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

Volume I
Analysis of Literature

Department of
Medicine and Surgery

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

Volume I

This report was prepared under a cost reimbursement-type contract awarded on a competitive bid basis on December 15, 1980. The contract will run for nine and one-half months and is funded at $111,743.

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FOREWARD

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. The need to conduct such a review 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.

This report was prepared wholly and exclusively by JRB Associates, Inc., and represents an independent assessment of the current state of science relating to the herbicides used in conjunction with the Vietnam conflict. The publication of this document does not signify that the contents necessarily reflect the views and policies of the Veterans Administration.

PREFACE

The controversy surrounding the tactical use of herbicides in Southeast Asia during the Vietnam conflict has now extended into its third decade. Few environmental or occupational health issues have received the sustained national attention that has been focused on "Agent Orange". The controversy centered first on the actual employment and subsequent ecological effects of herbicides in South Vietnam, then on the question of the safe disposal of surplus herbicide following the conflict, and lastly, on whether herbicides were responsible for health problems reported among Vietnam veterans. As each facet of the controversy came to the attention of the public, more government agencies were tasked to deal with the associated issues. Hence, today a great many branches and agencies of the Federal government are involved in seeking resolutions of the scientific, medical, legal and social problems surrounding "Agent Orange." In addition, many state governments have enacted legislation relating to this highly controversial issue.

The basis for resolving the Agent Orange controversy must in large measure stem from the results of scientific inquiry. Appropriately, the preparation of extensive reviews and summaries of the scientific literature have appeared in the past, e.g., Midwest Research Institute Report (1967), National Academy of Science Report (1974) and United States Air Force Technical Report (1978). The present report therefore has benefited from the information developed in previous reports as well as from recently published scientific data. The volume of available scientific literature pertaining to the herbicides used in Southeast Asia has increased almost geometrically since the 1967 report. Especially important has been the increase in the scientific data on the toxic contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The present report is published in two volumes. Volume I presents a detailed scientific assessment of the literature, and Volume II contains an annotated bibliography of the relevant literature.

Although much is known about the toxicity of the herbicides used in Vietnam, a number of gaps in the scientific knowledge are still present. It is hoped that this two-volume report may serve to focus the continuing public dialogue and future scientific inquiry on those aspects of the problem in which substantial doubt or gaps in information remain. This report should also assist researchers in identifying opportunities for the systematic development of new knowledge based on what is now known and accepted as fact.

VETERANS ADMINISTRATION
October 1981

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CHAPTER 1

INTRODUCTION

The potential long-term health consequences of exposure to herbicides used during the Vietnam Conflict has been an issue of increasing public concern for the past decade. Among the environmental and occupational health and safety issues raised during the 1960s and 1970s, few have received the sustained and growing national attention that has been focused on "Agent Orange."

The concern over U.S. military personnel exposures to herbicides in Vietnam has attained national significance for a variety of reasons. The problem was unexpected: the herbicides selected for use as defoliants in Vietnam had been widely used within the U.S. for many years, were considered only moderately toxic, and were believed to be safe and effective when handled with care. The presence of a highly toxic trace contaminant in one of the compounds was not recognized until the late 1960s, when the defoliation program was well established in Vietnam. The number of persons potentially exposed is almost unprecedented: an estimated 2.4-2.8 million U.S. military personnel served in Vietnam. The number and range of health effects attributed to exposure are great: from chloracne, to headaches, excessive tiredness, sleeplessness, and increased susceptibility to colds and flus, to birth defects in offspring, and cancer. The problem does not admit to a ready solution: in almost all cases, the magnitude and duration of individual exposures to herbicides in Vietnam cannot be determined, usually cannot even be estimated with accuracy, and may be confounded by the wide-spread use of the same herbicides within the U.S.

The search for answers to this dilemma has itself been complicated by the enormous public concern. The scientific literature varies widely in detail, quality, and findings. Researchers' suggestions and conclusions have been seized and debated, sometimes without sufficient attention to the data underlying those statements, the methods of collection used to obtain the data, and the approaches to analysis used to interpret them. All of these elements of scientific inquiry have limitations. "Good" research is necessarily careful research, and careful research is most often conservative of statement. Only recently have researchers addressing the problem of herbicide exposures in Vietnam had sufficient time since the recognition of the problem to begin to benefit from the opportunities for knowledge-development provided by lines of inquiry systematically pursued.

1.1 BACKGROUND OF THIS REPORT

Fifteen different herbicides were shipped to and used in Vietnam between January 1962 and September 1971. Eight of these formulations contained the phenoxy herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D)

or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), or both. The fifteen herbicides used in Vietnam included:

- PURPLE: a formulation of 2,4-D and 2,4,5-T used between January 9, 1962, and late 1964
- PINK: containing 2,4,5-T and used between 1962 and 1964
- GREEN: containing 2,4,5-T and used between 1962 and 1964
- ORANGE: a formulation of 2,4-D and 2,4,5-T used between January 1965 and April 1970
- WHITE: a formulation of picloram and 2,4-D
- BLUE: containing cacodylic acid
- ORANGE II: a formulation of 2,4-D and 2,4,5-T used in 1968 and 1969
- DINOXOL: a formulation of 2,4-D and 2,4,5-T; small quantities were tested in Vietnam between 1962 and 1964
- TRINOXOL: containing 2,4,5-T; small quantities were tested in Vietnam between 1962 and 1964
- DIQUAT: small quantities tested in Vietnam between 1962 and 1964
- BROMACIL: small quantities tested in Vietnam between 1962 and 1964
- TANDEX: small quantities tested in Vietnam between 1962 and 1964
- MONURON: small quantities tested in Vietnam between 1962 and 1964
- DIURON: small quantities tested in Vietnam between 1962 and 1964
- DALAPON: small quantities tested in Vietnam between 1962 and 1964.

Over 80 percent of the herbicides sprayed in Vietnam was "Agent Orange." After 1965, Herbicide Orange was used almost exclusively.

Those phenoxy herbicides containing 2,4,5-T also contained trace contaminants produced in the manufacturing process, including:

- 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD)
- 2,7-dichlorodibenzodioxin
- Penta-, hexa-, hepta-, and octachlorodibenzodioxins.

Phenoxy herbicides generally contain other dioxin contaminants in proportions of between 2-to-1 and 10-to-1 of the quantities of TCDD contained. The levels

of contamination of these dioxins in the phenoxy herbicides used in Vietnam is unknown. The U.S. Air Force (1979) estimates the maximum TCDD concentration in 2,4,5-T produced during the period was 15-47 parts per million (ppm); the Air Force estimates the mean concentration was 1.91 ppm.

It is the presence of the dioxin contaminant, TCDD, in 2,4,5-T that has been the focus of much of the debate about the health consequences of exposure to herbicides used in Vietnam. Although other dioxin contaminants were probably present in the phenoxy herbicides used, no other dioxin has been shown to be as toxic as 2,3,7,8-tetrachlorodibenzo-para-dioxin.

In January of 1980, in response to the national concern over exposures to herbicides in Vietnam, the U.S. Congress included provisions in Public Law 96-151 mandating that the Veterans Administration conduct a critical review of the worldwide literature on the phenoxy herbicides. In December of 1980, again in response to the national concern, the Veterans Administration expanded that mandate and initiated a critical review of the worldwide literature on all the herbicides used in Vietnam, including the phenoxy herbicides and their dioxin contaminant.

This volume and the accompanying annotated bibliography constitute the final report of that critical review.

For those who want to go beyond this report, several very good general reviews and references have been published, including: NRC, 1974; IARC, 1977; Joint IARC/NIEHS Working Group, 1978; National Research Council of Canada, 1978; Young et al., 1978; Bovey and Young, 1980; and Esposito et al., 1980.

1.2 METHODS

The methodology used in compiling this critical review of the literature included five principal activities:

- Literature identification
- Literature retrieval and control
- Document indexing and abstracting
- Literature analysis
- Report preparation.

Initially, three assumptions guided the development of the effort:

- The Congress was primarily interested in the health consequences of exposure to the herbicides used in Vietnam (i.e., the substances).
- The Congress was primarily concerned with health effects among otherwise healthy adult men (i.e., the population).

- The Congress was seeking information and scientific judgment on the state of knowledge concerning the likelihood and severity of health effects resulting from exposure to the herbicides used in Vietnam (i.e., the outcomes).

These assumptions led to the development of a set of relevancy criteria which guided the culling of the literature. The activity used both automated and manual search techniques, including tree-searches of cited references. It was facilitated by the willing cooperation of a number of involved Federal agencies, including the Department of Agriculture, the Environmental Protection Agency, the Food and Drug Administration, the Center for Disease Control, and the Department of Defense.

Using an expanding and reiterative search strategy and the relevancy criteria developed, over 1,400 documents were acquired and examined. About 1,200 were judged to fall within the relevant parameters, were thoroughly reviewed, and are included in the accompanying bibliography. Over 900 of these documents were annotated in detail by staff scientists; relevant abstracts, summaries, review articles, and editorials were included in the bibliography, but were not annotated.

This body of culled literature was used by staff scientists in the compilation of this report. Particular attention was payed to the quality of the data presented and the methods used in its collection and analysis. Attention was also directed toward conflicting findings and gaps in available information. The draft report, exclusive of final conclusions, was reviewed by a number of senior scientists and expert consultants.

This report was developed exclusively by the contractor, JRB Associates, without consultation, advice, or direction from the Veterans Administration. The conclusions, opinions, judgments, and recommendations presented are those of the contractor, and may not reflect the opinions, positions, or policies of the Veterans Administration.

The goal of this report is to present a balanced and critical review of the current state of published scientific knowledge relevant to the problem of exposures to herbicides, and particularly phenoxy herbicides, in Vietnam. This report cannot solve such a complex problem. It may, however, serve to focus and direct future discussion to those aspects of the problem in which substantial doubt or lack of information remains, and thereby contribute to the systematic pursuit of promising opportunities for knowledge-development.

1.3 LIMITATIONS

As this report devotes considerable attention to the limitations inherent in the work of others, it is important that one attempt to recognize the report's own inherent limitations.

First, no search of the literature in so volatile a subject area as this can in fact be complete. New research continues to be published, the

automated data bases used to search the literature are often as much as a year behind the journals they abstract, and any automated search is necessarily limited by the intelligence that originally abstracted and keyed the journals' contents. The contents of the accompanying bibliography have been brought as much up to date as possible by manual searches of recent issues of key journals and the advice and input of expert readers. It is inevitable that some published and many unpublished reports are not included here. It is hoped that few of these contain critically relevant or important, supported findings not duplicated elsewhere.

Second, all research efforts must yield to some limitations of time and resources. In this case, both the time (nine-and-a-half months) and the resource limitations were real concerns. There are documents that, although identified and considered relevant, could not be obtained within the time available. These are relatively few. There are documents that might have proven relevant if examined, but that were judged beyond the criteria of relevance when identified and so were not acquired. There are documents that, almost certainly relevant, simply were uncovered too late to be included in the schedule. Several reports recently declassified by the U.S. Air Force fall into this category. Ultimately, it is again hoped that few of these omissions contain new, important, supported findings not duplicated elsewhere.

Third, realities of time, resources, and practicality served to limit the scope of the literature search in very specific terms, as well:

- Substances similar in structure-activity relationships to the herbicides used in Vietnam were not considered. These substances include Silvex, paraquat, and dioxins other than 2,3,7,8-TCDD.

- The fate and effects of the metabolites of the Vietnam herbicides were not considered. For example, the fate and toxicological effects of cacodylic acid (Herbicide Blue) are described, but not the fate and toxicological effects of inorganic arsenic administered in the absence of cacodylic acid.

A final element critical to the veterans and public concern over exposures to herbicides in Vietnam was judged beyond the scope of the present study: the estimation of exposure potential inherent in the Vietnam experience.

Approximately 2.6 million U.S. military personnel served in South Vietnam between January 1, 1965, and March 31, 1973, the period of heaviest herbicide use (GAO, 1979a). The personnel that appear to have had the greatest risk of exposure were the people who took part in the actual spraying of the herbicides. This would include the flight mechanics of RANCH HAND aircraft who generally operated the spraying units; persons operating spraying units on helicoptors, trucks, and boats; and persons using back-pack sprayers. Although most of the herbicide spraying was done aerially, and up to 87 percent by RANCH HAND fixed-wing aircraft, up to 4 percent may have been applied in ground operations (Craig, 1975). Although it is apparent that exposures did

occur among spray operators and RANCH HAND aircrews (Young et al., 1978; GAO, 1979b), little can be said about the quantity and quality of such exposures.

Many variables alter the rate of absorption of 2,4,5-T by workers. Some of these factors, including type of occupation, rate of spraying, type of protective clothing, and rates of absorption by both dermal and inhalation routes, were considered in developing a model for estimating potential dosages of 2,4,5-T absorbed by workers (RPAR Assessment Team, 1979). The Assessment Team used several different values for each parameter, based on assumptions regarding the conditions of exposure. They then performed exposure assessments for occupational situations. However, Leng (1978) has challenged some of the assumptions used by the RPAR Assessment Team to calculate their exposure assessment, including the extent of skin exposure and dermal absorption rates. Nisbet (1980) has also presented estimates of human exposures in the general population. Since the assumptions used for these exposure assessments apply to occupational use of herbicides, but not military use, the results are not necessarily related to assessments of potential exposure in Vietnam.

The General Accounting Office (1979b) further attempted to estimate troop deployment in spray areas, and aborted missions and dumped herbicide cargos have also been reported. Once again, it is apparent that ground troop exposures occurred in Vietnam, but it is beyond the scope of this report to attempt to assess the magnitude and duration of such exposures; this work must be carried out by others.

1.4 CONCLUSIONS AND GAPS IN CURRENT KNOWLEDGE

This section presents summary statements of the conclusions supported by the available literature and gaps in current knowledge identified during the literature review. These summary statements are arranged by topic areas addressed in subsequent chapters of this report:

- Metabolism
- Human exposure to TCDD
- Acute toxicity
- Subacute and chronic toxicities
- Reproductive toxicity
- Mutagenicity
- Carcinogenicity.

1.4.1 Metabolism (Biodynamics and Biotransformation)

Conclusions

- Pharmacokinetics of 2,4-D and 2,4,5-T in humans have been described.

- Both 2,4-D and 2,4,5-T are cleared rapidly from the blood after they are absorbed, with half-lives for plasma clearance in humans of 12-23 hours.

- Both compounds are excreted by the kidney primarily as the unmetabolized compounds.

- Renal clearance rates for phenoxy acids in animals decrease at high doses that cause nephrotoxicity and saturate the renal transport system.

- The clearance rate of 2,4-D in humans decreases when the urinary pH is low.

- Neither 2,4-D nor 2,4,5-T has been shown to accumulate in animal fat.

- Both compounds reach fetal tissues after they are administered to pregnant animals.

- TCDD is cleared slowly, with half-lives for body clearance of 2-3 weeks in animals.

- TCDD undergoes biotransformation and the metabolites are rapidly excreted in bile.

- TCDD is retained in the liver of the rat, a species that shows an hepatotoxic response to TCDD, to a far greater extent than in the livers of two other species which do not show liver lesions after TCDD administration.

- Diquat is absorbed by the lung, but is not retained in the lung and is rapidly cleared by animals.

- Free radical formation does not appear to be diquat's mechanism of toxicity under conditions of normal oxygen tension.

- Diuron and bromacil undergo biotransformation prior to excretion; diuron is excreted by the kidney.

TCDD Enzyme Induction and Receptor Binding

- TCDD is a potent inducer of various microsomal enzymes; the induced enzymes show elevated levels over a long period of time.

- In certain strains of mice, TCDD binds to a cytosol receptor, the gene product of the Ah locus.

Gaps in Information

Information on the following topics is incomplete or missing in the literature reviewed for this analysis:

- Patterns of biotransformation, distribution, and excretion of TCDD in humans
- The chemical structures of TCDD metabolites in bile
- Differences in distribution and biotransformation of TCDD for a wide range of species
- Differences in TCDD-receptor binding capacity and extent of enzyme induction in a wide range of species
- Pathways for the biotransformation of cacodylic acid and the relative importance of each pathway
- Biodynamics, including pathways and rates of elimination in humans or animals for: bromacil, picloram, dalapon, monuron, tandex.

1.4.2 Incidents of Human Exposure to TCDD

Conclusions

- Chloracne is the most consistently reported health effect of TCDD exposure in humans; in severe cases, chloracne has lasted for 28 years; milder cases have gone undetected or have disappeared in less than a year.
- Neurasthenia, a series of subjective complaints including irritability, fatigue, and insomnia, has been reported after many industrial accidents and exposures; in 1 instance these complaints occurred in the absence of chloracne; a 2-year latency period between TCDD exposure and the onset of neurasthenia has been reported.
- Other neurological disorders (as peripheral neuritis) and hepatic disorders (as hepatomegaly) have been reported after several of the incidents.
- The earlier accidents and exposures were associated with a wider variety of symptoms and more severe symptoms than the later incidents.
- Porphyria cutanea tarda and gastrointestinal problems have not been commonly reported and seem to be associated with long-term exposure.
- An increased risk among exposed people has not been established in mortality studies; increases in any particular cause of death has not been observed for more than 1 study group, so far.

