. 1987. 2,3,7,8-tetrachlorodibenzo-p-dioxin causes elevation of the levels of the protein tyrosine kinase pp60c-src. J. Biochem. Toxicol. 2:141-154

Keywords: Acute toxicity; Mechanism of action; Other toxic effects; Injection; In vitro; Dioxins; Guinea pig; Mouse; Rat

The authors studied the effect of TCDD on levels of a protein tyrosine kinase in a variety of tissues in a variety of species. This effect appeared to be mediated by the Ah receptor.


Keywords: Acute toxicity; Mechanism of action; Other organ; Injection; Dioxins; Mouse
The authors investigated the mechanism by which TCDD causes thymic involution in Ah-responsive mice. Treatment with TCDD caused marked increases in the activity of several protein kinases in thymic epithelium and increased the expression of the c-erb-A gene in liver.


Keywords: Cancer; Epidemiological study; Occupational exposure; Dioxins; Other contaminating compounds; Human

See pages 42 and 107.


Keywords: Cancer; Epidemiological study; Occupational exposure; 2,4-D; Human

See page 40.


Keywords: Cancer; Epidemiological study; Environmental exposure; Occupational exposure; Phenoxy herbicide formulations; Human; Review article

The authors reviewed retrospective cohort and case control epidemiology studies of the possible association between exposure to phenoxy herbicides and cancer. They concluded that discordant results are the result of methodologic problems and that the total weight of evidence does not support an association (63 references).

Bookstaff, R.C., Moore, K.W., and Peterson, R.E. 1988. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the regulation of plasma leutinizing hormone (LH) concentration in male rats. Toxicologist. 8:230

Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes a study of the effect of TCDD on testosterone levels and the regulation of steroid hormones
in rats. The authors concluded that TCDD treatment enhanced the ability of testosterone to inhibit luteinizing hormone.


Keywords: Acute toxicity; Mechanism of action; In vitro; Dioxins; Mouse

The authors used a photo-affinity label to study the kinetics of the interaction between TCDD and the Ah-receptor from mouse liver in vitro. They found that the dissociation rate constant was biphasic, leading them to conclude that binding of TCDD to the receptor was a two-step process involving an initial rapid binding step followed by a slower transformation step.

Brandwene, D.S. and Kahn, P.C. 1988. The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3-dichlorodibenzo-p-dioxin (DCDD), and 2,3,7-trichlorodibenzo-p-dioxin (TrCDD) on cytochrome P450 generated reactive oxygen. Toxicologist. 8:91

Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat; Abstract

This abstract summarizes a study of hydrogen peroxide production in hepatic microsomes from TCDD-treated rats. TCDD did not stimulate peroxide production but did inhibit glutathione peroxidase activity.


Keywords: Immunotoxicity; Mechanism of action; Oral; Dioxins; Mouse; Abstract

This abstract summarizes experimental investigations of the effect of TCDD on surface marker expression of thymic and splenic lymphocytes in mice challenged with sheep red blood cells.

An unpublished preprint of this article was included in Volume XII of this review. This study was critically reviewed in Volume XI (see Clement 1988).

See pages 24 and 28.


The authors investigated the acute oral toxicity of 2,3,4,7,8-pentachlorodibenzofuran in male rats. The \( \text{LD}_{50} \) using a 35-day observation period was 916 \( \mu \text{g/kg} \). The types of toxic effects seen were very similar to those caused by 2,3,7,8-TCDD.


Keywords: Genetic toxicity; Hepatotoxicity; Mechanism of action; Injection; Dioxins; Mouse

See pages 56 and 105.


Keywords: Acute toxicity; Mechanism of action; Other toxic effects; Injection; Dioxins; Rat; Abstract

This abstract summarizes studies of the mechanism by which TCDD decreased the storage of vitamin A in the livers of rats. The findings were inconclusive.


Keywords: Absorption, distribution, metabolism, and excretion; Injection; Unspecified route of exposure; Dioxins; Rat; Abstract

Tissue distribution of PCDD/Fs were studied in rats following subcutaneous or intraperitoneal injection. Differences were found in adipose levels and the ratio of liver to adipose levels between routes. Analytical results were not provided.

The authors studied the kinetics of the binding of TCDD to the Ah receptor from rat hepatocytes and concluded that conventional Scatchard analysis of this interaction was inappropriate because of thermal degradation of the unoccupied receptor. They proposed a model in which the unoccupied receptor must undergo a slow conformational change before rapidly binding to TCDD or becoming inactivated.


The adverse human health effects, emphasizing those effects that might result from long-term, low-level exposure, of 2,3,7,8-TCDD were reviewed in this article (13 references).


The authors studied the mechanism by which TCDD causes porphyria using a subcellular fraction prepared from mouse liver cells. They found that TCDD inhibited uroporphyrinogen decarboxylase in this system.

Keywords: Porphyria cutanea tarda; Subchronic toxicity; Mechanism of action; Injection; Dioxins; Mouse

The authors studied the mechanism of the induction of hepatic porphyria in mice by TCDD. They found that iron plays an important role in this process and concluded that TCDD and related compounds inhibit uroporphyrinogen decarboxylase. This conclusion is in contrast to that of Lambrecht et al. 1988.


Keywords: Chloracne; Epidemiological study; Other skin effects; Environmental exposure; Dioxins; Human

See pages 105 and 108.


Keywords: Cancer; Epidemiological study; Environmental exposure; Occupational exposure; Phenoxy herbicide formulations; Human

See page 39.


Keywords: Environmental exposure; Occupational exposure; Phenoxy herbicide formulations; Dioxins; Review article

This review article represents the fifth complete update of scientific literature relevant to the health effects of phenoxy herbicides and their chlorinated dibenzo-p-dioxin
contaminants, and it covers the literature that became available in 1987.


Keywords: Tissue levels; Environmental exposure; Dioxins; Human; Abstract

PCDD/F levels were measured in milk from women in Norway and Sweden. Mean levels were: 2,3,7,8-TCDD = 2.7 - 3.3 pg/g milk fat; OCDD = 150 - 260 pg/g milk fat; 2,3,4,7,8-PeCDF = 12 - 20 pg/g milk fat; and TCDD equivalents = 15 - 25 pg/g milk fat.


Keywords: Reproductive toxicity; Phenoxy herbicide formulations; Human; Review article

This review article discusses the effects of Agent Orange on male reproductive function. The authors concluded that no definite associations between exposure of Vietnam vets to Agent Orange and birth defects in their children could be drawn.


Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human; Commentary or opinion

This letter to the editor disputed the authors' discussion and interpretation of a study of soft-tissue sarcoma among Vietnam veterans (Kang et al. 1987, see Volume XI of this review).


Keywords: Cancer; Enzyme induction or inhibition; Immunotoxicity; Mechanism of action; Other skin effects; Dioxins; Review article
The authors reviewed recent research on the nature of the cytochrome \( \text{P} \text{-} 450 \) gene in mouse and human epithelial cell and the regulation of that gene by the Ah receptor (41 references).


Keywords: Cancer; Epidemiological study; Occupational exposure; Dioxins; Other contaminating compounds; Human

This update of the mortality study of workers who may have been exposed to chlorinated dibenzo-\( \text{p} \)-dioxins at the Dow Chemical Company in Midland, Michigan was presented at the 6th International Symposium on Chlorinated Dioxins and Related Compounds held in Fukuoka, Japan in 1986. This study was critically reviewed in Volume IX of this review (see Clement 1987).


Keywords: Epidemiological study; Cancer; Occupational exposure; Phenoxy herbicide formulations; Human; Review article

This review addressed the possible relationship between human cancer and exposure to pesticides, reviewing both epidemiologic and experimental evidence. The review emphasized epidemiologic evidence suggesting elevated rates of certain types of cancer, such as lymphatic cancer, among farmers but concluded that the epidemiologic evidence for association of any form of cancer with exposure to pesticides including herbicides is inconclusive (42 references).


Keywords: Enzyme induction or inhibition; Hepatotoxicity; Absorption, distribution, metabolism, and excretion; Subchronic toxicity; Oral; Dioxins; Rat

See page 116.

The author of this letter to the editor criticized an earlier paper by Sielken (1987) (see Volume XII of this review) regarding the use of mathematical models to estimate risks of cancer from exposure to dioxins at environmentally relevant concentrations.

Cuthill, S. and Poellinger, L. 1988. DNA binding properties of dioxin receptors in wild-type and mutant mouse hepatoma cells. Biochem. 27:2978-2982

The authors studied the binding of Ah receptors taken from wild-type and mutant mouse hepatoma cells to DNA using DNA-cellulose column chromatography. Neither receptor bound to DNA without prior biochemical manipulation and then only the receptor from the wild-type cells was active, suggesting the need for some conformational change in the receptor prior to binding.