- No data have been systematically collected for a clearly defined study group from Vietnam; health effects are usually claimed by individuals, without documentation by health professionals; exposure to herbicides in Vietnam (and potentially TCDD) is presumed in these studies and exposure levels are unknown; these data have not been compared to any control groups, in general; and symptoms reported often have been nonspecific and may be associated with other factors present in combat situations.

Gaps in Information

The following information is missing from most accounts of human exposure to TCDD.

- The number of exposed people who were not affected
- Health status of exposed workers that did not develop chloracne
- Incidences of conditions other than chloracne and comparison of these data with data from control groups
- Standardization of methods of evaluating symptoms of neurasthenia for purposes of comparison among different studies
- Conditions that could be detected by the examination methods used, but which did not occur (especially for conditions reported in other incidents)
- Sufficient mortality data for analysis (due to the short period of time that has lapsed since some of the incidents occurred and the relatively small number of workers exposed)
- Exposure levels
- Human health effects from use of defoliants in Vietnam have not been systematically documented.

1.4.3 Acute Toxicity

Conclusions

- For both 2,4-D and 2,4,5-T, the single oral dose lethal to 50 percent of exposed animals (the oral LD_{50}) is between 350-800 mg, based on published data, almost all of which was published 20-30 years ago.
- The cause of death from lethal doses of 2,4-D or 2,4,5-T to animals is unknown; both compounds produce several non-specific effects, such as mild weight loss.
- 2,4-D produces neurotoxicity in humans and animals.

- The LD_{50} values for TCDD are extremely low (between 1-300 ug/kg) and vary widely among different species.

- A long latency period, of about 3 weeks, occurs between TCDD administration to test animals and death, and the cause of death is usually not known.

- The in vivo characteristics of TCDD intoxication suggest toxicity on a cellular level, although TCDD toxicity has not been demonstrated in cultured cells.

- Thymic atrophy (without a corresponding loss in immune function) and severe weight loss have been observed in many species after TCDD exposure.

- Weight loss does not result from decreased food consumption, disturbances in absorption of nutrients from the gastrointestinal tract, or a stress reaction mediated by endocrine glands.

- TCDD produces hepatotoxicity only in some species.

- The oral LD_{50}s for monuron and diuron in animals are about 1,000 mg/kg; both produce neurotoxicity; death usually occurs 1 day after exposure, from respiratory or cardiac failure.

- The oral LD_{50}s for picloram and dalapon in animals are between 2,000-8,000 mg/kg; death occurs within hours of a lethal dose of dalapon.

- The oral LD_{50} for diquat in animals is between 30 and 200 mg/kg; doses in this range produce severe gastrointestinal lesions and death within 2 weeks; doses 4-5 times higher produce neurotoxicity and death within several hours.

- Values ranging from 200 to 3,000 mg/kg have been reported for the oral LD_{50} for cacodylic acid in rodents.

Gaps in Information

The following information has not been reported or is not adequate in published literature:

- Effects of acute exposure to 2,4,5-T in humans

- LD_{50} values for 2,4,5-T samples with less than 0.1 ppm TCDD

- Verification of the LD_{50} values for 2,4-D that were published 20-30 years ago

- LD_{50} values for cacodylic acid, published in a refereed journal, with descriptions of details on sample purity, methods used, and patterns of toxicity that could be compared to those of inorganic arsenic poisoning

- The causes of death and target organs for picloram and dalapon

- Information on the acute toxicity of tandex.

1.4.4 Subacute and Chronic Toxicities

Conclusions

- 2,4-D and 2,4,5-T are not cumulative toxicants.

- Subacute toxicity of both compounds resemble their acute toxicities, except that subacute doses of 2,4-D do not produce myotonia, but cause bleeding of the gums in dogs.

- TCDD is a limited cumulative toxicant; cumulative effects of doses administered within a month of each other have been observed, but not for doses administered beyond about one month.

- The subacute effects of TCDD that are not observed after acute doses are porphyria and depletion of blood cells; iron deficiency protects TCDD-treated animals from the porphyrinogenic effects.

- Chronic doses of diquat cause cataracts in two species tested (dog and rat).

Gaps in Information

- The subacute effects of cacodylic acid, monuron, diuron, bromacil, and tandex have not been described thoroughly or at all.

1.4.5 Reproductive Toxicity

Conclusions

- No human reproductive effects have been verified to date from male or female exposure to 2,4-D, 2,4,5-T, or TCDD.

- In the two experiments that involved exposure of males only to phenoxy acids prior to conception, no evidence of reproductive effects was observed; (combinatinons of 2,4-D, 2,4,5-T, and TCDD were administered in one study and of 2,4,5-T with an unknown level of TCDD contamination in another study).

- After 2,4-D is administered to pregnant animals, decreased fetal growth rates have occurred.

- After 2,4,5-T (with less than 0.1 ppm TCDD) is administered to pregnant animals, decreased fetal growth rates have occurred and at higher doses in mice, cleft palate is produced; these effects are observed in the absence of maternal toxicity, this teratogenic effect of 2,4,5-T has not been observed in the rat, hamster, monkey, or rabbit.

- After TCDD is administered to pregnant mice, cleft palate and renal abnormalities is fetuses have occurred.

- Synergistic effects may occur in mice when the level of TCDD added to 2,4,5-T exceeds 5 ppm; this effect pertains to the incidence of cleft palate.

- Diquat, dalapon, and diuron produce adverse effects on development only when they are administered at doses that cause maternal toxicity.

- Bromacil and picloram have not produced effects on development at any doses tested.

Gaps in Information

The following types of studies have not been conducted and published to date:

- The effects of human male exposure during a limited time prior to conception on reproductive outcome of the resultant pregnancy, for documented exposure to 2,4-D, 2,4,5-T, and/or TCDD

- The effect of exposure of males of mammalian animal species to any single herbicide or dioxin, alone, on reproductive performance.

1.4.6 Mutagenicity

Conclusions

- 2,4-D and 2,4,5-T produce weak mutagenic effects.

- TCDD has shown mutagenic effects in bacteria and yeast systems, which have not been confirmed yet in mammalian in vivo tests.

- Cacodylic acid, bromacil, and monuron have not produced mutagenic effects in in vitro tests.

- Diquat and diuron have produced mutagenic effects in vitro, which have not been confirmed yet in vivo.

Gaps in Information

The following gaps in information remain:

- The in vivo mammalian mutagenic effects of TCDD, diquat, and diuron
- The mutagenic potential of dalapon, picloram, and tandex in any system.

1.4.7 Carcinogenicity

Conclusions

- Evidence from human studies suggest that exposure to phenoxy acids, with concomitant exposure to many other pesticides and to TCDD, may lead to an increased risk of soft-tissue sarcoma; the etiologic role specifically of phenoxy acids has not been elucidated.
- Mortality studies of groups of human workers exposed to TCDD has not revealed an increased carcinogenic risk in these people, although the numbers of deaths in these groups have been exceedingly small to date.
- Animal studies have not produced any evidence that 2,4-D, 2,4,5-T, cacodylic acid or picloram are carcinogenic.
- TCDD appears to act secondarily or indirectly in enhancing the carcinogenicity of other components (usually unidentified) in animal studies.
- Carcinogenic effects of monuron have been observed in animals; further studies of the carcinogenicity of this compound are being conducted.

Gaps in Information

Information on the carcinogenic potential of diquat, diuron, dalapon, bromacil, picloram, and tandex and on only 2,4-D, 2,4,5-T, or TCDD, without concomitant exposure to trichlorphenol or other herbicides in humans is missing.

1.5 RECOMMENDATIONS

This section presents recommendations for further study drawn from the review of the literature addressed in this report.

- Dalapon and bromacil are compounds that were used in small amounts in Vietnam and have not been shown to pose a significant risk; no further studies are recommended on these compounds.

- Picloram also has a low order of toxicity. The carcinogenic potential of monuron is currently under investigation. Monuron was not used extensively in Vietnam and, other than the carcinogenic potential, has a low order of toxicity. No additional studies are recommended for these compounds.

- Diquat has a moderate toxicity and has been well studied. The only study recommended on this compound is in vivo mammalian mutagenicity testing, in light of positive effects observed in in vitro tests. This compound does not produce effects that would be likely to place humans at high risk after exposure.

- The information on cacodylic acid is conflicting and not adequately documented. Its toxicity and metabolism in relationship to the extent of biotransformation to inorganic arsenic after absorption and the toxicological impact of this metabolism should be investigated.

- No information on the toxicology of tandex was found. Low usage of this compound in Vietnam, however, does not make it a likely target of concern.

- The effects of 2,4-D, 2,4,5-T, and TCDD administered in combination have generally not been compared to the individual effects to determine whether the combination produces additive, potentiating, or synergistic effects; an exception is the effect of cleft palate in mice by 2,4,5-T, which was potentiated by doses of TCDD. The effects of combined doses should be investigated.

The major concern of veterans in Vietnam that has not been adequately addressed in published literature to date is the potential for human exposure to TCDD to produce the same health effects with the same potency as those observed in animal studies. The wide variation of responses to TCDD among different species and a lack of understanding of the mechanisms of its toxicity and metabolism have led to this situation. The remaining recommendations address this issue.

- Procedures for evaluating both exposure levels and health effects from occupational exposures and accidents should be established by an international agency. These procedures should be available before another incident occurs, so the most useful types of information can be collected on a timely basis and the same type of data could be obtained from different accidents for purposes of comparison.

  Any protocol should consider the items listed above as Gaps in Information in previous accounts; information on cholesterol levels and other parameters discussed in other recommendatons should be studied.

- The relative importance of the rates and pathways of biotransformation and tissue distribution in various species should be addressed. Studies should be initiated to:

  - Identify the biliary metabolites of TCDD

  - Compare in various species the pathways of TCDD metabolism (based on the types of metabolites formed) and the rates of metabolism with TCDD toxicity in that species, as was done by Gasiewicz and Neal (1979) for the hamster

  - Determine the relative importance of the proportion of TCDD distributed to specific tissues with the toxicity in that tissue. (If disproportionate distribution to specific human tissues occurs, this should become apparent as TCDD levels in autopsy samples become available).

- The potential for the inductive effects of TCDD to alter lipid metabolism and cause depletion of fat stores has not been adequately considered. TCDD produces a long-term elevation of serum cholesterol (in animals and humans), a long-lasting induction of certain enzymes, and a long latency period after exposure and before death occurs, during which time animals become emaciated. The possibility that enzymes that degrade lipid stores are induced and no longer respond to regulatory mechanisms should be investigated.

- The biochemical events that precede chlorance have not been adequately considered and may in time lead to the development of useful therapy.

- Humans have been proposed to be less sensitive than animals to the toxic effects of TCDD (Crow, 1980). Recent experiments by Poland and Glover (1980) demonstrated that the presence of (1) cytosol receptors for TCDD, (2) sensitivity to enzyme induction by TCDD, and (3) sensitivity to the toxic effects of TCDD, including cleft palate and thymic atrophy, all segregated together in certain strains of mice and were all absent in others. If this approach were extended to different animals species and these parameters were shown to correlate in different species, a basis for extrapolating the inductive potential and receptor-binding capacity (which potentially could be measured in vitro in human tissue) to the likelihood of toxic effects in humans may be able to be established.

  By understanding the mechanisms of TCDD toxicity, the degree of correlation of receptor binding, enzyme induction and toxicity, and the role of metabolism in altering toxicity in animals, extrapolations of these parameters to man may become feasible.

CHAPTER 1.

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CHAPTER 2.

THE MILITARY USE AND APPLICATION OF HERBICIDES IN VIETNAM

This chapter will deal with the history of tactical herbicide use in Vietnam. The herbicides used and their various components are described, along with the distribution of the compound for defoliant or crop destruction purposes. Types of herbicidal missions, methods of application, and potential human exposures are described as well.

2.1 SUMMARY OF TACTICAL USE

In 1961, the military conducted tests in Vietnam on the feasibility of using commercially available herbicides for defoliation and crop destruction. Further tests were conducted in Vietnam from 1962 to 1968, in Thailand in 1964 and 1965, and at Eglin AFB in Florida from 1962 to 1970, on the effectiveness of various herbicide mixtures and for methods of herbicidal application (Young et al., 1978).

The three major military objectives of herbicide use in Vietnam were (Young et al., 1978; NRC, 1974):

- Defoliation for offensive purposes - removal of trees and vegetation from enemy controlled or heavily infiltrated areas, caches, supply routes, and communication lines to increase visibility prior to reconnaissance, air strikes, or ground operations
- Crop destruction - removal of food sources from enemy controlled or heavily infiltrated areas in order to hinder the establishment of permanent bases and large scale military offensives by the enemy
- Defoliation for defensive purposes - removal of trees and vegetation along major water transportation routes, highways, and communication lines, and around the perimeters of military bases, supply depots, and landing zones, to prevent cover for ambush.

Herbicides were sprayed in Vietnam for defoliation and crop destruction from 1962 to 1971 in a military operation named RANCH HAND. The total area sprayed between 1962 and 1965 was small, accounting for less than 7 percent of the total acreage sprayed during the Vietnam conflict. Rapid yearly increases in the annual number of acres sprayed occurred from 1962 to 1967. The annual number of acres sprayed reached a maximum in 1967, leveled off slightly in 1968 and 1969, and declined rapidly in 1970 prior to the termination of spraying in 1971. During this time more than 20 million gallons of herbicides were sprayed over 6 million acres, some of which were sprayed more than once. More than 3.5 million acres of South Vietnam--approximately 8.5 percent of the country--were sprayed one or more times. Herbicide was applied at about 1.0 to 1.5 gallons per acre from January 1962 to July 1964. After July 1964 until

the termination of spraying in 1971, herbicide agents were generally applied at approximately 3 gallons per acre (NRC, 1974).

The major herbicides sprayed in Vietnam were assigned code names corresponding to the color of identification bands painted on the storage drums. During the initial stages of light herbicide use in Vietnam, from 1962 through 1964, the most commonly used herbicides were Purple and Pink. Orange, White, and Blue, which were introduced into the Vietnam conflict after 1964, rapidly replaced the use of Purple and Pink, and became the most widely used herbicides in Vietnam (Young et al., 1978).

Heavily sprayed areas included inland forests near the demarcation zone; inland forests at the junction of the borders of Cambodia, Laos, and South Vietnam; inland forests north and northwest of Saigon; mangrove forests on the southernmost peninsula of Vietnam; and mangrove forests along major shipping channels southeast of Saigon. Crop destruction missions were concentrated in northern and eastern central areas of South Vietnam (NRC, 1974).

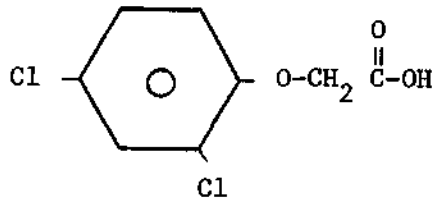
Most of the herbicides used in Vietnam were sprayed by fixed-wing aircraft, although a substantial number of missions were also carried out by helicopter, particularly after mid-1970. Only small amounts of herbicides were sprayed by ground sources such as river boats, trucks, and personnel wearing back-pack sprayers (NRC, 1974).

The aerial spraying of herbicides rapidly declined in 1970 after reports were released concerning the possible teratogenicity of 2,4,5-trichlorophenoxyacetic acid 2,4,5-T, a component of Orange. Some of the reported teratogenicity of 2,4,5-T was later attributed to 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD), a common contaminant of 2,4,5-T and its derivatives. The last spraying of herbicide by airplane in Vietnam occurred in January of 1971. After that, sprayings were done primarily for defensive defoliation purposes and were carried out by helicopter or on the ground; these operations also were terminated by the end of 1971. Military stockpiles of Orange were incinerated on shipboard in the Pacific Ocean near Johnston Island in 1977 (Young et al., 1978). Further details on the herbicides used in Vietnam and the quantitative, temporal, and geographical distribution of the herbicides are presented in subsequent sections of this chapter.

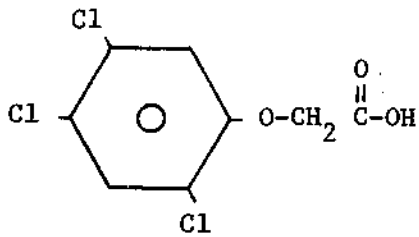
2.2 DESCRIPTION OF HERBICIDES USED IN VIETNAM

The herbicides most widely used in Vietnam, in terms of gallons sprayed and acres covered, were Orange, White, and Blue. Other color-coded herbicides used in Vietnam included Purple, Pink, Orange II, and Green (Young et al., 1978).

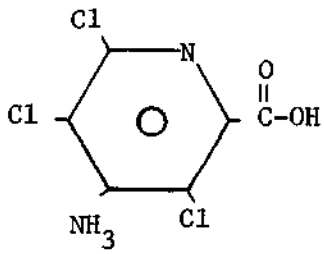
The major non-solvent chemical components of the color-coded herbicides were mixtures of esters or salts of herbicides widely used in agriculture and forestry in the U.S., such as 2,4-D, 2,4,5-T, picloram, and cacodylic acid. The structures of these herbicides are given in figure 2-1. The structures of the most important esters and salts of 2,4-D, 2,4,5-T, picloram, and cacodylic acid used in Vietnam are given in figure 2-2. The structure of TCDD, a toxic contaminant of 2,4,5-T and its derivatives is given in figure 2-3 (Young et al., 1978).



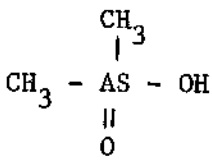
2,4-Dichlorophenoxyacetic Acid (2,4-D)



2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T)

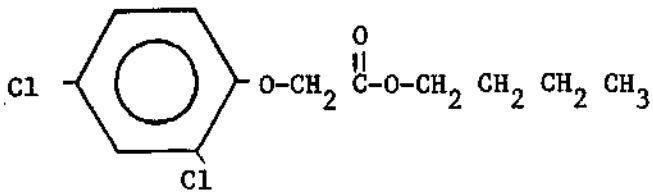


4-Amino-3,5,6-Trichloropicolinic Acid
(Picloram)

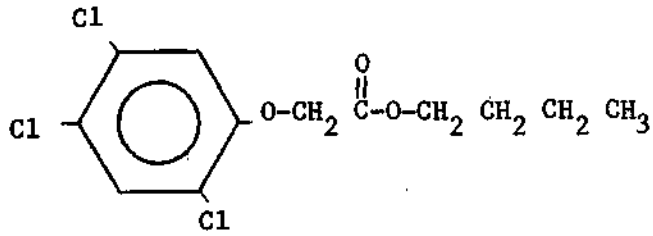


Hydroxydimethylarsine Oxide
(Cacodylic Acid)

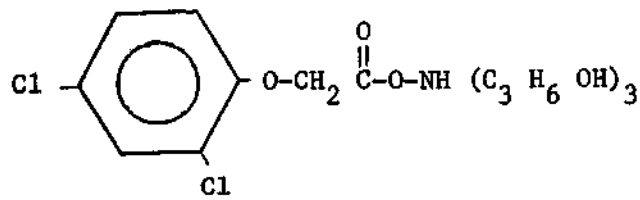
FIGURE 2-1. CHEMICAL STRUCTURES OF PARENT HERBICIDES



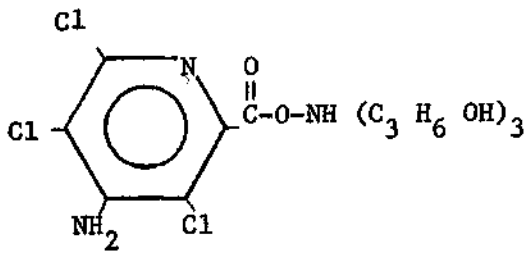
n-Butyl Ester of 2,4-D
(Orange Component
Orange II Component
Purple Component)



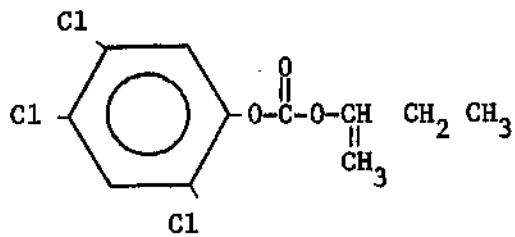
n-Butyl Ester of 2,4,5-T
(Orange Component
Purple Component
Pink Component)



Triisopropanolamine Salt of2,4-D
(White Component)

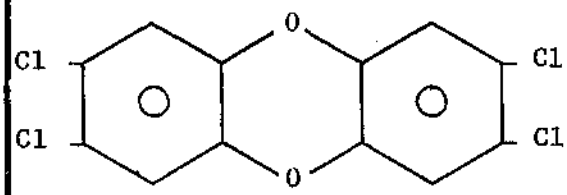


Triisopropanolamine Salt of
Picloram
(White Component)



Isobutyl Ester of 2,4,5-T
(Purple Component
Pink Component)

Figure 2-2: Chemical Structures of Herbicide Esters and Salts, Used in Vietnam



2,3,7,8-Tetrachlorodibenzo-para-Dioxin (TCDD)

Figure 2-3: Chemical Structure of 2,3,7,8-Tetrachlorodibenzo-para-Dioxin (TCDD)

The physical properties (e.g., solubility, volatility) of the esters and salts made them generally better suited than the parent herbicide acids for storage, application, or forest canopy penetration. However, the effectiveness of the esters and salts indicated that they either maintained most of the herbicidal activity of the parent herbicide or were rapidly transformed to the parent herbicide in the environment after application. The physical and chemical properties of 2,4-D, 2,4,5-T, picloram, cacodylic acid, corresponding esters and salts, and TCDD that are related to environmental fate or biological assimilation are discussed in chapter 3.

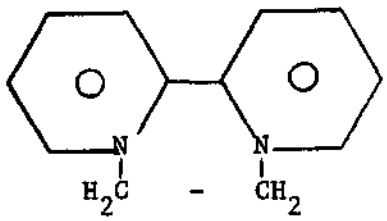
In addition to the color-coded herbicides, small amounts of the following herbicides were tested and possibly used to a limited extent in Vietnam (Young et al., 1978): The chemical structures of these compounds are given in figure 2-4.

- Dinoxol, mixture of the butoxyethanol esters of 2,4-D and 2,4,5-T
- Trinoxol, 40% butoxyethanol ester of 2,4,5-T
- Diquat, [6,7-dihydrodipyridol]pyrazidinium
- Bromacil, 5-bromo-3-sec-butyl-methyluracil
- Monuron, 3-[p-chlorophenyl]-1,1-dimethylurea
- Diuron, 3-[3,4-dichlorophenyl]-1,1-dimethylurea
- Tandex, [3,3-dimethylureido]-phenyltertbutyl carbamate
- Dalapon, 2,2-dichloropropionic acid.

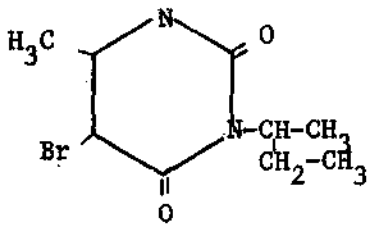
The physical descriptions and approximate chemical compositions of the major color coded herbicides used in Vietnam are presented below along with measured levels of TCDD in Orange and Purple.

Orange

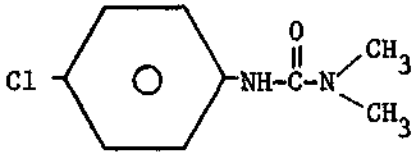
Orange is described by Young et al. (1978) as a reddish-brown or tan liquid which was essentially insoluble in water, but soluble in diesel fuel and various organic solvents. It had a specific gravity of approximately 1.28 at 25°C and was moderately volatile compared to White or Blue. The vapor pressure at 25°C of the n-butyl ester of 2,4-D, which made up close to 50 percent of Orange, is reported to be 8.4×10^{-6} mm (Hameker and Rerlinger (1969; in NRC, 1974) and 3.9×10^{-4} mm at 25°C (Zepp et al., 1975); the vapor pressure of the n-butyl ester of 2,4,5-T, which made up close to the other 50 percent of Orange, is estimated to be similar (Hameker and Rerlinger, 1969; in NRC, 1974).



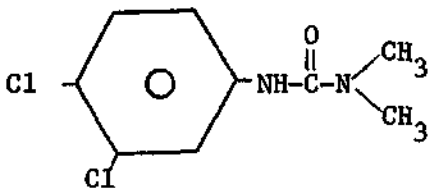
Diquat



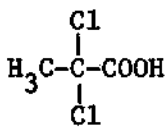
Bromacil



Monuron



Diuron



Dalapon

Figure 2-4. Chemical Structures of Herbicides Used in Small Quantities in Vietnam

The specified chemical formulation of Orange was 50:50 mixture by weight of the n-butyl esters of 2,4-D and 2,4,5-T (NRC, 1974). The military specifications were as follows (Young et al., 1978):

- n-butyl ester of 2,4-D 49.49%
- n-butyl ester of 2,4,5-T 48.75%
- 2,4,5-T 1.00%
- 2,4-D 0.13%
- inert compounds 0.62%

One gallon of Orange contained approximately 4.2 lbs. of active (as free acid plus acid part of the esters) 2,4-D and 4.4 lbs. of active 2,4,5-T (Young et al., 1978). However, the chemical composition of Orange varied somewhat for different lots from different manufacturers, due to the presence of unreacted precursors, chemical byproducts, and contaminants. Analysis of Orange samples from various lots stored at Gulfport, Mississippi and Johnston Island showed the following typical variations and mean composition (Fee et al., 1975).

Orange Sample Components	Variation	Mean
n-butyl ester of 2,4-D	42.6% - 46.2%	44.2%
n-butyl ester of 2,4,5-T	39.3% - 44.9%	42.1%
miscellaneous butyl esters of 2,4-D and 2,4,5-T	4.0% - 9.1%	7.0%
miscellaneous octyl esters of 2,4-D and 2,4,5-T	0.3% - 5.8%	2.0%
2,4-D	0.2% - 0.8%	0.5%
2,4,5-T	0.1% - 0.8%	0.6%
other components (e.g., butanol, toluene, trace TCDD)		3.6%

It should be noted that the analyses were performed on samples taken from lots which had often been stored for several years and which may have undergone more changes in chemical composition than most of the Orange sprayed in Vietnam.

Chemical formulations which contain 2,4,5-T or its derivatives are generally contaminated with the dioxin TCDD, which is substantially toxic in mammals. The concentration of TCDD in 42 samples of Orange taken from Gulfport in 1972 varied from 0.05-13.0 ppm with a mean concentration of 1.77 ppm. The concentration of TCDD in 238 samples of Orange taken from storage in

Gulfport, Mississippi in 1975 varied from 0.02-15.0 ppm with a mean concentration of 2.11 ppm. The concentrations of TCDD in samples taken from Orange that had been shipped to Johnston Island from Vietnam in 1972 were similar. The concentration of TCDD in 200 samples of Orange taken from Johnston Island in 1972 varied from 0.05-47.0 ppm with a mean concentration of 1.91 ppm. The concentration of TCDD in ten samples of Orange taken from Johnston Island in 1974 varied from 0.07-5.3 ppm with a mean concentration of 1.68 ppm. However, only four of the 200 samples contained TCDD concentrations greater than 15.0 ppm. Some of the Orange sampled at Johnston Island may have been Orange II (Young et al., 1978).

White

White is described by Young et al. (1978) as a dark brown liquid with high viscosity, which was essentially insoluble in diesel fuel and various organic solvents, but was soluble in water. White had a specific gravity of approximately 1.12 at 25°C and is described as non-volatile compared to Orange (Young et al., 1978).

The specified chemical formulation of White was an approximate 50:40:10 mixture by weight of the solvent triisopropanolamine, triisopropanolamine salt of 2,4-D, and the triisopropanolamine salt of picloram. The approximate military specifications were (Young et al., 1978):

- Triisopropanolamine, other inert components 50.2%
- Triisopropanolamine salt of 2,4-D 39.6%
- Triisopropanolamine salt of picloram 10.2%

The triisopropanolamine salts of 2,4-D and picloram have much higher aqueous solubilities but much lower vapor pressures than the parent compounds 2,4-D and picloram (NRC, 1974).

One gallon of White contained approximately 2.0 lbs. of active 2,4-D and 0.54 lbs. of active picloram (active meaning as free acid plus acid portion of the salts) (Young et al., 1978).

Blue

Blue is described by Young et al. (1978) as having been a clear yellowish-tan liquid that was soluble in water, but insoluble in diesel fuel and various organic solvents. Blue had a specific gravity of approximately 1.32 at 25°C and was involatile compared to Orange (Young et al., 1978).

The active components of Blue are primarily the sodium salt of cacodylic acid and cacodylic acid. The military specifications for Blue were (Young et al., 1978):

- Water 59.5%
- Sodium salt of cacodylic acid 26.4%
- Cacodylic acid 4.7%
- Sodium chloride 5.5%
- Surfactant 3.4%
- Antifoam agent 0.5%

One gallon of Blue contained approximately 3.1 lbs. of active (acid plus acid portion of salt) cacodylic acid (Young et al., 1978).

Orange II, Purple, Pink, Green

The specified chemical formulations of Orange II, Purple, Pink, and Green are given below. The chemical formulations of Orange II and Purple are similar to Orange (Young et al., 1978).

- Orange II - 50:50 by weight isooctyl ester of 2,4,5-T and n-butyl ester of 2,4-D
- Purple - 50:30:20 by weight n-butyl ester of 2,4-D, n-butyl ester of 2,4,5-T, and isobutyl ester of 2,4,5-T
- Pink - 60:40 by weight n-butyl ester of 2,4,5-T and isobutyl ester of 2,4,5-T
- Green - mostly n-butyl ester of 2,4,5-T.

Orange II, Purple, Pink, and Green all contain esters of 2,4,5-T. Therefore, all four herbicides were probably contaminated with TCDD. Of the four herbicides, only one sample of Purple has definitely been analyzed for TCDD. The one sample of Purple analyzed had a TCDD concentration of 45 ppm, which is generally much higher than for Orange samples. Young et al. (1978) postulate that both Purple and Pink generally may have contained higher levels of TCDD than Orange. They base this postulate primarily on the comparison of TCDD levels in the soils of test grid sites receiving predominantly Orange spray to those receiving predominantly Purple or Pink spray.

2.3 THE HERBS TAPE

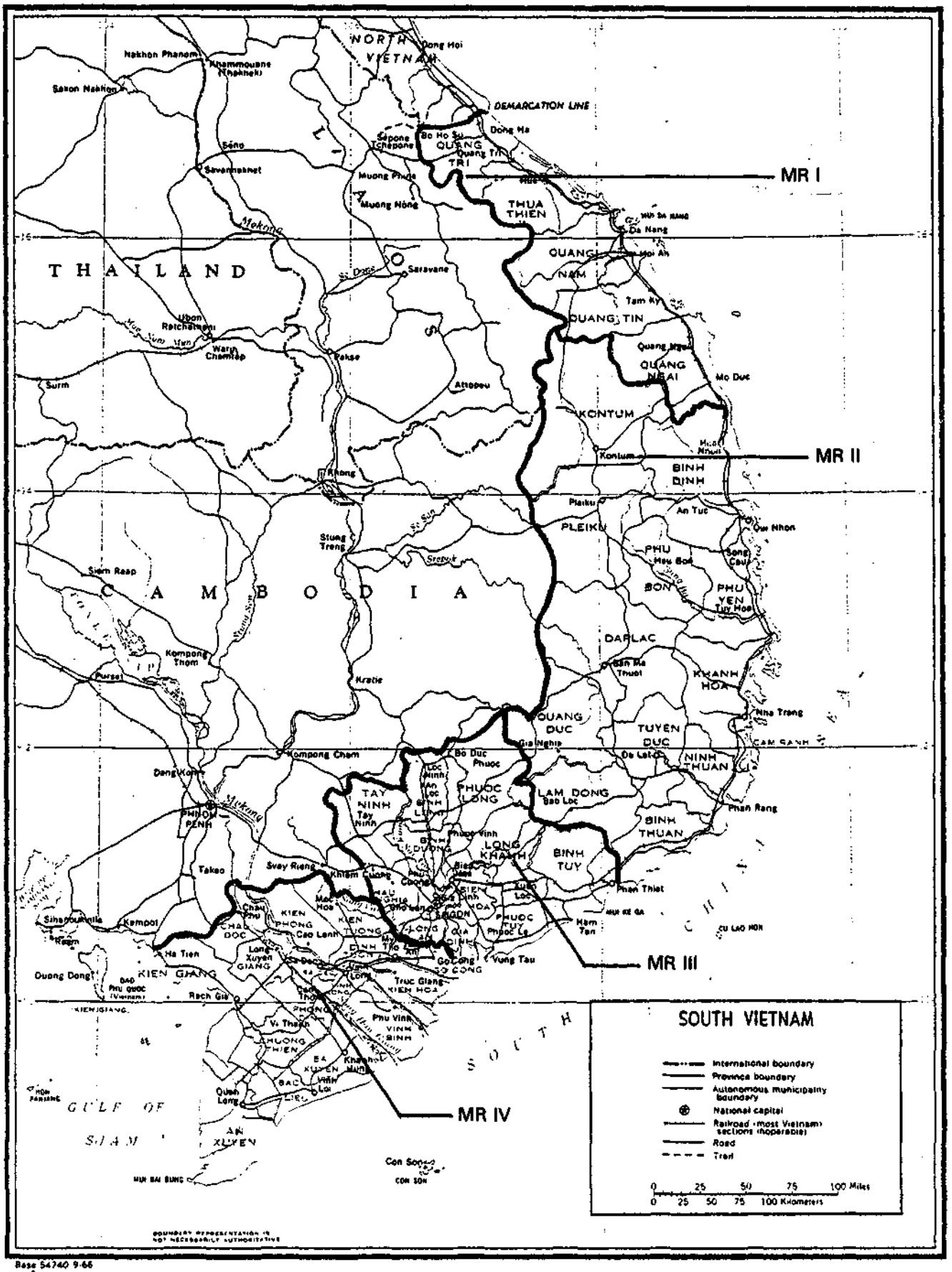
The most detailed information found in the literature on the distribution of herbicides was the National Research Council (NRC), National Academy of Sciences Report on the Effects of Herbicides in South Vietnam (1974). However, the information in the NAS report is based primarily on data stored on the HERBS computer tape, supplied by the Department of Defense, which is incomplete. The HERBS tape contains information on most of the herbicidal missions flown by fixed-wing aircraft from August 1965 to the last such flight in January 1971, and on crop missions flown by helicopter between June 1968 and March 1971. It does not include information on any herbicidal missions performed prior to August 1965, non-crop related herbicidal missions flown by helicopter, crop missions flown by helicopter prior to June 1968, nor any non-aerial herbicidal missions (e.g., river boat, truck, back sprayers). It also does not contain information on helicopter missions flown by the South Vietnamese military.