In this brief review article, the authors described the physico-chemical similarities between the "dioxin receptor" and the glucocorticoid receptor in rat liver and mouse hepatoma cells (11 references).


In this brief review article, the authors described the physico-chemical similarities between the "dioxin receptor" and the glucocorticoid receptor in rat liver and mouse hepatoma cells (11 references).

See page 59.


In this paper, the authors presented evidence that the dioxin (Ah) receptor in rats is associated in a complex with a protein having a molecular weight of approximately 90,000, known as the heat shock protein. This same protein forms a similar receptor complex with the glucocorticoid receptor.

The authors studied the interaction of the TCDD-Ah receptor complex with the cytochrome P₄₅₀ gene in mouse hepatoma cells and identified a dioxin-responsive element in the 5’-flanking region that functions as a transcriptional enhancer. The dioxin-responsive element was shown to be specific for the TCDD-receptor complex and to have a relatively high affinity for that complex.

Keywords: Mechanism of action; In vitro; Dioxins; Mammalian cells in culture

The authors studied the binding of the TCDD-Ah receptor complex to DNA in vitro and identified a "core" DNA sequence that was present in all three receptor-dependent enhancers at the Ah gene locus. However, they found that binding of the ligand-receptor complex to this sequence failed to generate an active enhancer, suggesting a possible role for nucleotide sequences in regions flanking the enhancer core sequence.


Keywords: Acute toxicity; Absorption, distribution, metabolism, and excretion; Oral; 2,4-D; Rat

This Polish language article (with English summary) describes a study of the metabolic fate of a single oral dose of 2,4-D in male Wistar rats. Most of an acutely toxic dose was eliminated in the urine within two days of administration but detectable concentrations were present in urine as much as 20 days later.


Keywords: Tissue levels; Occupational exposure; Environmental exposure; Phenoxy herbicide formulations; Dioxins; Human; Abstract

The association of serum 2,3,7,8-TCDD levels and various characteristics in Vietnam vets were studied. There was a significant positive relationship between body mass index and serum levels and age and serum levels, and veterans from the western United States had lower levels than those from other parts of the country. No other significant relationships were found.
Dierickx, P.J. 1988. Interaction of 1,4-benzoquinone and 2,4-dichlorophenoxyacetic acid with microsomal glutathione transferase from rat liver. Arch. Int. Physiol. Biochem. 96:1-5

Keywords: Hepatotoxicity; Mechanism of action; Absorption, distribution, metabolism, and excretion; In vitro; 2,4-D; Rat

The interaction of 2,4-D with microsomal and cytosolic glutathione transferase (GST) purified from rat liver was studied. 2,4-D inhibited purified microsomal GST in a non dose-dependent manner, whereas the crude microsomal GST was inhibited in a dose-dependent manner. The authors concluded that the properties of soluble GST can not be extrapolated to the microsomal GST.


Keywords: Porphyria cutanea tarda; Occupational exposure; Environmental exposure; Dioxins; Human; Review article

The author reviewed a number of reports of altered urinary porphyrin excretion patterns in workers and examined the evidence linking various chemical exposure to chronic hepatic porphyria (40 references).


Keywords: Hepatotoxicity; Porphyria cutanea tarda; Environmental exposure; Dioxins; Human

This report has been published previously (Doss et al. 1984) and was reviewed in Volume V of this review (Clement 1985). The authors concluded that dioxin exposure in Seveso resulted in porphyria cutanea tarda (PCT) in two individuals who were genetically predisposed to this condition.


Keywords: Cancer; Dioxins; Human; Review article; Commentary or opinion

The author of this editorial criticized the U.S. Environmental Protection Agency (EPA) for not incorporating all
relevant information on the mechanism of action into its recent reexamination of its estimate of human cancer risks from exposure to 2,3,7,8-TCDD (no references).


Keywords: Neurobehavioral effects; Reproductive toxicity; Other route of exposure; 2,4-D; Bird


Keywords: Enzyme induction or inhibition; Mechanism of action; Injection; Dioxins; Rat

The authors studied the induction of cytochrome P-450c and aldehyde dehydrogenase by TCDD in various tissues of rats. They found that the induction of these two responses differed in dose-response, time course, and tissue specificity, suggesting that TCDD may not modulate the expression of both these genes through the Ah receptor.


Keywords: Mechanism of action; Porphyria cutanea tarda; Oral; Dioxins; Mouse

The authors studied the effect of hexachlorobenzene on the specific activity of uroporphyrin decarboxylase in rat liver in order to investigate potential mechanisms by which halogenated aromatic hydrocarbons, including TCDD, cause hepatic porphyria.


Keywords: Acute toxicity; Cardiovascular toxicity; Neurobehavioral effects; Oral; 2,4-D; 2,4,5-T; Rat; Mouse; Guinea pig

The authors found that single oral doses of three different phenoxy herbicides that were sufficiently large to cause
near-lethal toxicity caused vascular damage, as indicated by Evans blue extravasations in specific areas of the brain. They found differences between species in both the extent and location of damage to the blood brain barrier.


Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human; Abstract

This abstract of a symposium presentation describes a study that was published as a full-length article (Hardell and Eriksson 1988).

See page 33.


Keywords: Epidemiological study; Immunotoxicity; Environmental exposure; Dioxins; Human

See page 85.


Keywords: Epidemiological study; Cardiovascular toxicity; Other toxic effects; Occupational exposure; Dioxins; Other contaminating compounds; Human; Abstract

See page 107.

The author reviewed evidence that links disturbances in urinary porphyrin excretion patterns in humans exposed to chemicals, including TCDD, and concluded that the available evidence is not adequate to support the use of urinary porphyrin levels to monitor TCDD exposure.


See page 126.


In this editorial, the author examined the rationale behind the revision of the cancer risk assessment for TCDD by the U.S. Environmental Protection Agency (EPA) (see Preuss et al. 1988), and concluded that it was politically motivated.


This abstract of a symposium presentation summarizes the results of studies of the ability of TCDD to influence the formation of enzyme altered foci in the livers of rats. The formation of enzyme-altered foci appeared to depend on the composition of the diet. TCDD was inactive as a promoter when a synthetic diet was used.

Keywords: Epidemiological study; Cancer; Other toxic effects; Occupational exposure; Phenoxy herbicide formulations; Human

This cohort mortality study among Australian National Servicemen who served in Vietnam was part of the Australian Veterans Health Studies. This and associated studies were reviewed in Volume V of this review (see Clement 1985).


Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; In vitro; Dioxins; Mammalian cells in culture

The authors reported the identification of a factor in the nucleus of mouse hepatoma cells that binds to two different responsive elements and activates cytochrome P,-450 gene transcription in the presence of TCDD and related AHH inducers. Experimental evidence was presented indicating that the factor consists, at least in part, of the cytosolic Ah receptor.


Keywords: Tissue levels; Environmental exposure; Dioxins; Other contaminating compounds; Human

PCDD/F levels in breast milk from West German women were analyzed. All samples contained a typical pattern of PCDDs and PCDFs. 2,3,7,8-TCDD could not be measured in any content, but the levels of other isomers decreased with decreasing chlorination. Other trends were discussed.

See page 130.

Keywords: Cancer; Mechanism of action; Dioxins; Review article; Abstract

This abstract of a symposium presentation summarizes an approach to incorporating information on postulated mechanisms of action into cancer risk assessments for TCDD.


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; In vitro; Dioxins; Human; Mammalian cells in culture

The authors investigated the mechanisms by which TCDD suppressed the estrogen enhancement of tissue plasminogen activator in human breast cancer cells. They found no effect on the number or affinity of estrogen receptors and concluded that the effect was secondary to the increased metabolism of 17β-estradiol by cytochrome P-450.


Keywords: Hepatotoxicity; Mechanism of action; Other toxic effects; In vitro; Dioxins; Rat

In this article, the author described the development of a method using polyacrylamide gel electrophoresis to study the physicochemical properties of the Ah receptor from rat liver.


Keywords: Epidemiological study; Neurobehavioral effects; Reproductive toxicity; Occupational exposure; Phenoxy herbicide formulations; Human; Commentary or opinion
This editorial served as a preface for the volume of Environmental Research in which the American Legion study (Stellman et al. 1988) was published.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat

The authors compared the effects of a series of halogenated hydrocarbons on lipid peroxidase and glutathione peroxidase activity in the livers of rats. Only CCl4, TCDD and lindane increased lipid peroxidation and only CCl4 and TCDD inhibited glutathione peroxidase activity. The effects of TCDD were much less than those of CCl4, consistent with the findings of Albro et al. 1988.


Keywords: Tissue levels; Environmental exposure; Dioxins; Human; Abstract

PCDD/F levels in blood, fat and breast milk from individuals who consumed contaminated eggs in Northern California were compared to matched controls. Analytical results were not provided.


Keywords: Acute toxicity; Lethality; Mechanism of action; Thyroid effects; Injection; Dioxins; Rat

The authors studied the effect of hypophysectomy on the acute lethal toxicity of TCDD in rats. Hypophysectomy caused an increased sensitivity to the lethal toxicity of TCDD but administration of corticosterone overcame the effect of hypophysectomy and protected against the acute toxic effects of TCDD.

Keywords: Acute toxicity; Lethality; Mechanism of action; Thyroid effects; Injection; Dioxins; Rat

The effects of lethal doses of TCDD on the thyroid gland and on levels of thyroid hormones were compared to the effects of food deprivation. The authors concluded that the effects of TCDD were different from those of food deprivation.


Keywords: Acute toxicity; Lethality; Mechanism of action; Injection; Dioxins; Rat

The authors studied the effects of adrenalectomy with and without subsequent steroid hormone replacement on the acute lethal toxicity of TCDD in rats. Adrenalectomy increased the toxicity of TCDD and corticosterone protected against this increased lethality.


Keywords: Acute toxicity; Lethality; Hepatotoxicity; Mechanism of action; Thyroid effects; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes studies of the effect of TCDD on fatty acid synthesis as measured by tritium uptake in rats. TCDD decreased fatty acid synthesis in intact but not thyroidectomized rats, leading the authors to conclude that this effect was secondary to effects on the thyroid.


Keywords: Cancer; Alterations in sex hormones; Mechanism of action; Oral; Dioxins; Rat
The authors investigated the induction of **estradiol 2-hydroxylase** activity by TCDD in the livers of male and female rats. Their studies suggested that TCDD increases estradiol metabolism by inducing the synthesis of cytochrome P-450d activity. Additional studies with diethylnitrosamine led the authors to conclude that the induction of cytochrome P-450d may be an important contribution to the mechanisms by which TCDD promotes the formation of liver tumors.


**Keywords:** Cancer; Epidemiological study; Neurobehavioral effects; Occupational exposure; Phenoxy herbicide formulations; Human; Commentary or opinion;

A cohort mortality study was conducted on forestry workers exposed to herbicides containing 2,4-D and 2,4,5-T. No excess cancer mortality and no deaths due to soft-tissue sarcoma (STS) or non-Hodgkin’s lymphoma (NHL) were reported. There was a statistically significant increase in deaths from suicide which the authors speculated may be due to pesticide-induced CNS toxicity.

See page 44.


**Keywords:** Cancer; Mechanism of action; other skin effects; Dioxins; Review article

The authors reviewed the results of research on the actions of TCDD on human keratinocytes in culture and discussed the implications of these results for such clinical endpoints as chloracne and cancer promotion (28 references).


**Keywords:** Mechanism of action; Porphyria cutanea tarda; Oral; Dioxins; Mouse

Using **Ah-responsive** and non-responsive mouse strains, the authors showed that the Ah receptor is probably involved in the pathogenesis of hepatic porphyria by hexachlorobenzene.

Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat; Abstract

This abstract summarizes a study that suggested that the dose-related decrease in hepatic vitamin A stores seen in rats treated with TCDD is not due simply to reduced feed intake.


Keywords: Acute toxicity; Mechanism of action; Other toxic effects; Injection; Oral; Dioxins; Rat; Abstract

This abstract summarizes studies of the dose-response and time course of the perturbation of vitamin A in rats treated with acutely toxic doses of TCDD.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes a study of the effect of single doses of TCDD on vitamin A storage and metabolism in two different strains of rats having different susceptibility to the acute toxic effects of TCDD. Slight differences in the vitamin A response were not adequate to account for the observed differences in sensitivity to other acute toxicity.

This abstract summarizes comparative studies of the acute toxicity of TCDD in rats, guinea pigs, hamsters, and mice. While the doses required to produce liver enlargement and thymic atrophy in mice, rats and guinea pigs are similar to those that produce lethality, this is not true for the hamster, where doses several orders of magnitude lower than the LD$_{50}$ produce these effects.

Hannah, R.R. 1988. DNA Binding forms of rat Ah receptor complex. Toxicologist. 8:35

In this abstract, the authors described the use of DNA-cellulose column chromatography to study the nuclear binding of the TCDD-Ah receptor complex. Two interconvertible forms of the receptor with different affinities for DNA were found.


In this book chapter, the authors commented on and defended the methods and conclusions of their epidemiologic studies that indicated associations between exposure to phenoxy herbicides and increased risks of soft-tissue sarcoma, non-Hodgkin's lymphoma and other forms of cancer (50 references).


See page 40.

Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

See page 33.


Keywords: Epidemiological study; Cancer; Dioxins; Human; Commentary or opinion

In this letter to the editor, the authors addressed the issue of a possible association between Kaposi sarcoma and exposure to chlorinated dibenzo-p-dioxins and/or phenoxy herbicides (see Vineis and Zahm 1988) and concluded that further epidemiologic investigation is desirable.


Keywords: Enzyme induction or inhibition; Mechanism of action; In vitro; Dioxins; Human

A human squamous carcinoma cell line was found to contain a receptor that possesses the physicochemical properties of the Ah receptor and which is present at concentrations comparable to those in rodent liver cells. The affinity of this receptor for TCDD was about an order of magnitude lower than that in mouse hepatoma cells. Nevertheless, the authors concluded that this cell line provides a good model system for studying the properties of the human Ah receptor.


Keywords: Enzyme induction or inhibition; Mechanism of action; Other toxic effects; Injection; Dioxins; Rat

By comparing responses to TCDD in two different substrains of Sprague-Dawley rats the authors showed that a 4-5S protein present in liver cytosol from one substrain played no role in the induction of aryl hydrocarbon hydroxylase.
activity by TCDD, despite a high affinity for TCDD. AHH induction which was similar in the two substrains is probably mediated by binding of TCDD to an 8–9S receptor protein present in both substrains.


Keywords: Mechanism of action; Reproductive toxicity; Birth defects; Injection; In vitro; Dioxins; Mouse

The effects of 2,3,7,8-TCDD and other ligands of the Ah receptor on fetal development and on primary cultures of thymocytes from C57BL/6 mice were studied. Benzo(a)pyrene increased TCDD-induced fetolethality but not the incidence of cleft palate. 2,3,7,8-TCDBF and 2,3,7,8-TCDD caused a decrease in lymphoid development in culture. The authors concluded that TCDD and other ligands act by a common mechanism which may involve direct interaction with receptors present in the thymus.

Hassoun, E.A. and Arif, A.T. 1988. Effect of D,L-α-difluoromethyl ornithine on cleft palate induced by the TCDD congener, 3,3′,4,4′-tetrachloroazoxybenzene, in the fetuses of mice. Toxicology. 51:77-85

Keywords: Mechanism of action; Reproductive toxicity; Injection; Other contaminating compounds; Mouse

See page 79.


Keywords: Absorption, distribution, metabolism, and excretion; Tissue levels; In vitro; Dioxins; Human

The distribution and extraction of exogenously added 2,3,7,8-TCDD from human whole blood and its association with various lipoproteins was studied. TCDD was totally recovered in plasma, and up to 5% was found in immunocytes. The bulk of 2,3,7,8-TCDD was found to be carried in the LDL fraction.

The authors investigated the effects of several compounds that modify sulfhydryl groups (-SH) on the binding of TCDD to Ah receptor from rat livers. All of these compounds inhibited TCDD binding, suggesting the involvement of -SH groups in the maintenance of the physicochemical properties of the Ah receptor. However, the authors found important differences between the effects of these compounds on the properties of the Ah receptor and those of the glucocorticoid receptor.


In this important study the authors cloned a 3574 base pair fragment from the human cytochrome P450IA1 gene. They identified three new regulatory elements and showed that the fragment was able to induce expression when treated with TCDD.


This article describes a study of the acute oral toxicity of TCDD in adult male mink. High single doses produced a characteristic wasting syndrome and liver toxicity. A 28-day LD_{50} of 4.2 jug/kg indicates that mink are relatively sensitive to the acute toxic effects of this compound.

Keywords: Acute toxicity; Lethality; Mechanism of action; Injection; Dioxins; Rat; Abstract

This abstract summarizes studies that are described in full in Gorski et al. 1988a.


Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; Injection; Dioxins; Other contaminating compounds; Guinea pig

The authors determined dose-response relationships for body weight loss and induction of microsomal aryl hydroxylase activity in guinea pigs for a series of chlorinated dibenzofurans and dibenzo-p-dioxins. The results were used to derive a quantitative structure-activity relationship for these compounds.


Keywords: Cancer; Mechanism of action; Dioxins; Mouse; Review article; Abstract

This abstract of a symposium presentation summarizes information on the mechanism by which TCDD caused increased incidences of cancer in experimental animals. The authors argued that TCDD is a complete carcinogen rather than a promoter.