Despite its deficiencies, the HERBS tape contains data on the spraying of approximately 17.6 million gallons of Orange, White, and Blue, 88.0 percent of the estimated total of 20 million gallons of herbicide spraying in Vietnam. The information on the tape accounted for the spraying of approximately 5.9 million acres, 92.2 percent of an estimated total of 6.4 million acres sprayed in Vietnam from 1962 through 1971. Based on other data supplied to NRC, it was estimated that approximately 1.25 million gallons of herbicides were sprayed on approximately 400,000 acres prior to August 1965, and that approximatley 600,000 gallons of herbicides were sprayed on crops by helicopter between August of 1965 and June of 1968. In addition, approximately 13,000 acres of crops were sprayed by helicopter with approximately 36,000 gallons of White and Blue between March and October of 1971 (NRC, 1974).

The data supplied in the remaining sections of this chapter are taken primarily from the NRC (1974) report. The numbers of gallons sprayed and acres covered were estimated by NRC (1974) from the numbers of missions and airplanes on the missions, as recorded on the HERBS tape. Estimates of gallons sprayed are based on tank capacities of aircraft taking part in the missions. Estimates of areas sprayed in defoliation and crop destruction methods were based on an assumed swath width of 80m and the length of spray line recorded on the HERBS tape. Estimates of areas sprayed during missions over perimeters, waterways, caches, etc., were based on the estimated number of gallons sprayed and an assumed application of three gallons per acre.

2.4 GEOGRAPHICAL AND TEMPORAL DISTRIBUTION OF HERBICIDES IN SOUTH VIETNAM

South Vietnam was divided into four major military regions (MR I through MR IV) north to south. A map of South Vietnam, presented in figure 2-5, shows the boundaries of the four military regions and the provinces. Figures 2-6 and 2-7 show the outline of Vietnam and darkened regions which represent approximate areas of defoliation and crop destruction missions, respectively. These maps were developed from maps supplied in the NAS report, which were based on the HERBS tape.



Base 54740 9-66

Figure 2-5. South Vietnam

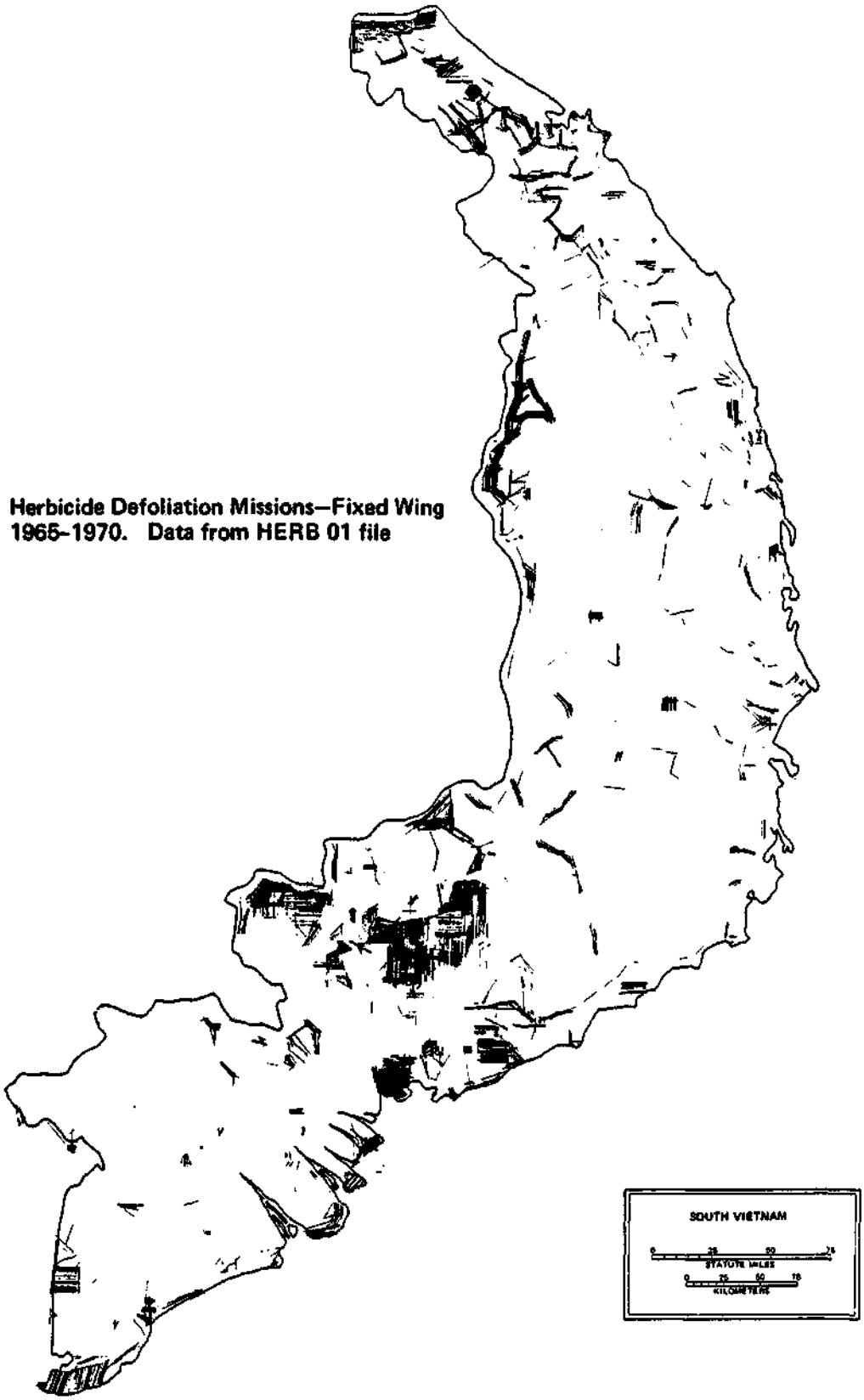


Figure 2-6. Defoliation Missions

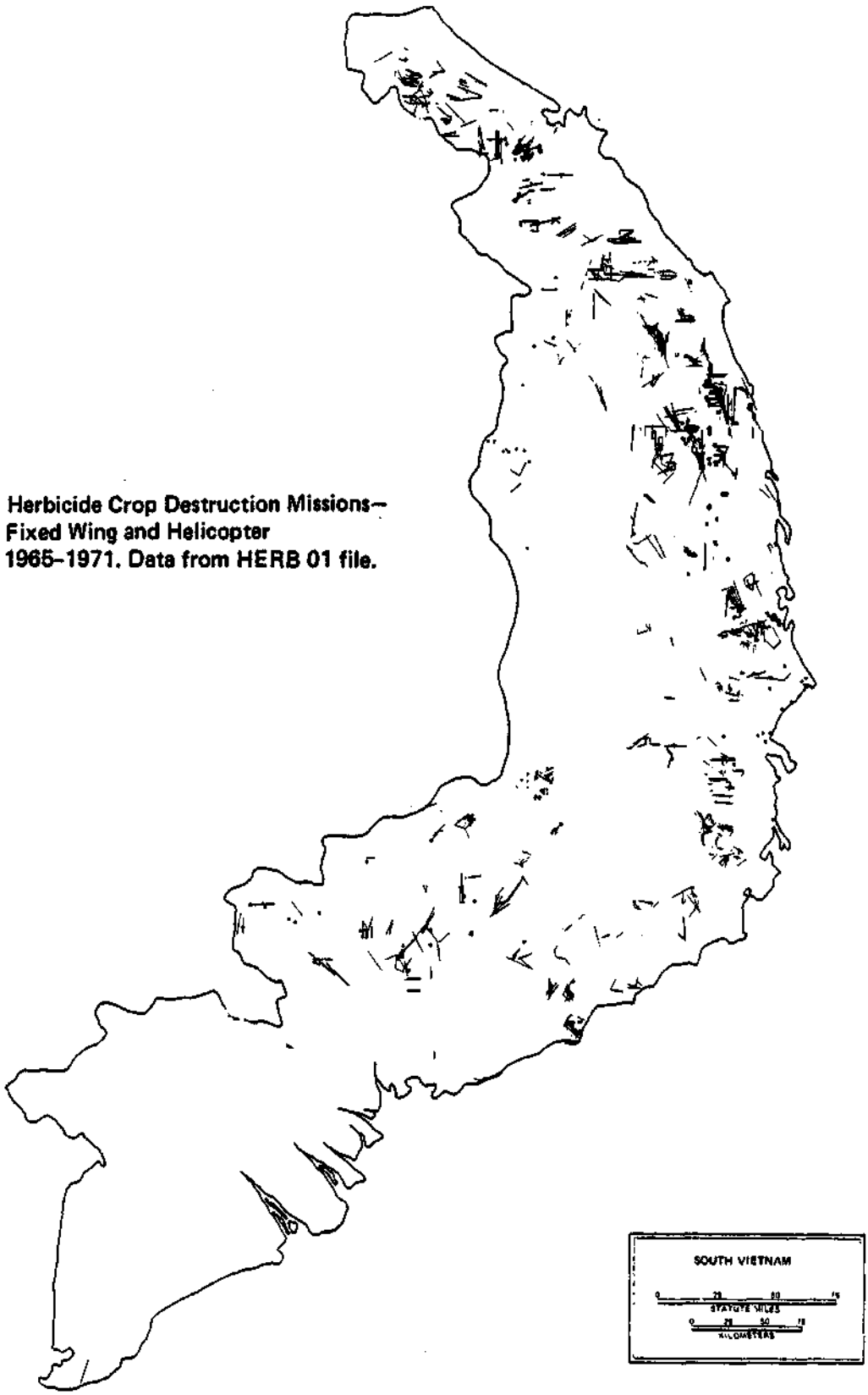


Figure 2-7. Crop Destruction Missions

Examination of figures 2-5, 2-6, and 2-7 indicates that the greatest number of defoliation missions flown between August 1965 and January 1971 were directed against:

- Inland forests in MR I, especially along the demarcation line
- Inland forests in MR II along the Laotian and Cambodian borders
- Inland forests in MR III northeast, north, and northwest of Saigon
- Mangrove forests in MR IV on the Ca Mau Peninsula
- Mangrove forests in the Rung Sat region of the province southeast of Saigon, which encompassed major shipping channels.

Crop destruction missions between August 1965 and February 1971 were primarily directed against fields in MR I and the eastern half of MR II. Crop destruction missions were generally prohibited in the Mekong Delta region, which makes up most of MR IV.

During the initial periods of Operation RANCH HAND, from January 1962 through 1964, Purple and Pink were the most widely used herbicides. According to Young et al. (1978), based on a DOD memorandum, approximately 145,000 gallons of Purple and 123,000 gallons of Pink were procured and disseminated in South Vietnam from January 1962 through December 1964. After 1964, the most widely used herbicides were Orange, White, and Blue. The approximate annual and total volumes of Orange, White, and Blue sprayed in Vietnam from August 1965 through February 1971 are listed in table 2-1. Table 2-1 is adapted from table S-I in the NRC report and is based only on the HERBS tape. Part of the 11.3 million gallons of Orange sprayed was probably Orange II. Approximately 950,000 gallons of Orange II were shipped to Vietnam during 1968 and 1969; some of this was not used in Vietnam and was shipped from Vietnam to Johnston Island in 1972.

Table 2-2 lists the estimated numbers of acres sprayed annually in Vietnam from January 1962 through December 1972. The values for 1962 through 1965 and for 1971 are taken from Westing (1976). The values for 1966 through 1971 are taken from table III-B-2 in the NAS report and are based on the HERBS tape. Acres which are sprayed more than once are counted as additional acres sprayed. It can be seen from Table 2-2 that the estimated acreage covered annually from January 1962 through December 1964, when Purple and Pink were the herbicides primarily used, is small compared to the acreage covered after 1965, when Orange/Orange II, White, and Blue were the herbicides primarily used. The annual number of acres sprayed approximately quadrupled from 1965 to 1966, and doubled from 1966 to 1967, when it reached a maximum. The maximum in 1967 was followed by a small decline in 1968, a leveling-off in 1969, and a rapid decline in 1970 prior to termination of spraying in 1971. The number of acres sprayed in 1970 was less than 20 percent of the acres sprayed in 1969.

TABLE 2-1: APPLICATION OF HERBICIDES IN THE VIETNAM WAR BY YEAR

Millions of Gallons

Year	1962-July 1965	Aug-Dec 1965	1966	1967	1968	1969	1970	1971	Total
Orange	NA[a]	.37	1.64	3.17	2.23	3.25	.57	.00	11.22
White	NA[a]	0	.53	1.33	2.13	1.02	.22	.01	5.24
Blue	NA[a]	0	.02	.38	.28	.26	.18	.00	1.12
Total	1.27	.37	2.19	4.88	4.63	4.53	.97	.01	18.95

[a]Not Available.

(Table S-I in NRC 1974)

TABLE 2-2: ANNUAL NUMBER OF ACRES SPRAYED IN VIETNAM[a]

Year	Acres[a]
1962	5,724[b]
1963	24,920[b]
1964	93,869[b]
1965	221,552[b]
1966	608,106[c]
1967	1,570,114[c]
1968	1,365,479[c]
1969	1,365,754[c]
1970	294,925
1971	

a Acres sprayed more than once are counted as additional acres sprayed.

b Westing (1976)

c NRC (1974)

2.5 QUANTITATIVE AND QUALITATIVE USE DISTRIBUTION OF HERBICIDES IN SOUTH VIETNAM

As mentioned in section 2.4, the major targets of herbicide spraying in South Vietnam were inland forests, crops (cultivated land), and mangrove forests. Table 2-3 lists the distribution of herbicide spraying among inland forests, cultivated land, and mangrove forests. Table 2-3 is taken completely from table S-III in the NRC report, and is based on the HERBS tape.

From August 1965 to February 1971, more than 11.3 million gallons of Orange/Orange II, 5.3 million gallons of White, and 1.1 million gallons of Blue were sprayed in South Vietnam. The volume use distribution that can be estimated for Orange, White, and Blue during the period August 1965 through February 1971 is presented below and is based on table III-B-1 in the NRC report, as derived from the HERBS tape.

- Orange/Orange II
  - 89.5% defoliation, 8.6% crop destruction
  - 0.9% other (cache searches, communication lines, etc.)
  - 0.9% perimeter defense, 0.3% waterway defense.
- White
  - 95.3% defoliation, 1.1% crop destruction
  - 1.9% perimeter defense, 0.3% waterway defense
  - 1.4% other
- Blue
  - 39.3% defoliation, 53.1% crop destruction
  - 4.2% perimeter defense, 0.4% water defense
  - 3.0% other
- Average
  - 87.9% defoliation, 9.2% crop destruction
  - 1.4% perimeter defense, 0.3% waterway defense
  - 1.2% other.

The HERBS tape does not include an estimated 600,000 gallons of herbicide agents sprayed on crops by helicopters from August 1965 to June 1968. When these are included, the percentages for the average are 85.4 percent defoliation, 12.2 percent crop destruction, 1.4 percent perimeter defense, 0.3 percent waterway defense, and 0.7 percent other. The HERBS tape also does not include an estimated 1.25 million gallons of herbicides sprayed prior to August of 1965, including 145,000 gallons of Purple and 123,000 gallons of Pink.

Both Orange/Orange II and White were used on woody species. White was preferred over Orange in areas where spray drift was a concern, as it is not volatile compared to Orange. However, Orange was often favored over White in

TABLE 2-3: ESTIMATED ACREAGE SPRAYED ONE OR MORE TIMES, 1965-1971[a]

Vegetation Type	Total in SVN in 1953		Number of Times Sprayed Aug. 1965-Mar. 1971				Total Sprayed One or More Times	
	Millions of Acres	Percent	Millions of Acres 1	2	3	4+	Millions of Acres	Percent
Inland forest	25.91	62.4	1.72	0.62	0.22	.11	2.67	10.3
Cultivated land	7.80	18.8	0.20	0.04	0.01	0.00	0.26	3.2
Mangrove forest	.72	1.7	0.14	0.07	0.03	0.02	0.26	36.1
Other	7.07	17.1	0.31	0.07	0.02	0.00	0.39	5.5
Total	41.50	100.0	2.37	0.80	0.28	0.13	3.58	8.6

[a]Does not include coverage of missions before August 1965 (1.27 million gallons) and missions after that date for which location information is incomplete (1.1 million gallons), representing about 12.5% of the total gallonage accounted for. Compare tables III C-1 and III C-2, and related text.