Keywords: Tissue levels; Phenoxy herbicide formulations; Dioxins; Human; Commentary or opinion

This commentary discusses the progress made and the challenges facing the quantification of toxic chemicals in selected human populations.

Keywords: Tissue levels; Environmental exposure; Phenoxy herbicide formulations; Dioxins; Human; Abstract

Levels of 2,3,7,8-TCDD in milk and fat from North and South Vietnamese were measured. No analytical results were presented.


Keywords: Cancer; Dioxins; Human; Review article; Commentary or opinion

This editorial suggested that humans are much less sensitive to the toxic (including carcinogenic) effects of TCDD than experimental animals.


Keywords: Acute toxicity; Lethality; Mechanism of action; Injection; Dioxins; Rat; Abstract

This abstract summarizes studies of the histopathology of various tissues in rats treated with acutely toxic doses of TCDD compared to pair-fed and ad libitum fed control rats. Histopathological alterations are caused both by decreased food intake and by TCDD but these changes are distinguishable.


Keywords: Mechanism of action; Birth defects; Injection; Dioxins; Mouse; Abstract

The effect of day of administration on the teratogenicity of TCDD in mice was studied. The maximum incidence of both cleft palate and dilated renal pelvis occurred when TCDD was given on days 10 and 11 of gestation, indicating that these two effects tended to occur together.

**Keywords:** Acute *toxicity*; Cancer; Reproductive toxicity; Mechanism of action; Dioxins; Human; Review article

This Japanese language article summarizes information on the health effects of 2,3,7,8-TCDD with emphasis on episodes of environmental contamination at Seveso and Niagara Falls (25 references).


**Keywords:** Mechanism of action; Other toxic effects; In vitro; Dioxins; Rat; Abstract

This abstract summarizes a study of the effect of molybdate ion on the DNA binding properties of the complex formed between TCDD and the Ah receptor in rat hepatic cytosol. Molybdate ion appeared to interfere with the activation of the TCDD-receptor complex, thus interfering with DNA binding.


**Keywords:** Epidemiological study; Enzyme induction or inhibition; *Hepatotoxicity*; *Immunotoxicity*; Dioxins; Occupational exposure; Human

See page 86.


**Keywords:** Tissue levels; Occupational exposure; *Phenoxy herbicide formulations*; Dioxins; Human

See pages 122, 123 and 125.


**Keywords:** Acute toxicity; Lethality; Mechanism of action; Oral; *Phenoxy herbicide formulations*; Human

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Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human; Commentary or opinion

This letter to the editor constitutes a reply by the authors of a case-control study of the relationship between soft-tissue sarcoma and military service in Vietnam to the critique of that study by Constable et al. (1987).


Keywords: Genetic toxicity; In vitro; 2,4-D; Single-celled animal


Keywords: Enzyme induction or inhibition; Mechanism of action; In vitro; Dioxins; Mammalian cells in culture

The authors studied the effect of TCDD on the expression of the cytochrome P450 gene in a series of mouse hepatoma cell lines, some of which were resistant to AHH induction by TCDD. Some resistant cell lines contained Ah receptor with undiminished affinity for TCDD, but with impaired ability to translocate to the nucleus.


Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

This study was presented at the 6th International Symposium on Chlorinated Dioxins and Related Compounds in Fukuoka, Japan in 1986 and was critically reviewed in Volume IX of this review (see Clement 1987).

Keywords: Acute toxicity; Absorption, distribution, metabolism, and excretion; Mechanism of action; Neurobehavioral effects; Injection; 2,4-D; Mouse; Rabbit

See pages 113, 114 and 115.


Keywords: Porphyria cutanea tarda; Dioxins; Human; Review article

In this careful and detailed review the author described the pathology of porphyrins focussing on the distinction between two different types of porphyria cutanea tarda (PCT) seen in humans. Morphological characteristics and animal models for these diseases were described (23 references).


Keywords: Neurobehavioral effects; Occupational exposure; Dioxins; Human

See page 97.

Kleeman, J.M., Moore, R.W., and Peterson, R.E. 1988. Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on testosterone (T) production by isolated perfused testes. Toxicologist. 8:230

Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Other organ; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes a study of the mechanisms by which TCDD decreases testosterone synthesis in rat testes. The results suggested that TCDD did not inhibit testosterone production, but instead, interfered with cholesterol-supported secretion of that hormone.

This is a publication of a mortality study among Vietnam veterans in the state of Massachusetts. This study was available in 1985 and was critically reviewed in Volume VII of this review (see Clement 1986).

See page 28.


Keywords: Absorption, distribution, metabolism, and excretion; Mechanism of action; Unspecified route of exposure; Dioxins; Rat; Abstract

The dose-dependency and half-life of 2,3,7,8-TCDD accumulation in rat liver and fat was studied following a single subcutaneous injection of 1 - 3,000 ng TCDD/kg body weight. TCDD content (%) in liver increased with increasing dose. (T₁/₂ in fat = 24.5 days; T₁/₂ in liver = 11.5 days).


Keywords: Absorption, distribution, metabolism, and excretion; Injection; Dioxins; Rat; Abstract

The route of administration and vehicle that produced maximum absorption of TCDD was studied in rats. The authors concluded that subcutaneous injection in toluene/DMSO (1:2 v/v) at a volume of 0.2 ml/kg b.w. yielded the best absorption. Analytical results were not provided.


Keywords: Cancer; Epidemiological study; Environmental exposure; Other contaminating compounds; Human

See page 45.

Keywords: Tissue levels; Environmental exposure; Other contaminating compounds; Human

See page 129.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Injection; Dioxins; Rat

Tritiated water was used to follow the incorporation of tritium during fatty acid and cholesterol synthesis in rat liver and adipose tissue. Single doses of TCDD strongly inhibited both fatty acid and cholesterol synthesis in these tissues. The authors concluded that this inhibition may contribute to the wasting phenomenon.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes studies that were published in full as Lakshman et al. 1988a.


Keywords: Acute toxicity; Mechanism of action; Porphyria cutanea tarda; In vitro; Dioxins; Bird; Abstract

In this abstract the authors summarized extensive experimental evidence that the porphyria caused by TCDD in mice, Japanese quail and cultured chicken embryo hepatocytes is not due to a decrease in uroporphyrinogen decarboxylase.
Some of these experiments are described in full in Lambrecht et al. 1988.


Keywords: Hepatotoxicity; Porphyria cutanea tarda; Mechanism of action; In vitro; Dioxins; Bird

The authors described the results of studies of the mechanism by which TCDD causes an accumulation of porphyrins (porphyria) in cultured hepatocytes from chicken embryos. They concluded that the porphyria is the result of induction of cytochrome P450 leading to increased uroporphyrinogen oxidation.


Keywords: Hepatotoxicity; Mechanism of action; In vitro; Dioxins; Guinea pig; Hamster; Mouse; Rat; Abstract

This abstract summarizes studies of the in vitro thermal inactivation of Ah receptor from the livers of different species. The authors found significant interspecies differences in the thermodynamic properties of the receptor, but these differences were not sufficient to account for interspecies differences in Ah-receptor mediated responses to TCDD.


Keywords: Cancer; Absorption, distribution, metabolism, and excretion; Dioxins; Review article

The authors used experimental data developed by Gasiewicz et al. to develop a physiologically based pharmacokinetic model for the uptake and tissue distribution of TCDD in responsive and non-responsive strains of mice and suggested that pharmacokinetic considerations may play a role in strain differences in sensitivity to the toxic effects of these compounds.

Keywords: Cancer; Genetic toxicity; Reproductive toxicity; Occupational exposure; Dioxins; Human; Review article

In the absence of a regulatory standard for occupational exposure of workers to 2,3,7,8-TCDD, the authors present the rationale for their recommendation of an occupational exposure limit of 0.2 ng/m of air as an 8-hour time weighted average concentration. The basis for this limit is the absence of acute toxic effects in workers as well as chronic toxicity data from experimental animals (74 references).


Keywords: Epidemiological study; Neurobehavioral effects; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 95 and 96.

Li, M.A., Denomme, M.A., Towner, R., Leece, B., and Safe, S. 1988. Synergistic toxic interactions of hexachlorobenzene (HCB) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. Toxicologist. 8:35

Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; Other toxic effects; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract describes a study of the interaction of TCDD and hexachlorobenzene in causing acute toxic effects in rats. The authors found that HCB interacted synergistically with TCDD in producing weight loss and thymic atrophy.


Keywords: Acute toxicity; Enzyme induction or inhibition; Hepatotoxicity; Mechanism of action; Unspecified route of exposure; Dioxins; Mouse; Abstract

This abstract describes a study of the comparative dose-response and time course of the induction of EROD activity, inhibition of the glucocorticoid receptor and inhibition of
the EGF receptor in mouse livers after treatment with TCDD. The authors concluded that there were small but important differences between these three separate effects.


Keywords: Cancer; Mechanism of action; In vitro; Dioxins; Mammalian cells in culture

See page 48.


Keywords: Tissue levels; Environmental exposure; Dioxins; Other contaminating compounds; Human

The authors used multivariate statistical methods to analyze data on PCDD/F levels in human milk. They found that there is little overall relationship between the personalia variables and chemical variables.


Keywords: Renal toxicity; Subchronic toxicity; Injection; 2,4-D; Rat

See page 109.


Keywords: Acute toxicity; Immunotoxicity; Mechanism of action; Other organ; Unspecified route of exposure; Dioxins; Mouse; Abstract

See page 88.

Keywords: Immunotoxicity; Mechanism of action; In vitro; Dioxins; Mouse

See page 87.


Keywords: Acute toxicity; Lethality; Mechanism of action; Other organ; Unspecified route of exposure; Dioxins; Rat

Abstract

This abstract summarizes studies of the effect of TCDD on the gastric mucosa of rats. TCDD caused gastric hyperplasia in the later stages of the wasting syndrome. The authors concluded that this was secondary to decreased gastric acid secretion.


Keywords: Enzyme induction or inhibition; Mechanism of action; In vitro; Dioxins; Mammalian cells in culture

The authors investigated reasons for the inability of TCDD to induce aryl hydrocarbon hydroxylase (AHH) activity in a rat hepatoma cell line. The lack was not caused by either a deficiency of Ah receptor or a defect in the induction of NADPH-cytochrome c reductase. The authors postulated a defect in either the Ah receptor-DNA binding or in the structural gene coding for cytochrome P-450.


Keywords: Chloracne; Cancer; Porphyria cutanea tarda; Occupational exposure; Dioxins; 2,4,5-T; Human
See page 47.


Keywords: Epidemiological study; Occupational exposure; Dioxins; 2,4,5-T; Human

See page 47.


Keywords: Hepatotoxicity; Mechanism of action; In vitro; Dioxins; Rat

The authors compared the DNA binding properties of the dioxin (Ah) receptor and the glucocorticoid receptor. Both receptors bound to DNA only when bound to their specific ligands, suggesting to the authors that both receptors possess structural similarities in their functional domains.


Keywords: Epidemiological study; Birth defects; Environmental exposure; Dioxins; Other contaminating compounds; Human

See page 47.


Keywords: Tissue levels; Dioxins; Human; Review article

This Japanese language review article is the second in a series of five and reviews scientific information on concentrations of chlorinated dibenzo-p-dioxins and dibenzofurans in tissues of humans both with and without excessive exposure to these compounds (38 references).

Keywords: Dioxins; Human; Review article

This Japanese language article is the first of a series of five articles reviewing the current state of knowledge on the toxicity of, and environmental exposure to, chlorinated dibenz-p-dioxins and dibenzofurans (4 references).


Keywords: Acute toxicity; Lethality; Mechanism of action; Other organ; In vitro; Dioxins; Rat

The authors studied the effects of TCDD on primary cultures of immature rat thymocytes, finding that TCDD increased protein synthesis, increased DNA fragmentation, and increased intracellular calcium levels. They concluded that TCDD stimulates a cellular suicide process similar to one found for glucocorticoid hormones.


Keywords: Genetic toxicity; In vitro; 2,4-D; 2,4,5-T; Single-celled animal

This German language article discusses the results of genotoxicity studies conducted on 2,4-D and 2,4,5-T. Both compounds were nonmutagenic in Salmonella typhimurium strains TA 97, TA 98, TA 100 and TA 102 when tested with and without metabolic activation.

See page 58.

Mohammad, F.K. and St. Omer, V.E.V. 1988a. Effects of prenatal exposure to 2,4-D/2,4,5-T mixture on postnatal changes in rat brain glutamate, GABA, protein, and nucleic acid levels. Bull. Environ. Contam. Toxicol. 40:294-300

Mohammad, F.K. and St. Omer, V.E.V. 1988b. Behavioral and neurochemical alterations in rats prenatally exposed to 2,4-dichlorophenoxyacetate (2,4-D) and 2,4,5-trichlorophenoxyacetate (2,4,5-D) mixture. Teratology. 37:515


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Other organ; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes studies that are published in full in Moore and Peterson 1988.

Mullison, W.R. 1986. An interim report summarizing 2,4-D toxicological research sponsored by the industry task force on 2,4-D research data and a brief review of 2,4-D environmental effects. The Technical and Toxicology Committees of the Industry Task Force on 2,4-D Research Data. July, 1986, 7 pages

Keywords: Cancer; Chronic toxicity; Oral; 2,4-D; Rat; Review article

See page 47.


Keywords: Acute toxicity; Lethality; Mechanism of action; Thyroid effects; Injection; Dioxins; Rat

The authors studied the role of dietary composition in the acute lethal toxicity of TCDD to cold-adapted rats. The relevance of their findings to human health is unclear.

Nagayama, J. 1987a. Some of the recent findings and comments on pharmacokinetic, biochemical effects and animal toxicology of dioxins and dibenzofurans. Chemosphere. 16:2191-2192

Keywords: Enzyme induction or inhibition; Mechanism of action; Absorption, distribution, metabolism, and excretion; Dioxins; Review article

The author summarized information on the metabolic fate and mechanism of action of the PCDDs/PCDFs that was presented at the 6th International Symposium on Chlorinated Dioxins and Related Compounds held in Fukuoka, Japan in September, 1986 (no references).
Nagayama, J. 1987b. AHH Inducibility of dioxins and dibenzofurans and possible mechanisms of their toxicity. Toxicol. Forum (Japan). 10:591-600

Keywords: Enzyme induction or inhibition; Hepatotoxicity; Mechanism of action; Dioxins; Review article

This Japanese language article is the fifth in a series of five and it assesses the state of knowledge of the mechanism(s) by which chlorinated dibenzo-p-dioxins and dibenzofurans elicit responses in experimental animals (31 references).


Keywords: Enzyme induction or inhibition; Mechanism of action; Other skin effects; Environmental exposure; Other contaminating compounds; Human

See page 105.


Keywords: Enzyme induction or inhibition; Hepatotoxicity; Immunotoxicity; Mechanism of action; Subchronic toxicity; Other organ; Injection; Dioxins; Mouse; Abstract

This abstract summarizes a study in which TCDD was administered weekly by intraperitoneal injection to Ah-responsive and Ah-nonresponsive strains of mice. Based on the differential responses seen, the authors concluded that some, if not all, of the acute toxic effects of TCDD are mediated by the Ah receptor.


Keywords: Cancer; Absorption, distribution, metabolism, and excretion; Environmental exposure; Dioxins; Human; Review article

The authors reviewed pathways by which humans might be exposed to 2,3,7,8-TCDD and related PCDDs/PCDFs as a result of environmental contamination and described how to
calculate average daily intakes for estimating population cancer risks (34 references).


Keywords: Tissue levels; Epidemiological study; Environmental exposure; Dioxins; Other contaminating compounds; Human

See page 128.


Keywords: Birth defects; Reproductive toxicity; Injection; Dioxins; Monkey; Rat; Abstract

The reproductive toxicity of 2,3,7,8-TCDD in both male and female rodents and non-human primates was studied.


Keywords: Absorption, distribution, metabolism, and excretion; Unspecified route of exposure; Dioxins; other contaminating compounds; Monkey; Rat; Abstract

The distribution of PCDD/Fs was studied after a single dose (route not specified) in rats and monkeys. 2,3,7,8-substituted derivatives but not non-substituted derivatives were found in fat and liver. The authors concluded that this had important implications with regard to the application of TCDD-equivalents. Analytical results were not provided.

Keywords: Tissue levels; Environmental exposure; Dioxins; Phenoxy herbicide formulations; Human; Abstract

2,3,7,8-TCDD was measured in the fat of Vietnamese women with a history of exposure to Agent Orange. Elevated levels (as high as 103 ppt) were detected in some women. Analytical results were not provided.


Keywords: Immunotoxicity; Mechanism of action; Reproductive toxicity; Other route of exposure; Other contaminating compounds; Bird

See page 88.


Keywords: Acute toxicity; Immunotoxicity; Mechanism of action; Other route of exposure; Dioxins; Bird; Abstract

See page 88.


Keywords: Acute toxicity; Cancer; Enzyme induction or inhibition; Hepatotoxicity; Lethality; Dioxins; Review article

This Japanese language article is the third in a series of five and summarizes information on the toxicity of chlorinated dibenzo-p-dioxin and dibenzofurans from studies in experimental animals (59 references).

Keywords: Tissue levels; Environmental exposure; Dioxins; Other contaminating compounds; Human

The levels of PCDD/Fs in the milkfat from Swedish women were analyzed over time. The authors found that the levels of these compounds in milk decreased from the period of 1972 to 1985, possibly reflecting a lower level of environmental pollution. They also found that PCDD/F levels decreased over successive nursing periods (i.e. number of infants nursed), which suggested that nursing serves as a route of excretion for these compounds.