[b]Inland forests include those areas classed as dense forest, secondary forest, swidden zones, bamboo forests, open dipherocarp, Lagerstroemia and Leguminosae forests. "Other" include pine forests, savanna and degraded forests, grasslands and steppes in higher elevations, dunes and brushland, grass and sedge swamps and areas of no vegetation (urban areas, roads, water courses, etc.). Classification and area figures follow Bernard Rollet (1962). See tables II-E and III B-3 and the accompanying text.

(Table S-III in NRC, 1974)

areas where spray drift was of little concern because it is a faster defoliant than White. Orange was also used on a wide variety of broadleaf crops. The persistence of picloram in soil made the use of White for crop destruction generally disadvantageous. A large percentage of Blue was used for crop destruction, particularly on cereal or green crops.

2.6 RELEASE OF TCDD IN SOUTH VIETNAM DURING SPRAYING OF HERBICIDES

TCDD is a byproduct of the alkaline hydrolysis (under high temperature and pressure) of 1,2,4,5-tetrachlorobenzene to form 2,4,5-trichlorophenol, which is the industrial precursor of 2,4,5-T (Young et al., 1978; Esposito, 1980). TCDD is, therefore, a common contaminant of 2,4,5-T and its various esters. Conditions such as those used to synthesize 2,4,5-trichlorophenol favor the formation of highly chlorinated dioxins such as TCDD (Young et al., 1978; Esposito, 1980). However, 2,4-dichlorophenol (which is the industrial precursor of 2,4-D) and picloram are not synthesized under alkaline conditions at high temperature and pressure (Young et al., 1978). Therefore, contamination of 2,4-D or picloram by TCDD is unlikely. TCDD was not detected to a detection limit of .0005-.02 ppm in samples of 2,4-D or picloram (Young et al., 1978), but other dibenzo-p-dioxins have been detected in both 2,4-D and 2,4,5-T (Esposito, 1980).

Although a mechanism has been proposed for the possible photochemical generation of TCDD from 2,4,5-T, Crosby et al. (1973) could not detect TCDD in 2,4,5-T that had been irradiated with sunlight or simulated sunlight. The failure to detect TCDD may be due to a more rapid rate of TCDD photodecomposition than rate of formation (Crosby et al., 1973).

The major source of release of TCDD in South Vietnam appears to have been as a contaminant of herbicides such as Orange/Orange II, Purple, and Pink, all of which contained 2,4,5-T and various esters of 2,4,5-T.

The spraying of herbicides in Vietnam was occasionally done as a drying step prior to deforestation by fire (Collins, 1967). Therefore, reports that TCDD can be produced from the pyrolysis of 2,4,5-T or its butyl ester are of interest. Buu-Hoi et al. (1971) reported that TCDD was formed during the pyrolysis of 2,4,5-T, its butyl ester, and vegetation previously treated with 2,4,5-T or its butyl ester. Saint-Ruf (1972) reported that TCDD was formed from the pyrolysis of the herbicide Silvex which contains 2,4,5-T. However, Langer et al. (1973) could not detect TCDD in the sodium salts of either 2,4,5-T or Silvex that had been heated to 300°C and 350°C respectively. Stehl and Lamparski (1977) detected TCDD in the burned residues of grass that had been previously treated with approximately 12 lbs per acre of 2,4,5-T. Ahling et al. (1977) detected TCDD levels in the burned residues of wood chips covered with 2,4,5-T prior to burning at 500°C.

Close to 500 samples of Orange/Orange II have been analyzed for TCDD. The concentration of TCDD in the samples varied from 0.02 to 15.0 ppm (except for four samples greater than 15.0 ppm). The mean concentration of TCDD in the Orange/Orange II was approximately 2.0 ppm. One gallon of Orange/Orange II (specific gravity of Orange 1.28 at 25°C, density of Orange II similar)

weighed approximately 10.7 lbs. Assuming that the Orange/Orange II sampled was representative of the Orange/Orange II sprayed in South Vietnam, one gallon of Orange/Orange II sprayed in Vietnam could be expected to have generally contained between 2.1×10^{-7} lbs and 1.6×10^{-4} lbs of TCDD or an the average of 2.1×10^{-5} lbs of TCDD. Therefore, during one spraying of Orange/Orange II at three gallons/acre, forests or crops would theoretically be expected to have received generally between 6.3×10^{-7} lbs/acre and 4.8×10^{-4} lbs/acre or an average 6.3×10^{-5} lbs/acre TCDD. However, the targeted forests and crops would probably generally receive less TCDD than calculated above due to drifting of spray. Furthermore, the amount of TCDD reaching ground level in heavily forested areas would be far less than the amount striking the upper canopy.

Both Purple and Pink contained esters of 2,4,5-T and were apparently contaminated with TCDD. Although only one sample of Purple (45.0 ppm TCDD) and none of Pink had been analyzed for TCDD, Young et al. (1978) presented circumstantial evidence that both Purple and Pink generally contained much higher levels of TCDD than Orange/Orange II. The comparison of TCDD residues in soil from adjacent test grids receiving Orange, Purple, or Pink, supports that postulate, however, evidence they present to support their estimate of TCDD levels in Purple and Pink greater than 30.0 ppm and 60.0 ppm respectively is somewhat weaker and is, therefore, discussed below.

Young et al. (1978) based their estimate on the average TCDD concentration in Purple on the assumption that the four samples of Orange/Orange II with levels of TCDD greater than 15.0 ppm were actually Purple labeled as Orange by mistake. They averaged the TCDD levels in those four samples with the TCDD level in the one sample of verified Purple that was analyzed. Their assumption that the four samples of Orange/Orange II with TCDD levels greater than 15.0 ppm (all taken from Johnston Island) were actually Purple was based on several factors. These included: the high TCDD level in the one Purple sample analyzed; reports that as many as 20 drums of Purple were redrummed into cans labeled as Orange just prior to the shipping of Orange/Orange II from Vietnam to Johnston Island; reports that substantial quantities of the isobutyl ester of 2,4,5-T, a component of Purple but not of Orange/Orange II, had been detected in a few of the samples from Johnston Island; and the fact that all four samples of Orange/Orange II with levels of TCDD greater than 15.0 ppm were taken from Johnston Island.

The estimate of the average TCDD concentration in Pink was based on the relative percentages of the acid equivalent of 2,4,5-T in Purple and Pink and the assumption that both Purple and Pink were formulated from batches of 2,4,5-T with similar TCDD levels. Based on the TCDD levels in 2,4,5-T manufactured from 1958 to 1963, the authors also estimated that the average concentration of TCDD in Purple formulated prior to 1964 may have been as low as 5.0 ppm. Since the spraying of Purple in Vietnam was essentially terminated after 1964, most of the Purple sprayed would have been formulated prior to 1964.

If the levels of TCDD in Purple and Pink were generally as high as those estimated by Young et al. (1978), the amount of TCDD released per acre during the spraying of Purple or Pink would have been far greater than during the

spraying of Orange/Orange II. This is despite the fact that the application rates for most of the Purple and Pink sprayed (1-1.5 gallons per acre prior to July 1964) were lower than for Orange/Orange II (three gallons per acre). However, as pointed out by Young et al. (1978), most of the Purple and Pink were sprayed prior to 1965 before the massive build-up of U.S. military personnel. Furthermore, only an estimated 90,000 acres were sprayed with Purple or Pink compared to over three million acres sprayed with Orange/ Orange II (Young et al., 1978).

Overall, the spraying of more than 11.3 million gallons of Orange/Orange II from August 1965 through February 1971 is estimated to have released close to 240 pounds of TCDD, assuming that the average concentration of TCDD in Orange/Orange II was 2 ppm (2.1×10^{-5} pounds per gallon).

2.7 RELEASE OF ESTERS OF 2,4,5-T

The major herbicides sprayed in South Vietnam that contained esters of 2,4,5-T were Orange/Orange II, Purple, and Pink. One gallon of Orange contained approximately 4.6 pounds of the acid equivalent of 2,4,5-T. The specified formulation for Orange was 50 percent by weight of the n-butyl ester of 2,4,5-T. Assuming one gallon of Orange weighed 10.7 pounds, one gallon of the specified formulation of Orange would contain approximately 5.4 pounds of the n-butyl ester of 2,4,5-T. Therefore, Orange applied at three gallons per acre is estimated to have released a maximum of 13.8 pounds per acre of the acid equivalent of 2,4,5-T and 16.2 pounds per acre of the n-butyl ester of 2,4,5-T per spraying. The actual amount of 2,4,5-T released per acre was probably less than estimated because, actual samples of Orange contained less than 50 percent by weight of the n-butyl ester of 2,4,5-T. Furthermore, spray drift and volatilization generally reduced the amount deposited per acre. Canopy cover in heavily forested areas would, as in the case of other chemicals, greatly reduce the amount of ester reaching ground level.

As stated previously, some of the approximately 11.3 million gallons of Orange sprayed in Vietnam was probably Orange II, since 950,000 gallons of Orange II were shipped to Vietnam during 1968 and early 1969 (Young et al., 1978). The specified formulation for Orange II was 50 percent by weight of the isooctyl ester of 2,4,5-T, instead of 50 percent of the n-butyl ester of 2,4,5-T, as in Orange. Assuming that one gallon of Orange II, like Orange, weighed approximately 10.7 pounds, one gallon of the specified formulation of Orange II would contain approximately 5.4 pounds of the isooctyl ester of 2,4,5-T. Therefore, Orange II applied at three gallons per acre is estimated to have released a maximum of 16.2 pounds per acre of the isooctyl ester of 2,4,5-T per spraying.

The densities of Purple and Pink could not be found in the literature. Assuming the densities of Purple and Pink were similar to Orange, one gallon of Purple or Pink would have weighed approximately 10.7 pounds. The specified formulation of Purple was 30 percent by weight the n-butyl ester of 2,4,5-T and 20 percent by weight the isobutyl ester of 2,4,5-T, so one gallon of the specified formulation of Purple would contain approximately 3.2 pounds of the n-butyl ester of 2,4,5-T and 2.1 pounds of the isobutyl ester of 2,4,5-T.

Therefore, Purple applied at 1.5 gallons per acre is estimated to have released a maximum of 4.8 pounds per acre of the n-butyl ester of 2,4,5-T and 3.2 pounds per acre of the isobutyl ester of 2,4,5-T.

The specified formulation of Pink was 60 percent by weight the n-butyl ester of 2,4,5-T and 40 percent by weight the isobutyl ester of 2,4,5-T, so one gallon of the specified formulation of Pink would contain approximately 6.4 pounds of the n-butyl ester of 2,4,5-T and 4.3 pounds of the isobutyl ester of 2,4,5-T. Therefore, Pink applied at 1.5 gallons per acre is estimated to have released a maximum of 9.6 pounds per acre of the n-butyl ester of 2,4,5-T, and 6.5 pounds per acre of the isobutyl ester of 2,4,5-T.

2.8 RELEASE OF ESTERS OF 2,4-D AND THE TRIISOPROPANOLAMINE SALT OF 2,4-D

The major herbicides sprayed in South Vietnam that contained esters or salts of 2,4-D were Orange/Orange II, White, and Purple. One gallon of Orange/Orange II weighed approximately 10.7 pounds and contained approximately four pounds of the acid equivalent of 2,4-D. The specified formulation of both Orange and Orange II was 50 percent by weight the n-butyl ester of 2,4-D, so one gallon of the specified formulation of Orange/Orange II would contain approximately 5.4 pounds of the n-butyl ester of 2,4-D. Therefore, Orange/Orange II applied at three gallons per acre is estimated to have released a maximum of 12 pounds per acre of the acid equivalent of 2,4-D and 16.2 pounds per acre of the n-butyl ester of 2,4-D.

One gallon of White (density of 1.12 at 25°C) weighed approximately 9.3 pounds, and contained approximately 2 pounds of the acid equivalent of 2,4-D. The specified formulation of White was 40 percent by weight of the triisopropanolamine salt of 2,4-D, so one gallon of the specified formulation of White would contain approximately 3.7 pounds of the salt. Therefore, White applied at three gallons per acre is estimated to have released a maximum of 11.1 pounds per acre of the triisopropanolamine salt of 2,4-D per spraying.

The specified formulation of Purple was 50 percent by weight the n-butyl ester of 2,4-D. Assuming that one gallon of Purple weighed approximately the same as one gallon of Orange (10.7 pounds), one gallon of the specified formulation of Purple would contain 5.4 pounds of the n-butyl ester of 2,4-D. Therefore, Purple applied at 1.5 gallons per acre is estimated to have released a maximum of 8.1 pounds per acre of the n-butyl ester of 2,4-D per spraying.

2.9 RELEASE OF THE TRIISOPROPANOLAMINE SALT OF PICLORAM

One gallon of White weighed approximately 9.3 pounds and contained approximately 1.54 pounds of the acid equivalent of picloram. The specified formulation of White was approximately 10 percent by weight the triisopropanolamine salt of picloram, so one gallon of the specified formulation of White would contain approximately 0.93 pounds of the triisopropanolamine salt of picloram. Therefore, White applied at three gallons per acre is estimated to have released a maximum of 2.8 pounds per acre of the triisopropanolamine salt of picloram.

2.10 RELEASE OF CACODYLIC ACID AND ITS SODIUM SALT

One gallon of Blue (specific gravity 1.32 at 25°C) weighed approximately 11 pounds and contained approximately 3.1 pounds of the acid equivalent of cacodylic acid. The approximate formulation of Blue was 5 percent by weight cacodylic acid and 26.5 percent by weight the sodium salt of cacodylic acid, so one gallon of Blue contained approximately 0.5 pounds of cacodylic acid and 2.9 pounds of the sodium salt of cacodylic acid. Therefore, Blue applied at three gallons per acre is estimated to have released a maximum of 9.3 pounds per acre of the acid equivalent of cacodylic acid, 1.5 pounds per acre of cacodylic acid, and 8.7 pounds per acre of the sodium salt of cacodylic acid.

CHAPTER 2.

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CHAPTER 3

ENVIRONMENTAL FATE AND MONITORING

In order to assess environmental effects of herbicide use, this chapter looks at the mechanisms by which herbicides are distributed in the air, soil, and water. These include wind drift after initial spraying, and contamination of waters through leaching of treated soils, soil or sediment/water partitioning, or water erosion of contaminated soil particulates. Transformation of the chemicals in the environment by such processes as photolysis, hydrolysis, and volatilization are studied. Finally, the ultimate impact of the distribution of herbicides in the environment is investigated: the biological accumulation, concentration, and magnification of the chemicals in terrestrial and aquatic plants and animals.

3.1 ENVIRONMENTAL FATE AND MONITORING OF 2,4-D, 2,4,5-T, PICLORAM, AND ASSOCIATED ESTERS AND SALTS

3.1.1 Physical and Chemical Properties

The physical properties of 2,4-D, 2,4,5-T, picloram, and associated esters and amine salts are listed in table 3-1. The chemical structures of 2,4-D, 2,4,5-T, and picloram are depicted in figure 2-1. The chemical structures of the esters and salts are depicted in figure 2-2.

The n-butyl esters of 2,4-D and 2,4,5-T, which are the primary components of Orange, undergo nonbiological and biological hydrolyses to 2,4-D and 2,4,5-T, respectively (Zepp et al., 1975; Smith, 1972; Grover, 1973 in Crosby, 1976; Smith, 1976). Picloram, 2,4-D, and 2,4,5-T are susceptible to photodecomposition by radiation at wavelengths above 290 nm which is the approximate ground-level cutoff of solar radiation (Mosier and Guenzi, 1973; Crosby and Tutas, 1966; Baur and Bovey, 1974; Zepp et al., 1975; Baur et al., 1973; Crosby and Wong, 1973). They are also susceptible to various biological transformations in plants and soil, with transformation rates generally in the order 2,4-D > 2,4,5-T > picloram (Young et al., 1978; NRC, 1974).

3.1.2 Hydrolysis

Zepp et al. (1975) determined the hydrolysis rates of the methyl and n-butoxyethyl esters of 2,4-D in water as a function of pH. In general,

TABLE 3-1. PHYSICAL PROPERTIES OF HERBICIDES

Compound	Vapor Pressure	Aqueous Solubility
2, 4-D	Much lower than Ester	620 mg/l
n-Butyl Ester	3.9×10^{-4} mm[2] at 25° C,[1] 8.4×10^{-6} mm at 25° C	1 mg/l at 25°C
Triisopropanolamine Salt	Lower than Ester	>620 mg/l
2,4,5-T	Lower than Ester	250 mg/l
n-Butyl Ester	Comparable to n-Butyl Ester of 2,4-D[1]	<250 mg/l
Picloram	6.2×10^{-7} mm[1] at 35° C	430 mg[1]/l at 25° C
Triisopropanolamine Salt	Lower than Picloram	430 mg[1]/l at 25° C

1. NRC (1974)
2. Zepp et al. (1975)

the overall rate of hydrolysis of the esters was given by Zepp et al. (1975) as:

$$\frac{d[C]}{db} = -(k_A [H^+] + k_N + k_B [OH^-])[C] \qquad (3\text{-}1)$$

Where

k_A, k_N, k_B = rate constants for acid catalyzed, neutral and base catalyzed hydrolysis

$[C]$ = ester concentration

$[H^+]$, $[OH^-]$ = hydrogen, hydroxide ion concentration.

The measured rates indicated that $k_B[OH^-]$ was much greater than k_N or $k_A[H+]$ over the normal pH range for natural waters of 6-9. Therefore, in natural waters, the hydrolysis rate for the esters can probably be given approximately by:

$$\frac{d[C]}{dt} = -k_B [OH^-][C] \qquad (3\text{-}2)$$

Assuming that the pH of a well-buffered natural water remains constant, $[OH^-]$ remains constant and the hydrolysis rate becomes pseudo-first order with a half-life independent of concentration and given by

$$t_{1/2} = \ln 2/k_B[OH^-] \qquad (3\text{-}3)$$

Using the measured value of k_B for the methyl ester of 2,4-D and assuming that structural reactivity relationships for the esters of 2,4-D were similar to those observed for acetic acid esters, Zepp et al. (1975) estimated that the value of k_B for the base-catalyzed hydrolysis of the n-butyl ester of 2,4-D was approximately 3.7 molL-1 sec^{-1}. Substituting the estimated value of k_B into equation 3-3 along with hydroxy ion concentrations at pH 6 and 9, Zepp et al. estimated that the hydrolysis half-life of the n-butyl ester of 2,4-D in natural water would range from 220 days at pH 6 to 5.2 hours at pH 9. The estimated hydrolysis half-lives at pH 7 and 8 are respectively 22 days and 52 hours.

Smith (1972) and Grover (1973) in Crosby (1976) experimentally determined the hydrolysis half-life of the n-butyl ester of 2,4-D to be 100 hours in neutral water and much less in aqueous soil suspensions. Smith (1976) reported that the n-butyl ester of 2,4-D undergoes almost complete hydrolysis to 2,4-D in less than 24 hours in moist soil. Similar results were reported for the hydrolysis of the n-butyl and isooctyl esters of 2,4,5-T to 2,4,5-T. Aly and Faust (1969) reported that esters of 2,4-D were completely hydrolyzed to 2,4-D within 9 days in lake water. Therefore, rates of biological hydrolysis appear to be greater than rates of chemical hydrolysis.

Based on the above discussion, the esters of 2,4-D and 2,4,5-T released to the soil or biologically active water in Vietnam probably underwent rapid hydrolyses to 2,4-D and 2,4,5-T.

3.1.3 Photodecomposition

Phenoxy herbicides such as 2,4-D and 2,4,5-T can undergo several different photo-reactions in water, including photo-oxidation of the phenoxy side chain to form chlorophenols, photo-nucleophilic displacement of Cl by OH to form chlorophenols, and photo-reductive dechlorination involving the replacement of Cl with H to form phenoxyacetic acid (Crosby 1976; Akermark, 1978).

Zepp et al. (1975) estimated the average photolytic half-life of 2,4-D in shallow clear water as follows. The rate of photolysis was assumed to be given by

$$\frac{d[C]}{dt} = - k_A \bar{\Phi} [C] \qquad (3\text{-}4)$$

Where

k_A = average rate of sunlight absorption

$\bar{\Phi}$ = quantum yield.

The average rate of sunlight absorption was calculated by integrating sunlight intensity data over a 12-hour period of sunlight for September on a clear day at latitude 34°N. If the quantum yield is assumed to be independent of wavelength, or is an average value, the rate of photolysis becomes psuedo-first order with a half-life given by (Zepp et al., 1975):

$$t_{1/2} = \ln 2/k_a \bar{\Phi} \qquad (3\text{-}5)$$

Substituting the value of k_A and a reported quantum yield for 2,4-D in water into equation 2-5, Zepp et al. estimated that the photolytic half-life of 2,4-D in clear, shallow water in September at latitude 34°N, exposed to 12 hours of unobstructed sunlight, would be 20 days. Estimates of half-lives under cloud cover or in deeper water would be much greater.

Crosby and Wong (1973) determined the photodecomposition rate of 2,4,5-T in water exposed to summer sunlight at Davis, California. Aqueous solutions of 2,4,5-T were irradiated with sunlight for approximately 12 hours per day for 4 days. After 48 hours of irradiation, approximately 17 percent of tthe 2,4,5-T had undergone photodecomposition. Studies on the photodecomposition rates of 2,4-D and 2,4,5-T exposed to artificial light at wavelengths above 290 nm showed that the photodecomposition rate of 2,4-D was generally well over twice that of 2,4,5-T under the experimental conditions used.

Although there have been reports that 2,4-D and 2,4,5-T are relatively stable to photolysis under dry conditions, Baur et al. (1974) and Baur et al. (1973) have shown that substantial losses of dried films of 2,4-D, 2,4,5-T,

and picloram on glass occur over a period of several days when the films are irradiated with low intensity light (700 to 1,100 uW/cm^2) at 356 nm. For example, after 7 days of irradiation, 41-79 percent of the picloram, 57-97 percent of the 2,4,5-T, and 30-70 percent of the 2,4-D were lost from petri dishes. The percentage lost for each compound depended upon the amount initially applied, which varied from approximately 10 ug to 100 ug. Other experiments conducted in the dark indicated that volatilization could not be responsible for the large losses observed during irradiation.

Experiments by Mosier and Guenzi (1973) indicate that picloram in aqueous solution exposed to radiation at wavelengths above 300 nm will undergo rapid photodecomposition. Irradiation of a 2.1 x 10^{-3} M aqueous solution of the sodium salt of picloram resulted in a 99 percent loss of picloram in less than 72 hours. The intensity of the radiation was not given.

3.1.4 Biological Transformations

2,4-D undergoes a wide variety of relatively rapid biological transformations in plants and in soil, including cleavage of the ether bond, hydroxylation, and ring cleavage (Allebone, 1975). Picloram and 2,4,5-T also undergo biological transformation in plants and soils, but transformation rates are generally lower than for 2,4-D (Young et al., 1978; NRC, 1974). The persistence of picloram, 2,4-D, and 2,4,5-T in soil and plants is discussed in section 3.1.7

3.1.5 Volatility

The n-butyl esters of 2,4-D and 2,4,5-T, which were the primary components of Orange, possess much higher vapor pressures than 2,4-D, 2,4,5-T, picloram, or associated amine salts. Therefore, they are generally much more volatile. Grover et al. (1972) reported that 25 to 30 percent of the n-butyl ester of 2,4-D applied to the ground for testing was collected as vapor drift up to 246 feet downwind from the application point, within 30 minutes after application. However, only negligible amounts of the dimethylamine salt of 2,4-D were detected as vapor drift.

Zepp et al. (1975) used the following equation from Mackay and Leinonen (1975) to estimate the evaporative half-life of the n-butyl ester of 2,4-D as a function of depth:

$$t_{1/2} = d \ln 2/k_L \qquad (3\text{-}6)$$

where

d = depth in meters

K_L = liquid mass transfer coefficient (in m/hr).

The liquid mass transfer coefficient is related to Henry's constant and the molecular weight. Henry's constant was estimated from the ratio of the vapor pressure to the aqueous solubility. They estimated the evaporative half-life of the n-butyl ester of 2,4-D from water in days to be approximately 1.1d, where d is the depth of the water in meters. For example, the estimated evaporative half-life from water that is 1-10 m deep was 1.1 to 11 days for the n-butyl ester of 2,4-D. Evaporative half-lives of the n-butyl ester of 2,4,5-T from water should be comparable. However, the n-butyl esters of 2,4-D and 2,4,5-T also undergo hydrolysis in water to 2,4-D and 2,4,5-T. Baur et al. (1973) observed negligible volatilization of 2,4,5-T or picloram from aqueous solution of the sodium salts incubated at 30°C for 7 days. Baur et al. (1974) observed similar results from the sodium salt of 2,4-D in aqueous solution. Therefore, the evaporative removal of the 2,4-D and 2,4,5-T moieties from water may depend upon the relative rates of evaporation and hydrolysis of the n-butyl esters.

3.1.6 Adsorption to Soils, Leaching, and Water Transport

Studies have indicated that the acidic herbicides picloram, 2,4-D, and 2,4,5-T are weakly adsorbed to soil (O'Connor and Anderson, 1974; Weber, 1972). The pK_a values for 2,4-D and 2,4,5-T are, respectively, 2.80 and 2.84. Since the pH of most soils is greater than 4.5, and that of most natural waters is greater than 6 (Tinsley, 1979; Stumm and Morgan, 1970), the acid herbicides will exist primarily in anionic forms in the environment. Therefore, the negatively charged anionic herbicide molecules are probably not readily adsorbed to soil because of the overall negative charge on the soil surface (Weber, 1972). Studies by Weber (1972) on the adsorption of 14 herbicides to soil indicated that the acidic herbicides 2,4-D and picloram were not adsorbed to soil as much as basic or cationic herbicides.

O'Connor and Anderson (1974) measured the adsorption of 2,4,5-T from water to four soils taken from the western United States. Adsorption to the soils fit Freundlich isotherms of the form

$$X = KC^{1/n} \qquad (3\text{-}7)$$

Where

X = amount of 2,4,5-T adsorbed in units of ug/g soil

C = concentration of 2,4,5-T in units of ug/ml

K = binding constant

1/n = empirical constant.

Although values of 1/n were not reported, they appeared from the graph to be fairly close to 1. Therefore, the observed binding constant K approximated sediment/water equilibrium partition coefficients. The value of K varied from 0.31 to 3.0 and was generally greatest for soils with the highest organic content. Chemicals having sediment/water partition coefficients under 10^2 are

classified as being weakly adsorbed (Mill, 1980). The values for the adsorption of 2,4,5-T to soils was obviously much less than 10^2, so the adsorption of 2,4,5-T to the soils tested was extremely weak.

Herbicides such as picloram, 2,4-D, and 2,4,5-T, which possess moderate aqueous solubilities (430 mg/l, 620 mg/l, 251 mg/l, respectively) but weakly adsorb to soil, should have some potential for leaching to surface runoff or groundwater (Tinsely, 1979; Bovey and Young, 1980). Although several field studies have indicated that 2,4-D and 2,4,5-T are susceptible to moderate leaching, this does not appear to be a major factor in the distribution of 2,4-D and 2,4,5-T in the environment since the highest concentrations of the compounds generally remain in the upper layers of the soil (Bovey and Young, 1980, in Associate Committee, 1978). For example, in tests conducted by the U.S. Air Force, 4,000 pounds per acre of Herbicide Orange were applied at a depth of 15 cm to soil plots in Utah. Although 2,4-D and 2,4,5-T residues were detected to a depth of 90 cm after 282 days, more than 90 percent of the residues remained in the top 30 cm of soil (Young et al., 1974, in Associate Committee, 1978).

Studies by Barnett et al. (1967; in Associate Committee, 1978) on the movement of 2,4-D residues in soil under simulated rainfall showed that most of the 2,4-D remained at a depth of 0-8 cm, although some was present at a depth of 8-15 cm. Only negligible amounts were detected below 15 cm.

The vertical displacement and leaching potential of picloram in some soils appears to be somewhat greater than for 2,4,5-T. Scifres et al. (1977) reported that no residues of 2,4,5-T could be detected in soil at a depth greater than 15 cm in a Southwest United States watershed that receives annual sprayings of 1:1 picloram and 2,4,5-T at approximately 1 pound per acre. However, low levels of picloram were detected to a depth of 60 cm. Although most of the 2,4,5-T detected was in the upper 2.5 cm of soil, substantial levels of picloram were detected to a depth of 15 cm.

Lutz et al. (1973) reported similar results for a North Carolina watershed receiving between 2 and 4 pounds per acre of 2,4,5-T and picloram. One hundred days after spraying, most of the 2,4,5-T residues remained in the top 8 cm of soil during the study, although some 2,4,5-T was detected to a depth of 45 cm. Although close to 70 percent of the picloram residues also remained in the top 8 cm of soil, a substantially higher proportion of picloram was detected at lower depths than for 2,4,5-T.

Based on studies performed on the movement of picloram in Texas and Puerto Rico soils, Bovey et al. (1969) concluded that substantial quantities of picloram can be leached from soils, particularly under conditions of heavy rainfall. The leaching of picloram and 2,4,5-T may lead to trace contamination of groundwater, as evidenced by the detection of low levels (less than 1 ppb) of picloram and 2,4,5-T in groundwater underlying an area of Texas that had received picloram and 2,4,5-T spray every 6 months for 2 1/2 years, at 2 pounds per acre.

There have been a number of studies on the removal of 2,4-D, 2,4,5-T, and picloram from soils by surface runoff water, either through leaching or by

erosion of soil particulates to which the herbicides are adsorbed (Barnett et al., 1967; Trichell et al., 1968; Edward and Glass, 1971; Sheets and Lutz, 1969; all in Bovey and Young, 1980; Lutz et al., 1973; Lawson, 1976). Although substantial quantities of the herbicides are often detected in runoff occurring soon after herbicide applications, concentrations rapidly decline in succeeding runoffs. Total losses of herbicides due to runoff over substantial periods of time do not generally account for more than 5 percent of the applied herbicide. For example, although concentrations of picloram, 2,4-D, and 2,4,5-T in initial runoff from recently sprayed experimental plots in North Carolina were often relatively high (0.3-4.2 ppm), concentrations in succeeding runoffs rapidly declined (Sheets and Lutz, 1969, in Bovey and Young, 1980). Total herbicide loss after several runoffs typically accounted for less than 1 percent of the applied herbicides. Nevertheless, substantial quantities of herbicides can be transported by surface runoff from watersheds receiving heavy herbicide spraying and heavy rainfall.

3.1.7 Environmental Persistence and Monitoring in Soil and Water

Several studies have shown that the relative persistence in soils of picloram, 2,4-D, and 2,4,5-T is generally picloram > 2,4,5-T > 2,4-D (Yoshida and Castro, 1975; Lutz et al., 1973; Altom and Stritzke, 1973). For example, Lutz et al. (1973) studied the persistence of picloram and 2,4,5-T applied at approximately 2 pounds per acre to North Carolina soils. Fifteen days after spraying, an average of approximately 40 percent of the picloram remained, compared to 10 percent of the 2,4,5-T. After 100 days only negligible amounts of 2,4,5-T remained, whereas approximately 10 percent of the original picloram remained. Persistence was related to initial concentration. Altom and Stritzke (1973) reported that the average half-lives of picloram, 2,4,5-T, and 2,4-D in three Oklahoma soils were, respectively, >100 days, 20 days, and 4 days.

Byast and Hance (1975) studied the degradation of ^{14}C-labeled 2,4,5-T in four South Vietnamese soils under laboratory conditions. Two soils were taken from an agricultural area in Bien Hoa Province. The other two soils were mangrove swamp soil from either the Rung Sat region or the Can Maw pennisula. After 49 days of incubation of the 2,4,5-T with the various soils, between 64.5 percent and 69.5 percent of the ^{14}C applied as 1 ppm ^{14}C-2,4,5-T had evolved to $^{14}CO_2$. After 168 days' incubation, between 76 percent and 79 percent of the ^{14}C applied as 15 ppm ^{14}C-2,4,5-T had evolved to $^{14}CO_2$. The initial concentration of 15 ppm 2,4,5-T is comparable to the estimated concentration the acid equivalent of 2,4,5-T would have if it were applied as the n-butyl ester of 2,4,5-T in Orange at 3 gallons per acre to a depth of 3 inches and assumed to be uniformly mixed (NRC, 1974).

Blackman et al. (1974) analyzed 11 soil samples taken in 1971 from a site in the Rung Sat region of Vietnam which received heavy herbicide spraying. The site had received at least 86 pounds per acre 2,4-D, 79 pounds per acre 2,4,5-T, 3 pounds per acre picloram, and 9 pounds per acre cacodylic acid. Four of the 11 samples contained 2,4-D concentrations equivalent to an application rate between 0.007 and 0.04 pounds per acre. All 11 samples contained 2,4,5-T concentrations equivalent to an application rate between 0.005 and

0.079 pounds per acre and picloram concentrations equivalent to an application rate between 0.002 and 0.01 pounds per acre. Picloram was not detected in filtered water samples taken from the lower part of the main shipping channel to Saigon in 1972 but was detected in suspended sediments removed from the water.

Schultz and Harman (1971) determined the persistence of 2,4-D in the water and mud of nine ponds located in Florida, Georgia, and Missouri. The ponds were sprayed with the dimethylamine salt of 2,4-D at the rate of 2, 4, or 8 pounds per acre acid equivalent of 2,4-D. Residues of 2,4-D declined in Florida and Georgia ponds, from maximums of 0.35 and 0.69 mg/l, respectively, observed 3 days after spraying, to less than 0.005 mg/l within 14 and 28 days, respectively, after spraying. Residues in Missouri ponds declined from a maximum of 0.63 mg/l to less than 0.005 mg/l within 56 days after spraying. 2,4-D residues in the mud of the Georgia and Florida ponds never exceeded 0.05 mg/kg and declined to less than 0.005 mg/kg within 56 days after spraying. A maximum value of 0.170 mg/kg 2,4-D residue was found in the mud of Missouri ponds but no 2,4-D residues could be detected in the mud of the Missouri ponds past 28 days after spraying.