Keywords: Tissue levels; Environmental exposure; Dioxins; Human

Levels of PCDD/Fs in milk, liver and fat from the general Japanese population were measured. Mean levels in milk of primipara’s were 37 ppt (TCDD) and 789 ppt (OCDD). Mean levels in milk of multipara’s were 19 ppt (TCDD) and 518 ppt (OCDD). These trends were similar in liver and fat samples, but the absolute amounts differed. Residents of urban areas had higher PCDD/F levels than residents of rural areas.


Keywords: Epidemiological study; Cancer; Occupational exposure; Phenoxy herbicide formulations; Human

See page 38.


Keywords: Chloracne; Epidemiological study; Occupational exposure; Dioxins; Human; Abstract
In this abstract of a symposium presentation, the authors reported finding 48 cases of chloracne among 637 workers employed in the manufacture of pentachlorophenol at a single plant. The risk of chloracne was associated with duration of employment. This survey was conducted as part of the ongoing NIOSH study of dioxin exposed workers in the United States.


Keywords: Tissue levels; Occupational exposure; Dioxins; Other contaminating compounds; Human; Abstract

See page 126.


Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; In vitro; Dioxins; Human

The authors studied the induction of aryl hydrocarbon hydroxylase (AHH) activity by TCDD in seven human breast cancer cell lines. They found that the different cell lines varied considerably in both their baseline AHH activity and the inducibility of that activity by TCDD. This may reflect an underlying variability in the amount or properties of Ah receptor.


Keywords: Enzyme induction or inhibition; Hepatotoxicity; Mechanism of action; Injection; In vitro; Dioxins; Rat

The authors studied the transcription of cytochrome P-450 when rats were treated in vivo or primary cultures of rat hepatocytes were treated in vitro with polycyclic aromatic hydrocarbons including TCDD. They found that post-transcriptional processes were important in the regulation of expression of this gene.

Keywords: Tissue levels; Epidemiological study; Environmental exposure; Dioxins; Human

Paired human serum and adipose tissue samples from 50 Missouri residents were analyzed for 2,3,7,8-TCDD content. After adjusting for total lipid content in both types of samples, a good correlation was obtained between serum and adipose levels of 2,3,7,8-TCDD. The authors concluded that serum 2,3,7,8-TCDD concentration is a valid measurement of 2,3,7,8-TCDD total body burden.


Keywords: Enzyme induction or inhibition; Mechanism of action; Hepatotoxicity; In vitro; Dioxins; Mouse

The authors achieved an approximately 20,000 fold enrichment of two peptides corresponding to the Ah receptor and a fragment thereof from mouse liver cytosol.


Keywords: Tissue levels; Environmental exposure; Phenoxy herbicide formulations; Dioxins; Human; Abstract

PCDD/F levels were measured in tissue samples taken from Vietnamese patients (clinical pilot-case report study). Analytical results were not provided.

This abstract describes the results of studies of the effect of salt concentration in the buffer on the outcomes of studies of the physico-chemical properties of the Ah receptor in various species.


Keywords: Hepatotoxicity; Lethality; Other organ; Other toxic effects; Subchronic toxicity; Oral; Other contaminating compounds; Rat

The authors fed rats diets containing 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) or 2,3,7,8-TCDD for 13 weeks. The toxic effects produced by the PeCDF were similar to those produced by TCDD. PeCDF appeared to have 0.4 times the potency of TCDD.


Keywords: Mechanism of action; Dioxins; Review article

This article is a summary of talks presented at the 6th International Symposium on Chlorinated Dioxins and Related Compounds in Fukuoka, Japan in September, 1986 on the structure and function of receptors for 2,3,7,8-TCDD (no references).


Keywords: Mechanism of action; In vitro; Dioxins

The authors compared the physicochemical properties of the "dioxin receptor" with those of the steroid hormone receptor in rat liver. Limited hydrolysis of the dioxin receptor with trypsin diminished its ability to bind to DNA without altering the gross structural properties of the receptor.

Keywords: Acute toxicity; Enzyme induction or inhibition; Lethality; Mechanism of action; Injection; Dioxins; Rat

The authors investigated the effects of TCDD on various components of the microsomal mixed function oxidase systems in two strains of rats that differ significantly in their sensitivity to the acute toxic effects of TCDD. The induction of cytochrome P-450 and several different oxidative enzyme activities was similar in the two strains, suggesting no important differences in Ah receptor content and function in these rat strains.


Keywords: Acute toxicity; Lethality; Mechanism of action; Other toxic effects; Injection; Dioxins; Rat

The authors used a battery of drugs that modulate adrenergic neurotransmission to study the mechanism of the wasting syndrome that characterizes the acute lethal toxicity of TCDD in rats. None of the drugs tested protected against the wasting effect suggesting that TCDD probably does not act by altering adrenergic neurotransmission.


Keywords: Absorption, distribution, metabolism, and excretion; Oral; Dioxins; Human; Abstract

The toxicokinetics of 2,3,7,8-TCDD in humans (fecal excretion, fat content) was studied. No analytical results were provided.

The authors had previously reported on the use of photo-affinity label to identify the Ah-receptor in the livers of dioxin-responsive mice. In this paper they described further studies indicating that one form of the receptor described earlier is actually a proteolytic fragment produced by a Ca-dependent proteinase that is present in liver cytosols from several species.


This abstract summarizes a study of the effect of TCDD on vitamin A metabolism in the liver of rats. The study and the results were very similar to those described in Bank et al. 1988.


This abstract summarizes a symposium presentation by an EPA official discussing the reason for and the product of a revised cancer risk assessment for TCDD. EPA recommended that a dose of 0.1 pg/kg/day be regarded as a dose associated with an upper limit incremental cancer risk of one in one million.


The authors studied the induction of aryl hydrocarbon hydroxylase activity in a mouse hepatoma cell line and in responsive and non-responsive mouse strains using a number
of inducers including TCDD. They used the results to derive a model explaining two different mechanistic categories of AHH inducers.


Keywords: Acute toxicity; Mechanism of action; Other skin effects; Dermal; Dioxins; Mouse

The authors examined the epidermal proliferative response to the topical application of TCDD in responsive and non-responsive mouse strains. TCDD caused involution of the sebaceous glands and stimulated transglutaminase activity in both strains but caused epidermal proliferation and hyperkeratinization only in the responsive strain.


Keywords: Cancer; Hepatotoxicity; Mechanism of action; Renal toxicity; Oral; Dioxins; Other contaminating compounds; Rat

See page 49.


Keywords: Acute toxicity; Cancer; Hepatotoxicity; Other toxic effects; Other organ; Injection; Dioxins; Hamster

The authors observed the formation of hepatocytes in the pancreas (transdifferentiation) given large doses of TCDD by intraperitoneal injection at four week intervals. The significance of this finding is unclear.


Keywords: Cancer; Injection; Dioxins; Hamster

See page 48.

Keywords: Cancer; Epidemiological study; Other toxic effects; Environmental exposure; Dioxins; Human; Commentary or opinion

This article presents preliminary results of an effort to develop mathematical models to indicate the extent and intensity of dioxin contamination in the vicinity of the ICMESA plant in Seveso, Italy as the result of an accident there in 1976.


Keywords: Acute toxicity; Enzyme induction or inhibition; Hepatotoxicity; Injection; Picloram; Rat

Evidence is presented that suggests that picloram inhibits cytochrome P-450h and induces cytochrome P-450d in male rat liver.


Keywords: Epidemiological study; Reproductive toxicity; Birth defects; Environmental exposure; Other contaminating compounds; Human

See page 71.


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Reproductive toxicity; Injection; Dioxins; Rat

See pages 76 and 77.

Keywords: Acute toxicity; Mechanism of action; Unspecified route of exposure; Dioxins; Rat

The authors studied the binding affinity for the Ah receptor in rat liver of a series of substituted trichlorodibenzo-p-dioxins and derived a quantitative structure-activity relationship for this binding.


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Injection; Dioxins; Rat

The authors studied the effects of TCDD on cytosolic and nuclear estrogen and progesterone receptor levels in the uterus of Long-Evans rats. TCDD caused a dose-dependent decrease in these receptors, which persisted up to seven days. TCDD also inhibited estrogen-induced increases in these receptors. The mechanism for this effect was not clear.

Romkes, M. and Safe, S. 1988b. Comparative effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and progesterone as antiestrogens in the female rat uterus. Toxicologist. 8:92

Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Other organ; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes studies that were described in full in Romkes and Safe 1988a.


Keywords: Birth defects; Mechanism of action; Oral; 2,4-D; Rat; Abstract

The maternal toxic, fetotoxic and developmental effects of 2,4-D and 2,4,5-T were studied in rats orally administered these chemicals on days 7-16 of pregnancy. 2,4-D induced significant maternal weight loss, maternal lethality,
embryo/fetal lethality, reduced fetal weights and an increased incidence of supernumerary ribs. 2,4,5-T induced only maternal lethality. The authors concluded that maternal toxicity does not induce a specific syndrome of developmental toxicity in the rat.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Thyroid effects; Oral; Dioxins; Rat

The authors studied the nature and mechanism of the effects of TCDD on the thyroid and on thyroid hormone levels in rats.


Keywords: Acute toxicity; Lethality; Mechanism of action; Injection; Dioxins; Rat; Abstract

This abstract summarizes studies that were described in full in Gorski et al. 1988c.


Keywords: Acute toxicity; Mechanism of action; other skin effects; Other toxic effects; In vitro; Dioxins; Mammalian cells in culture

The authors found that TCDD inhibited the induction of transglutaminase by vitamin A in cultured keratinocytes and that the dose-response relationship for this effect was nearly superimposable with that for AHH induction. The authors concluded that TCDD may decrease the sensitivity of tissues to vitamin A through the Ah receptor.


Keywords: Hepatotoxicity; Enzyme induction or inhibition; Mechanism of action; Injection; Dioxins; Hamster
The authors studied the kinetics of TCDD binding to the Ah receptor and subsequent nuclear translocation of the TCDD-receptor complex after in vivo administration to hamsters. In vitro experiments revealed two distinct forms of the TCDD-receptor complex, only one of which appeared to bind to DNA. Maximal AHH induction occurred and was sustained when only a small percentage of the available Ah-receptor was bound to TCDD and present in the nucleus.


Keywords: Acute toxicity; Alterations in sex hormones; Neurobehavioral effects; Mechanism of action; Injection; Dioxins; Rat

The authors conducted studies of the mechanism by which TCDD decreases serum levels of prolactin in rats. They interpreted their results as indicating that TCDD increases the dopaminergic activity of the tuberoinfundibular nucleus in the hypothalamus and the increased levels of dopamine decrease the release of prolactin by the adenohypophysis.


Keywords: Tissue levels; Occupational exposure; Environmental exposure; Dioxins; Human; Review article

This summary presents a review of methods and results for analysis of PCDD/Fs in human tissues.


Keywords: Tissue levels; Environmental exposure; Dioxins; Other contaminating compounds; Human

See page 129.

This abstract describes the use of cultured rat hepatoma cells as a bioassay for "dioxin-like" activity of complex mixtures of halogenated aromatic hydrocarbons. The degree of aryl hydrocarbon hydroxylase inhibition in these cells is a measure of TCDD equivalent activity.


The author provides a comprehensive, though brief, summary of the state of knowledge regarding human health risks from exposure to 2,3,7,8-TCDD and related PCDDs/PCDFs. This discussion was part of the proceedings of the 6th International Symposium on Chlorinated Dioxins and Related Compounds held in Fukuoka, Japan in September, 1986.


These authors had previously described characteristic alterations in hepatocyte morphology in rats and humans exposed to mixtures containing TCDD and related compounds but had not observed such an effect in cultured mouse hepatoma cells. In this paper, this latter finding was further confirmed by observing the hepatoma cells using scanning electron microscopy.


Keywords: Tissue levels; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Dioxins; Human; Abstract

PCDDs and PCDFs were measured in human adipose tissue and blood from American Vietnam veterans to determine if a constant ratio exists between levels in fat and blood. Analytical results were not provided.


Keywords: Tissue levels; Environmental exposure; Dioxins; Human; Abstract

Levels of PCDD/Fs in human milk samples from industrialized as compared to less industrialized nations were compared. Analytical results were not provided.

Schulze, G.E. 1988. 2,4-D-n-butyl ester (2,4-D ester) induced ataxia in rats: Role for n-butanol formation. Neurotoxicol. Teratol. 10:81-84

Keywords: Acute toxicity; Mechanism of action; Neurobehavioral effects; Injection; 2,4-D; Rat

See page 98.
Schulze, G.E. and Dougherty, J.A. 1988a. Neurobehavioral toxicity of 2,4-D-n-butyl ester (2,4-D ester): Tolerance and lack of cross-tolerance. Neurotoxicol. Teratol. 10:75-79

Keywords: Mechanism of action; Neurobehavioral effects; Subchronic toxicity; Injection; 2,4-D; Rat

See page 98.

Schulze, G.E. and Dougherty, J.A. 1988b. Neurobehavioral toxicity and tolerance to the herbicide 2,4-dichlorophenoxy-acetic acid-n-butyl ester (2,4-D ester). Fund. Appl. Toxicol. 10:413-424

Keywords: Absorption, distribution, metabolism, and excretion; Neurobehavioral effects; Subchronic toxicity; Injection; 2,4-D; Rat

See pages 98 and 113.


Keywords: Porphyria cutanea tarda; Mechanism of action; In vitro; Dioxins; Bird

The authors studied the induction of hepatic porphyria by polychlorinated biphenyls (PCBs) using chicken embryo hepatocytes in culture. Also included in this paper are the results of the analysis of urine from 20 Yu-Cheng victims (consumed PCB-contaminated cooking oil) for uroporphyrin and coproporphyrin two years after the incident. Three subjects had slightly elevated uroporphyrin levels.


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Dioxins; Commentary or opinion

This brief essay is an introduction to a series of articles on the mechanism of action of TCDD. It provides a general overview of the various theories regarding mechanisms of acute toxicity (8 references).

The author presents a critical review of the statistical procedures used to analyze human adipose 2,3,7,8-TCDD level data. The author concluded that the levels of 2,3,7,8-TCDD in human adipose tissue are lognormally distributed and positively correlated with age.


In this letter to the editor, the author responds to a concurrent letter by Crump (1988) that criticized an earlier publication. At issue is the selection of dose-response data and a mathematical model for low dose extrapolation in estimating the risks of cancer from exposure to TCDD.


This case-control study of soft-tissue sarcoma among employees of the Dow Chemical Company was described at the 6th International Symposium on Chlorinated Dioxins and Related Compounds held in Fukuoka, Japan in 1986. It was critically reviewed in Volume IX of this review (see Clement 1987) and was also published in the Journal of Occupational Medicine (see Sobel et al. 1986 in Volume XII of this review (Clement 1988).


See page 57.

In this letter to the editor, the author comments on an article about the health effects of Agent Orange in the Vietnam veterans that appeared in an earlier issue of this journal. This comment points out the lack of factual basis for many of the assumptions in the original article (see West and Leon 1986 in Volume XII of this review).


Keywords: Epidemiological study; Reproductive toxicity; Other skin effects; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 13, 19, 63 and 65.


Keywords: Epidemiological study; Reproductive toxicity; Birth defects; Environmental exposure; Dioxins; Human

See page 69.

Stohs, S.J. and Mohammadpour, H. 1988. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced lipid peroxidation in responsive and non-responsive mice. Toxicologist. 8:91

Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Mouse; Abstract

This abstract summarizes studies that were published in full in Mohammadpour et al. 1988.


Keywords: Porphyrin cutanea tarda; Dioxins; Human; Review article

The author briefly reviewed studies of human populations in which urinary porphyrin levels were measured as indicators of exposure to "porphyrinogenic" chemicals including TCDD and PBBs. These studies indicated changes in the pattern of porphyrin excretion but not in the amount (5 references).


Keywords: Chloracne; Epidemiological study; Absorption, distribution, metabolism, and excretion; Reproductive toxicity; Birth defects; Occupational exposure; Environmental exposure; Other contaminating compounds; Review article

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This paper summarizes studies of the human health effects of exposure to chlorinated aromatic hydrocarbons including PCDDs/PCDFs that were presented at the 6th International Symposium on Chlorinated Dioxins and Related Compounds held in Fukuoka, Japan in September, 1986.


Keywords: Cancer; Epidemiological study; Other toxic effects; Occupational exposure; Dioxins; Human; Abstract

See page 47.


Keywords: Cancer; Epidemiological study; Reproductive toxicity; Occupational exposure; Dioxins; Phenoxy herbicide formulations; Human; Review article

This Japanese language article is fourth in a series of five and reviews the results of epidemiologic studies of humans who were probably exposed to phenoxy herbicides and/or chlorinated dibenzo-p-dioxins and dibenzofurans (47 references).


Keywords: Tissue levels; Environmental exposure; Dioxins; Human; Abstract

The levels of PCDD/Fs in human milk samples across Europe were compared. The highest levels of TCDD equivalents were found in Belgium, Germany, the Netherlands, the United Kingdom and parts of South Vietnam. The lowest levels were found in Austria, Scandinavia and Yugoslavia.

This abstract summarized studies of the effect of TCDD on the gastric mucosa in rats. The gastric atrophy seen in pair-fed controls was not present in TCDD treated animals. The mechanism for this effect has not been determined.