(Norris, 1966) studied the persistence of 2,4-D and 2,4,5-T in forest floor litter under laboratory conditions. Approximately 85 percent of 2,4-D applied to red alder forest floor litter was decarboxylated within 300 hours. Less than 25 percent of the applied 2,4,5-T was decarboxylated within 300 hours, but 53 percent was degraded after 690 hours. Norris et al. (1977) studied the persistence of 2,4,5-T in a forest in the Northwest United States that had been treated with 2 pounds per acre of the isooctyl ester of 2,4,5-T. Residues of 2,4,5-T in vine maple, blackberry vines, grass, and Douglas Fir branches declined from minimum concentrations of from 11 ppm to 115 ppm (obtained almost immediately after spraying) to less than 0.5 ppm within 1 year after spraying. Maximum residues of 2,4,5-T on the forest floor (obtained approximately 1 month after spraying) declined 50 percent within 6 weeks and 90 percent within 6 months.

3.2 ENVIRONMENTAL FATE AND MONITORING OF TCDD

3.2.1 Physical and Chemical Properties

TCDD is a colorless crystalline solid at 25°C, with a melting point at 305°C and a molecular weight of 322 (Crummett and Stehl, 1973, in Esposito, 1980). The solubility of TCDD in water is approximately 0.2 ppb (0.2 ug/l); this is presumably at 20-25°C, but no temperature is given (Crummett and Stehl, 1973, in Esposito, 1980). The solubility of TCDD in Herbicide Orange is approximately 580 ppm, again presumably at 20-25°C (NRC, 1974). The vapor pressure and octanol/water partition coefficient could not be found in the literature. However, the low volatility of dried films of TCDD on glass and soil (Crosby et al., 1971) indicate that the vapor pressure is extremely low.

The chemical structure of TCDD is depicted in figure 2-3. TCDD is stable to acid and base treatment and its thermal decomposition temperature is above 750°C (NRC, 1974). Significant rates of nonbiological hydrolysis or oxidation

under normal environmental conditions are unlikely due to its chemical stability (EPA, 1979). The major routes of environmental transformation for TCDD appear to be photolysis and biotransformation. Rapid rates of TCDD photolysis by reductive dechlorination are observed in the presence of such adequate hydrogen donors as apparently are contained in Herbicide Orange (see section 3.2.2). Rates of biotransformation are slow by comparison but apparently can lead to substantial transformation over a period of several months to a year (see section 3.2.3).

3.2.2 Photolysis

TCDD has a solar radiation absorption maximum of 307 nm (Crosby et al., 1971), which is well above the approximate 290 nm limit of solar radiation which actually reaches the earth's surface (Tinsley, 1979). The compound exhibits a wide range of photolysis rates which are dependent upon the surrounding medium.

Crosby and Wong (1977) performed important photolysis experiments relative to the environmental fate of TCDD in Vietnam. They determined the photolysis rate of 15 ppm TCDD in Herbicide Orange irradiated by summer sunlight in California. The Herbicide was spread in thin layers (5 mg/cm^2) over borosilicate glass, as drops over excised rubber plant leaves (1.7 mg/cm^2 and 6.7 mg/cm^2), and on the surface of Sacramento loam soil (10 mg/cm^2). Samples were taken after various periods of irradiation of up to 6 hours, and benzene extracts were analyzed by gas chromatography for TCDD levels. After 6 hours of irradiation by sunlight, the following percentages of the TCDD originally in the Herbicide Orange remained:

- In layers over glass: 40 percent
- As drops on rubber plant leaves at 1.3 mg/cm^2: 25 percent
- As drops on rubber plant leaves at 6.7 mg/cm^2: negligible
- On Sacramento loam soil: 85 percent.

The authors postulate that the slower photolysis rate of TCDD in Orange spread on soil compared to that spread on glass or rubber plant leaves is due to shielding of soil-absorbed Herbicide Orange from the sunlight. The time required to remove 50 percent of the Orange on rubber plant leaves in both cases was less than 4 hours. The authors postulate that the apparent difference in photolysis rates for TCDD in Orange spread at different levels on the rubber plant leaves is primarily due to daily variations in sunlight intensity. Controls incubated in the dark were used to check on possible loss of TCDD by volatilization or through absorption to leaves or soil. The recovery of TCDD from controls stored in the dark for 6 hours was generally well over 98 percent, which indicated that neither volatilization nor poor extraction efficiency contributed significantly to the observed losses of TCDD in Orange irradiated by sunlight.

Crosby et al. (1971) determined the apparent photolysis rates of TCDD at 5 mg/l methanol, irradiated by sunlight and by UV light that approximately simulated sunlight. After various periods of irradiation, they analyzed samples by gas chromatography for TCDD levels. After 9 hours of irradiation by the UV lamp, less than 30 percent of the original TCDD in solution remained, and after 24 hours of irradiation only negligible amounts of TCDD remained. The major product of the TCDD photolysis was 2,3,7-trichlorodibenzo p-dioxin, although small amounts of a dichlorodibenzo-p-dioxin were also detected. Similar but faster TCDD photolysis rates were observed during irradiation by sunlight. After 4 hours of irradiation by sunlight, less than 40 percent of the original TCDD in solution remained, and after 7 hours only negligible amounts of TCDD remained. The results for experiments carried out in open flasks in sealed tubes were similar, which indicates that the contribution of volatilization to the removal of TCDD was negligible. Stehl et al. (1973, in Esposito, 1980) reported that TCDD in isooctane and octanol irradiated by artificial sunlight had a half-life of less than 40 minutes.

In contrast to the rapid photolysis rates observed for TCDD in Orange or various organic solvents, Crosby et al. (1971) observed negligible photolysis rates for TCDD in aqueous suspensions or on soil after solvent evaporation. They applied 2.4 ppm TCDD in methanol on 2.5 cm layers of soil. After the methanol was evaporated, both dry and water-moistened soil layers were irradiated with artificial sunlight for 96 hours. In another experiment, aqueous suspensions of TCDD were irradiated. The decomposition of TCDD was reported to be negligible in both the soil and the water.

TCDD spread on glass irradiated for 14 days was also stable to photolysis. Similar results were reported by Ward and Matsumura (1978). Twenty samples of lake water and sediment were incubated with ^{14}C-labeled TCDD for 39-40 days. Twelve samples were irradiated and eight samples were incubated in the dark. After the incubation period, the sediment and water were separated and extracted with various organic solvents. Thin layer chromatography was used to determine the percentage of recovered radioactivity that was in the form of ^{14}C-TCDD. The average recovery of TCDD from the samples incubated in the dark was 93.3 percent $\pm$ 7 percent. For samples irradiated with light, it was 89.5 percent $\pm$ 17.7 percent (sic). The difference in the recovery percentages does not appear to be statistically significant, so photolysis over the 39-40 day incubation period appears to have been negligible. However, the authors note that over 90 percent of the TCDD was recovered in sediments; thus, the sediment could have shielded much of the TCDD from the light. Also, although the authors do not specify the type of light used, they do indicate that the light intensity was low (50 to 80 foot-candles).

The products of TCDD photolysis typically appear to be less chlorinated and generally less toxic dioxins than TCDD, and continued irradiation can lead to decomposition of the dibenzo-p-dioxin structure. Crosby et al. (1971) indicated that continued irradiation of a methanol solution by sunlight for 26 hours after the complete photoreductive dechlorination of TCDD led to the decomposition of the dibenzo-p-dioxin structure as evidenced by the loss of solar radiation absorption above 290 nm.

The available literature indicates that TCDD undergoes rapid photolysis by direct sunlight when it is associated with Orange or various organic solvents, which can act as hydrogen donors during reductive dechlorination. This fact has prompted several groups to use organics on TCDD-contaminated soils to increase the TCDD photolysis rate (Esposito, 1980). In contrast, TCDD in water or left as a film on glass or soil after the evaporation of hydrogen-donating solvents appears to undergo negligible rates of photolysis. Therefore, TCDD released in Vietnam probably underwent rapid photolysis as long as it was associated with Orange or other herbicidal agents and was exposed to direct sunlight. However, based on the slow photolysis rate of TCDD not associated with organic solvents, the photolysis rate for any TCDD remaining after the evaporation of the more volatile n-butyl ester components of Orange probably would be much slower, and possibly negligible.

Another important aspect of predicting the rate of TCDD photolysis in Vietnam is the effect of shade. Orange, as well as other herbicide agents containing TCDD, did not cause complete defoliation until 1 to 2 months after application (Young et al., 1978). Therefore, TCDD in herbicidal agents penetrating the upper canopy of dense forests or vegetation may have remained shaded for several weeks. Unfortunately, little is known about the effect of shade on the rate of TCDD photolysis. Nash and Beale (1978) reported that TCDD is emulsifiable and that granular formulations of Silvex underwent photolysis in both shade and direct sunlight. However, they present only an average time required after volatilization to remove 50 percent of the TCDD for all conditions combined (7.7 days in emulsion formulation, 13.5 days in granular formulation).

3.2.3 Microbiological Degradation

The percentage of microorganisms capable of biodegrading TCDD appears to be low. Matsumura and Benezet (1973) tested the ability of approximately 100 microbiological strains to degrade TCDD. All of the strains previously had been shown to degrade pesticides normally considered persistent. However, only five of the strains tested were shown to have the ability to biodegrade TCDD.

Although no direct measurements of biotransformation rates for TCDD could be found in the literature, the rates appear to be slow based on reported persistence of TCDD in soil, sediments, and water (Kearney et al., 1972; Ward and Matsumura, 1978). Since the persistence of TCDD depends not only upon the rate of biotransformation, but also on photolysis rates and rates of transport, persistence studies are discussed separately in section 3.2.7.

The major products of the biotransformation of TCDD are apparently unknown. However, studies by Kearney et al. (1972) indicate that complete oxidation to CO_2 is negligible. They applied 1.78, 3.56, and 17.8 ppm ^{14}C-labeled TCDD to two Maryland soils. After a year of incubation, 52-89 percent of the original ^{14}C was recovered by combustion, the recovery percentage varying with soil type and initial TCDD application. According to the authors, the experimental design was such that loss of ^{14}C could occur only through volatilization of TCDD or its metabolites or the evolution of

$^{14}CO_2$ after complete oxidation of the ^{14}C-labeled TCDD. However, only negligible amounts of $^{14}CO_2$ were detected in the trapping solutions.

3.2.4 Leaching

The characteristics of a compound which primarily determine its leaching potential are its aqueous solubility and its adsorption to soil (Tinsely, 1979). TCDD has a low aqueous solubility (approximately 0.2 ppb), but its adsorption to soil is moderately strong (section 3.2.5). Therefore, the potential for substantial leaching of TCDD appears to be low. One method of experimentally estimating the leaching potential of a chemical is to determine its R_f value in thin layer chromatography using soil as the adsorbent layer, water as the solvent, and the chemical as the solute (Tinsley, 1979). Helling (1970, in Helling et al., 1973) studied the leaching potential of TCDD in five soils of various organic content with thin layer chromatography. He reported that the leaching potential for TCDD in all five soils was low.

Studies by the Air Force on the vertical distribution of TCDD in soils that received large quantities of herbicide agents during tests tend to confirm that significant leaching of TCDD in soils does not generally occur. For example, samples from a soil plot in Utah, 282 days after the application of approximately 4,000 pounds per acre of Orange (3.7 ppm TCDD average) to a depth of 6 inches, were analyzed for TCDD at various depths. The analysis showed the following average concentrations of TCDD: 0-6 inches in depth, 15,000 ppt; 6-12 inches, 3,000 ppt; 12-18 inches, 90 ppt; and 18-24 inches, 120 ppt. Therefore, over 82 percent of the TCDD detected remained at the 6-inch depth to which it had been originally applied. Furthermore, the detection of some, and perhaps most, of the TCDD present at lower depths was due to contamination during sampling. Analysis of the same plot 4 years later showed a reduction in average TCDD concentrations at all depth intervals, but most of the TCDD detected was still in the upper 0-6 inch level (Young et al., 1978): 0-6 inches, 6,600 ppt (56 percent reduction); 6-12 inches, 200 ppt (93 percent reduction); 12-18 inches, 14 ppt (88 percent reduction).

Analysis of soil samples taken from the Utah plot indicated that substantial leaching had not occurred over a 4-year period; but the average annual rainfall at the Utah plot was only 10 inches/year (Young et al., 1978). However, analysis of soil samples taken from plots receiving TCDD at Eglin AFB in Florida, where the average annual rainfall is 60 inches/year, were similar (Young et al., 1978). Samples taken 414 days after the application of Orange at 4,000 pounds per acre to a depth of 6 inches (TCDD concentration not specified) showed the following average concentration of TCDD (Young et al., 1976): 0-6 inches, 250 ppm; 6-12 inches, 50 ppm; 12-36 inches, <25 ppm (which was detection limit). Again, most of the TCDD was in the original 6-inch surface layer to which it had been applied.

Finally, Young (1975) determined the concentration of TCDD as a function of depth in soil samples taken in 1974 from a testing grid at Eglin AFB that had received 1,900 pounds per acre of Purple in various sprayings 10-12 years earlier. The analysis showed the following average TCDD concentrations as a function of depth: 0-1 inch, 150 ppt; 1-2 inches, 160 ppt; 2-4 inches,

700 ppt; 4-6 inches, 44 ppt; 6-36 inches, <10 ppt (which was the detection limit). Therefore, even after 10 to 12 years, at an average of 60 inches of rainfall per year, most of the TCDD detected was in the upper 6 inches of soil.

Leaching experiments performed by Matsumura and Benezet (1973) on soil columns, and by Nash and Beall (1978) on a model ecosystem, also indicate limited vertical dislocation of TCDD in soil to which water is continually applied. However, the experiments were not as long-term as the Air Force studies and significant quantities of TCDD were detected in the leachate. For example, levels of TCDD in the leachate from the ecosystem studied by Nash and Beall (1978) may have been as high as .06 ppb, which is approximately 25 percent of the aqueous solubility of TCDD. Levels of the TCDD in eluates from the soil column studied by Matsumura and Benezet (1973) were as high as 0.8 ppb, which is four times the aqueous solubility of TCDD and indicates that the leachate may have contained suspended soil matter to which TCDD was adsorbed. Therefore, the amount of TCDD that actually orginated from leaching was probably far less than the total TCDD in the leachate.

Although TCDD appears to have a very limited leaching potential, there is evidence that it may have leached from a landfill in New York containing 3,300 tons of trichlorophenol, from a landfill in Arkansas containing wastes from 2,4,5-T production, and from a dump site of the Hooker Chemical Company in Michigan (Esposito, 1980). However, the TCDD has been found primarily in the water and sediment of surrounding surface waters, indicating that TCDD transport may have occurred by soil erosion from contaminated surface layers instead of by leaching.

The results of the Air Force studies indicate that substantial contamination of groundwater in Vietnam by leaching of TCDD from soil was unlikely, even for periods of several years after spraying. Although the rainfall in Vietnam, particularly in the rainy season, may have been generally heavier than in Florida, the applications of TCDD in the Air Force experiments were far greater even than in areas of Vietnam receiving multiple spraying. Although there is evidence of some leaching of TCDD in the work of Matsumura and Benezet (1973), Nash and Beall (1978), and in reports of TCDD in the water and sediments of water bodies adjacent to dumping sites, most of the TCDD involved may have been transported by erosion of soil particulates to which TCDD was adsorbed.

3.2.5 Sediment or Soil/Water Partitioning

Isensee and Jones (1975) studied the partitioning of ^{14}C-labeled TCDD between soil, water, and aquatic species in a model aquatic ecosystem after equilibrium had been obtained. In various experiments, they added between .01 and 149 ug of TCDD to between 20 and 420 g of Lakeland sandy loam soil, Matapeake silt loam soil, or a mixture of the two soils at the bottom of an aquarium. They then added 4 liters of water and various aquatic species. After 30 days, they removed samples of soil and water from the aquarium. They removed suspended soil from water samples by centrifuge, and water from soil by drying, and analyzed the samples for ^{14}C. Thin layer chromatography of

extracts from some of the samples indicated that most of the ^{14}C was still in the form of ^{14}C-labeled TCDD. Therefore, estimates of the TCDD concentration in soil and water samples are based on the measured ^{14}C activity.

Data for the equilibrium adsorption of a chemical from water to soil can frequently be fitted to an empirical Freundlich equation of the form (EPA, 1979):

$$C_s = K_{sw} C_w^{1/n} \tag{3-8}$$

where

C_s = equilibrium concentration of chemical in soil in mass/mass units

C_w = equilibrium concentration of chemical in water in the same mass/mass units

K_{sw} = dimensionless sediment/water equilibrium partition coefficient

$1/n$ = empirical exponent.