This German language article describes a study in which adipose tissue samples from 19 patients at the University of Munich health research institute were analyzed for polychlorinated dibenzo-p-dioxins and dibenzofurans. Concentrations of dioxins increased with increasing degree of chlorination. Concentrations of total tetrachlorodibenzo-p-dioxins ranged from <1 to 18.2 pg/g.


Concentrations of PCDD/Fs in adipose and liver tissues from Munich residents reported the average concentration of TCDD was 8.0 ppt (2.6 - 18.0 ppt) in adipose and 16.4 ppt (1.0 - 88.9 ppt) in liver. The congener found at the highest concentration was OCDD, with average concentrations in adipose of 373.2 ppt and in liver of 4416.2 ppt.

Tomar, R.A. and Kerkvliet, N.I. 1988. Inhibition of anti-hapten antibody response in adoptive host reconstituted with T cells from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposed mice. Toxicologist. 8:149

See page 88.

Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat

The authors investigated the effect of DEHP, a hypolipidemic agent, on the acute toxicity of TCDD in rats. Pretreatment and cotreatment of rats with DEHP protected against some but not all of the fatty liver, hyperlipidemia and mortality induced by TCDD.


Keywords: Absorption, distribution, metabolism, and excretion; Hepatotoxicity; Mechanism of action; In vitro; Dioxins; Rat

TCDD was one of several chemicals whose uptake by an isolated perfused rat liver was studied. TCDD was efficiently taken up by the liver.


Keywords: Acute toxicity; Alterations in sex hormones; Mechanism of action; Dioxins; Review article

The authors reviewed the available information on the effect of TCDD on estrogen receptors and concluded that down-regulation of the estrogen receptor and the cascade of physiological responses to that down-regulation can explain many of the toxic responses caused by TCDD in experimental animals (72 references).


Keywords: Acute toxicity; Alterations in sex hormones; Lethality; Mechanism of action; Dioxins; Abstract; Commentary or opinion

This abstract summarizes information that was published in full in Umbreit and Gallo 1988a.

Keywords: Reproductive toxicity; Oral; Dioxins; Other contaminating compounds; 2,4,5-T; Mouse

See page .75.


Keywords: Acute toxicity; Enzyme induction or inhibition; Alterations in sex hormones; Reproductive toxicity; Mechanism of action; Oral; Dioxins; Mouse

The authors compared the effects of TCDD and estradiol on uterine weights in three strains of mice including Ah responsive and non-responsive strains. They found that TCDD inhibited the ability of estradiol to increase uterine weight in all these strains regardless of Ah responsiveness.


Keywords: Epidemiological study; Neurobehavioral effects; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 13, 15, 16, 92 and 93.


Keywords: Other skin effects; Cardiovascular toxicity; Respiratory toxicity; Neurobehavioral effects; Immuno-toxicity; Porphyria cutanea tarda; Hepatotoxicity; Reproductive toxicity; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 13, 15 and 16.


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Keywords: Reproductive toxicity; Birth defects;
Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 13, 15, 16 and 63.


Keywords: Epidemiological study; Tissue levels; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 14 and 120.


Keywords: Epidemiological study; Cardiovascular toxicity; Immunotoxicity; Neurobehavioral effects; Reproductive toxicity; Other skin effects; Occupational exposure; Phenoxy herbicide formulations; Human

See pages 13, 15, 16, 17, 63, 84, 92 and 93.


Keywords: Hepatotoxicity; Mechanism of action; Absorption, distribution, metabolism, and excretion; Oral; Dioxins; Other contaminating compounds; Mouse; Abstract

The distribution and retention of PCDD/Fs in mouse liver was studied in two different strains: C57BL (high affinity Ah receptor) and DBA/2 (low affinity Ah receptor). No analytical results were provided.


Keywords: Cancer; Epidemiological study; Immunotoxicity; Phenoxy herbicide formulations; Dioxins; Human; Commentary or opinion

Keywords: Acute toxicity; Enzyme induction or inhibition; Mechanism of action; Other toxic effects; Other route of exposure; Dioxins; Rat; Abstract

This abstract describes studies designed to determine the effects of TCDD on vitamin A levels in the liver and kidneys of neonatal rats and to compare those effects with effects on growth and AHH induction. No results or conclusions were included in this abstract.


Keywords: Acute toxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat; Abstract

This abstract describes studies designed to determine whether the Ah receptor is involved in the depletion of vitamin A by TCDD. No results or conclusions were included in this abstract.


Keywords: Acute toxicity; Hepatotoxicity; Mechanism of action; Oral; Dioxins; Rat.

The authors investigated the effect of three consecutive daily oral doses of TCDD on the concentrations of several metals in the livers of rats. The effects of TCDD on different metals were inconsistent. The significance of these results is not clear.

Keywords: Cancer; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat

See page 49.


Keywords: Acute toxicity; Cancer; Hepatotoxicity; Mechanism of action; Other toxic effects; Oral; Dioxins; Rat; Abstract

See page 49.


Keywords: Hepatotoxicity; Mechanism of action; Reproductive toxicity; Injection; Dioxins; Rat; Abstract

This abstract summarizes investigations of the effect of treatment of pregnant rats with TCDD on days 10–14 of gestation on the EGF receptor in fetal livers on day 20. TCDD decreased the number but not the affinity of EGF receptors. These functional alterations were not related to changes in kinase activity.


Keywords: Environmental exposure; Dioxins; Human; Review article

The authors reviewed the literature in order to define the clinical symptomatology resulting from acute intoxication by TCDD. They concluded that the long-term effects are uncertain (25 references).


Keywords: Acute toxicity; Lethality; Mechanism of action; Unspecified route of exposure; Dioxins; Rat; Abstract

This abstract summarizes studies of the effects of acute lethal doses of TCDD on gluconeogenesis in rats. TCDD markedly decreased the conversion of alanine to glucose, and corticosterone was partially protective against this effect.

Keywords: Absorption, distribution, metabolism, and excretion; Oral; Dioxins; Human; Abstract

The nature of the fecally excreted "TCDD equivalents" following "self-dosing" in humans was studied. 53% of recovered radioactivity was TCDD metabolites. No analytical results were presented.


Keywords: Neurobehavioral effects; Reproductive toxicity; Occupational exposure; Phenoxy herbicide formulations; Commentary or opinion

This is a reply to a letter to the editor commenting on an earlier article by these authors (see Stauber 1987).


Keywords: Epidemiological study; Reproductive toxicity; Birth defects; Environmental exposure; Phenoxy herbicide formulations; Human

See page 66.


Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

This study of cancer among licenced pesticide applicators in Sweden was published in abstract form in 1987 and was critically reviewed in Volume XI of this review (see Clement 1988).

A report of this study was available in 1986 and the study was critically reviewed in Volume IX of this review (see Clement 1987).

See page 36.


Keywords: Cancer; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human

See page 36.


Keywords: Tissue levels; Epidemiological study; Occupational exposure; Phenoxy herbicide formulations; Human; Abstract

Dioxin levels in the serum of Vietnam veteran personnel averaged 49.4 ppt as compared to 5.2 ppt in control samples. The authors concluded that this was a valuable tool to assess dioxin exposure in military personnel.

See page 121.

Wroblewski, V.J. and Olson, J.R. 1988. Effect of monooxygenase inducers and inhibitors on the hepatic metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat and hamster. Drug Metab. Dispos. 16:43-51

Keywords: Enzyme induction or inhibition; Absorption, distribution, metabolism, and excretion; In vitro; Dioxins; Rat; Hamster

The effect of different monooxygenase inducers and inhibitors of the hepatic metabolism of 2,3,7,8-TCDD by isolated rat and hamster hepatocytes was studied in an attempt to elucidate the difference between these two species with regard to sensitivity to 2,3,7,8-TCDD toxicity.
The authors concluded that 2,3,7,8-TCDD is metabolized by a form of cytochrome P-448 by both species, but differences in substrate specificity appear to exist between rat and hamster.


Keywords: Acute toxicity; Enzyme induction or inhibition; Hepatotoxicity; Mechanism of action; Injection; Dioxins; Rat; Hamster

The authors conducted a comparative study of the induction of AHH and other microsomal enzyme activities by TCDD and 3-MC in rats and hamsters. There were a number of differences in the manner in which enzyme activities responded to TCDD treatment in these species.

Yao, c. and Safe, S. 1988. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) antagonists protect against TCDD-mediated porphyria. Toxicologist. 8:65

Keywords: Hepatotoxicity; Porphyria cutanea tarda; Mechanism of action; Unspecified route of exposure; Dioxins; Mouse; Abstract

This abstract summarizes studies showing that Ah receptor antagonists block the alterations in hepatic porphyrin metabolism that are caused by TCDD in mice, providing additional evidence that the porphyrogenic effects of TCDD are mediated by the Ah receptor.