Isensee and Jones (1975) did not attempt to fit their data to a Freundlich equation, so 1/n was not determined. However, in many cases 1/n = 1 (EPA, 1979). JRB calculated soil/water equilibrium partition coefficients from the data presented by Isensee and Jones (1975) by assuming 1/n = 1 in equation 3-8 and rearranging the equation to give

$$K_{sw} = C_s / C_w \tag{3-9}$$

The calculated values of K_{sw} for six sets of data varied from 1.1×10^4 to 2.1×10^4.

Karickhoff et al. (1979) derived the following relationship from a linear least squares analysis of the binding of several organics with low aqueous solubilities to soils or sediments with varying organic content:

$$\log K_{oc} = -0.54 \log S + 0.44 \tag{3-10}$$

where

$K_{oc} = K_{sw}/oc$

K_{sw} = soil or sediment/water equilibrium partition coefficient

oc = organic fraction of the soil or sediment

s = aqueous solubility in mole fraction.

TCDD has an aqueous solubility of approximately 0.2 ppb (0.2 ug/l) and a molecular weight of 322, so the molar solubility is approximately 6.2×10^{-10} moles/l. Therefore, the mole fraction solubility of TCDD in water is approximately $6.2 \times 10^{-10}/55.6$, or 1.1×10^{-11}. Substituting that value for the

aqueous mole fraction solubility in equation 2-10 and solving for K_{oc} gives $K_{oc} = 2.3 \times 10^6$. The organic fractions of the Lakeland sandy loam soil and the Matapeake silt loam soil used by Isensee and Jones (1975) were respectively 0.009 and 0.015. Therefore, the theoretical values for K_{sw} for Lakeland and Matapeake soil are 2.1×10^4 and 3.5×10^4, respectively, which are within a factor of 2 of the ones calculated from the data presented by Isensee and Jones (1975).

The model aquatic ecosystem of Isensee and Jones (1975) was allowed to approach at least an approximate equilibrium. However, most natural aquatic systems are far from equilibrium (Stumm and Morgan, 1970), so care must be taken in predicting typical soil or sediment/water partitioning from soil or sediment/water equilibrium partition coefficients. Nevertheless, some general guidelines have been suggested based on an empirical comparison of actual soil or sediment/water partioning to soil or sediment/water equilibrium partition coefficients (Mill, 1980). If a compound has a soil or sediment/water partition coefficient greater than 10^3 for a given soil or sediment it will generally exhibit high soil or sediment/water partitioning in natural systems where the given soil or sediment predominates. If the equilibrium partition coefficient is less than 10^2 for a given soil or sediment, the compound will exhibit low soil or sediment/water partitioning.

The K_{sw} values calculated for TCDD above were for soils with moderately low organic contents and ranged from 1.1×10^4 to 3.5×10^4. Soils with higher organic contents would be predicted to exhibit proportionately higher soil or sediment/water equilibrium partition coefficients for the adsorption of TCDD. Therefore, based on the guidelines presented by Mill (1980), TCDD would be predicted to exhibit moderately high to high soil or sediment/water partitioning in most natural systems, depending upon the organic content of the predominant soil or sediment.

The apparently moderately high soil or sediment/water partitioning of TCDD probably inhibits the leaching of TCDD from soil to groundwater or surface water, as discussed in section 3.2.4. However, it may occasionally lead to transport of larger quantities of TCDD to surface water than would be predicted from its low aqueous solubility and leaching potential, by waterborne transport of suspended soil or sediment particulates to which TCDD is adsorbed. Matsumura and Benezet (1973), Ward and Matsumura (1978), and Isensee and Jones (1975) have detected TCDD levels several times greater than its aqueous solubility in unfiltered or filtered but uncentrifuged water samples from model ecosystems. Therefore, most of the TCDD in the water samples may have been adsorbed to suspended particulate matter. The model ecosystems were probably somewhat more static than most natural water systems, so the concentration of suspended particulate matter in those model systems was probably far less than would be observed in natural aquatic systems such as rivers, or in flood or rain water flowing over areas contaminated with TCDD. Therefore, it is conceivable that some TCDD could be transported from contaminated areas to rivers, streams, lakes, or ponds by water erosion of soil particulates to which TCDD is adsorbed. Furthermore, once the TCDD-contaminated particulates reached a river or stream, they could be transported farther downstream, or deposited as sediments which could be resuspended and transported downstream during periods of heavy flow.

As mentioned previously, the TCDD detected in the water and sediments of surface waters surrounding landfills and dump sites could have been transported by water erosion of TCDD-contaminated soil. Young et al. (1978) indicate that 10-35 ppt levels of TCDD in the silt of a pond and stream adjacent to a test area in Eglin AFB, Florida that had received heavy herbicidal spraying was probably primarily due to soil erosion; the TCDD was detected only at points where eroded soil entered the water. Bartleson, Harrison, and Morgan (1975; in Esposito, 1980) measured TCDD levels in the soil of the test area at Eglin AFB and reported that the highest levels of TCDD in the soil were generally found in low-lying areas and the lowest levels in areas of loose soil. Again, this supports the postulate that TCDD transport had occurred by water erosion of TCDD-contaminated soil (Esposito, 1980).

Shadoff et al. (1977) measured an average 5 ppt TCDD in the mud and 0.2 ppt TCDD in the water (each based on two samples) of a pond in Arkansas that received rainfall and irrigation runoff from rice fields treated with 1.25 pounds per acre of 2,4,5-T at various times for 18 years prior to the study. However, the pond water was repeatedly reused for irrigation of recently sprayed rice fields, so the low levels of TCDD detected could be explained by leaching alone, although some of the TCDD may have been transported by soil erosion. They also measured an average 3 ppt TCDD in the mud and 0.1 ppt TCDD in the water (each based on two samples) of an impoundment in Texas that received drainage from large areas receiving between 0.5 and 4 pounds per acre of 2,4,5-T for mesquite and brush control. Again, however, although some TCDD transport by water erosion of soil could have occurred, the low levels of TCDD measured from a large drainage area could probably be accounted for by leaching alone.

3.2.6 Volatilization and Transport in Air

Volatilization does not appear to be a significant short-term removal pathway for TCDD in the environment. Although the vapor pressure of TCDD could not be found in the literature, several short-term experiments have indicated that it is non-volatile.

Crosby et al. (1971) applied 2.4 ppm TCDD in methanol to soil and, after evaporating the methanol, irradiated the soil for 96 hours with a UV lamp. After the irradiation, almost all of the TCDD was recovered from the soil. The authors concluded that TCDD losses due to both photolysis and volatilization were negligible. Crosby and Wong (1977) applied 15 ppm TCDD in Orange to soil and exposed rubber plant leaves. Some of the soil and leaf samples were irradiated with sunlight for 6 hours and some were kept in the dark as controls. Although there were substantial losses of TCDD by photolysis from samples exposed to sunlight, TCDD losses from the dark controls were negligible. Therefore, the authors concluded that losses due to volatilization were negligible. The time period between application of the Orange and extraction for the control samples was not given, but it was at least 6 hours.

Although there have been suggestions in the literature that volatilization may contribute significantly to the removal of TCDD over longer time periods, the evidence is inconclusive since it does not take into consideration other possible routes of removal. For example, Isensee and Jones (1971)

applied ^{14}C-labeled TCDD in an aqueous surfactant to the leaves of a soybean plant and to oats. The leaves and oats were analyzed for ^{14}C content 2, 7, 14, and 21 days after application. After 2 days, the soybean leaves contained 92 percent and the oats contained 83 percent of the applied ^{14}C. No further loss of ^{14}C was observed in the soybean leaves, but oats continued to lose ^{14}C slowly, and contained 63 percent of the applied ^{14}C after 21 days. Although the authors attribute ^{14}C losses to the volatilization of TCDD, other routes of removal were possible, including photolysis, as suggested by Crosby and Wong (1977), volatilization of TCDD metabolites, and translocation in the plants.

Ward and Matsumura (1978) added ^{14}C-labeled TCDD to samples of lake sediment and incubated the sediments with lake water in 20-ml glass culture tubes with loosened screw caps. Different samples were incubated for different lengths of time, up to 589 days. After incubation, sediments were separated from water (usually by filtration), the sediment and water were extracted with organic solvents, and the radioactivity of the solvents was measured to determine the loss of radioactivity from the sediment/water samples. Water loss from the samples was also measured. The authors detected a direct relationship between the total loss of radioactivity and the loss of water from various samples. The authors suggested that the observed relationship indicated that the loss of radioactivity may have been related to water-mediated evaporation of TCDD. However, the loss of radioactivity may have been due primarily to volatilization of metabolites of ^{14}C-TCDD, which are more volatile than TCDD, or to the evolution of $^{14}CO_2$ during metabolism of ^{14}C-TCDD. The graph the authors present depicting loss of radioactivity versus water loss is similar to the graphs they present depicting radioactivity loss versus incubation time. Therefore, the observed relationship between radioactivity loss and water loss could be due to the dependence of both water loss and production of volatile metabolites from TCDD on length of incubation.

Transport of TCDD in the air is not dependent upon volatilization alone. Other possible mechanisms of air transport include wind erosion of TCDD-contaminated soil, spray drift of herbicides such as Orange that contain TCDD, and the introduction of TCDD-contaminated particulates into the air by burning. Some of the horizontal dispersion of TCDD (discussed in the previous section) that was attributed to leaching or water erosion of soil could have been due to wind erosion of TCDD-contaminated soil (Esposito, 1980). Tests by Harrigan (1970) indicated that as much as 13 percent of the Orange sprayed at targets under operational parameters similar to those used in Vietnam missed test areas because of both spray drift and volatilization. As mentioned previously, some of the herbicide missions flown in Vietnam were for the purpose of drying areas prior to massive fire bombing. Reports of problems in the fly ash of municipal incinerators (Esposito, 1980) and the relatively high thermal decomposition temperature of TCDD indicate that introduction of some TCDD-contaminated particulates into the atmosphere during the burning of forests and crops was possible.

3.2.7 Environmental Persistence

The persistence of TCDD at a given location in a single phase of the environment will depend upon its tranformation rate within the phase, its rate of transport within the phase, and its rate of transport to other phases.

Although it is sometimes possible to estimate qualitatively the relative importance of various removal processes, it is rarely possible to determine quantitatively the relative contribution of each process to the overall rate of removal. Therefore, determinations of the persistence of TCDD in the environment are discussed separately from discussions of specific removal processes.

Kearney et al. (1972) determined the persistence of TCDD in Lakeland, Maryland loamy sand soil (organic matter 0.9 percent) and in Hagerstown, Maryland silty clay loam soil (organic matter 2.5 percent) under laboratory conditions. Initial concentrations of 1, 10, and 100 ppm TCDD were established in both soils by application of benzene solutions of TCDD to 100 g samples of soil. Soils were sampled on the day of application and 20, 40, 80, 160, and 350 days afterwards. The samples were extracted with hexane-acetone and analyzed for TCDD by gas chromatography. The percentage recovery of applied TCDD decreased with time. After 350 days, the percentages of TCDD recovered from initial concentrations of 1, 10, and 100 ppm in Lakeland soil were, respectively, 54, 57, and 56 percent. Therefore, the persistence of TCDD in the Lakeland soil appeared to be independent of initial concentrations of TCDD, which would be consistent with first order and pseudofirst order kinetics. After 350 days, the percentages of TCDD recovered from initial concentrations of 1, 10, and 100 ppm in Hagerstown soil were, respectively, 54, 63, and 71 percent. Therefore, in Hagerstown soil, there did appear to be some dependence of persistence on the intial concentrations of TCDD. Also, the persistence of TCDD applied at 10 and 100 ppm in Hagerstown soil appeared to be slightly longer than in Lakeland soil, even though the authors postulated that the microbiological population in Hagerstown would be greater than in Lakeland soil because of the higher percentage of organic matter in Hagerstown soil. However, the apparent dependency of TCDD persistence on initial TCDD concentrations or on soil type may instead be due to differences in efficiency of extraction. For example, soils with higher organic content, such as Hagerstown soil, would be expected to bind TCDD more stongly that soils with lower organic content, such as Lakeland soil (Karickhoff et al., 1979). Therefore, the efficiency of extraction may have been lower for Hagerstown soil than for Lakeland soil. Although the authors give an average extraction efficiency of 85 percent, they do not give extraction efficiencies for each initial TCDD concentration in each soil. Since the average extraction efficiency was 85 percent, the persistence of TCDD in the soil tested was considerably greater than indicated by the recovery percentages. Therefore, the time required to remove 50 percent of the applied TCDD in the soils tested was much greater than 1 year. Furthermore, if the removal processes approximate first order or pseudo-first order kinetics, the time required to remove 50 percent of the applied TCDD will be independent of applied concentration and, therefore, always greater than 1 year.

Ward and Matsumura (1978) determined the persistence of TCDD in Lake Menelota, Wisconsin water and sediment under laboratory conditions. They applied ^{14}C-labeled TCDD to glass tubes containing approximately 5 grams of wet sediment and 18 ml of water. After incubation, the sediment and water were separated by filtration and extracted with organic solvents. The ^{14}C content of the extracts was determined by liquid scintillation counting. The ^{14}C content of the sediments was also determined by counting the $^{14}CO_2$ evolved and trapped during combustion. Some of the sediment and water organic extracts were evaporated and the remaining residues were dissolved by ether or

acetone and analyzed by thin layer chromatography (TLC) to determine the percentage of the recovered ^{14}C that was still ^{14}C-TCDD. After 588 days of incubation, between 44.5 and 52.2 percent of the applied ^{14}C was recovered from the sediment by combustion and between 42.1 and 49.3 percent by extraction. Analysis by TLC indicated that between 95.6 and 99 percent of the ^{14}C recovered from sediments was still ^{14}C-TCDD. The results of the analyses of the water over the sediment after 588 days of incubation were not given. However, after 167 days of incubation with another type of Lake Menelota sediment, less than 2 percent of the orginally applied ^{14}C was in the aqueous phase. Therefore, it is probable that most of the ^{14}C remaining after 588 days incubation was in the sediment phase. Since slightly less than 50 percent of the applied ^{14}C was recovered from the sediment after 588 days and since most of the recovered ^{14}C was ^{14}C-TCDD it appears that the time required under laboratory conditions to remove 50 percent of the applied TCDD in the Lake Menelota sediment tested was approximately 600 days.

Ward and Matsumura (1978) also determined the persistence of TCDD in Lake Menelota water without sediment. After approximately 590 days of incubation, between 67.1 and 75.8 percent of the applied ^{14}C was recovered, of which over 98.4 percent was shown by TLC to be TCDD. Therefore, the persistence of TCDD in the tested lake water was longer than in the tested lake sediment under laboratory conditions.

In 1972, the U.S. Air Force began field tests on soil plots in Utah and in Florida to determine the persistence of TCDD in soil (Young et al., 1978; Young et al., 1976; Commoner and Scott, 1976). The Utah soil was clay loam with 1.4 percent organic content and a pH of approximately 7.8 (Young et al., 1976). The Florida soil was sandy loam with 0.5 percent organic content and a pH of approximately 5.6. Approximately 4,000 pounds per acre of Orange that contained TCDD were applied to the plots in both Utah and Florida. However, Commoner and Scott (1976) point out that the actual application level was higher, since the Orange was deposited uniformly, to a depth of six inches, in narrow swaths within the plots. The average concentration of TCDD in the Orange used in Utah was 3.7 ppm, but the concentration of TCDD in the Orange used in Florida was not reported (Commoner and Scott, 1976). As mentioned previously in section 3.2.4, most of the recovered TCDD remained in the top 6 inches of soil even after several years. The concentration of TCDD in composite soil samples taken from the plots in Utah and Florida at various times after Orange application are given below (Young et al., 1976):

Days After Applications	Florida	Utah
5	375 ppt	—
282	—	15 ppb
414	250 ppt	—
513	75 ppt	—
637	—	7.3 ppb
707	46 ppt	—
780	—	5.6 ppb
1,000	—	3.2 ppb
1,150	—	2.5 ppb

Under conditions of low pollutant concentration, the kinetics of biodegradation will sometimes approximate a second order reaction of the form (EPA, 1979):

$$\frac{dc}{dt} = - k_b [C] [X] \qquad (3\text{-}11)$$

Where

k_b = second order rate constant

C = pollutant concentration

X = cell population.

Under conditions of low pollutant concentration and high cell population, the cell population can sometimes be assumed to remain relatively constant (after the log period) to give a pseudo-first order equation of the form:

$$\frac{dc}{dt} = k_b - k_b [C] \qquad (3\text{-}12)$$

Where

k_b^1 = pseudo-first order rate constant = $k_b [X]$

Integration of equation 2-12 gives

$$\ln C = -k_b^1 t + \ln C \qquad (3\text{-}13)$$

Where

C_o = Initial pollutant concentration.

Therefore, a plot of ln C versus t for a process following pseudo-first order kinetics should be linear with a slope equal to (k_b^1).

Figure 3-1 is a plot of ln/2.3 versus t for both the Utah (concentrations in ppb) and Florida (concentrations in ppt) experiments with TCDD persistence in soil. The plot is derived from the kinetic data given above and is taken from the semi-logarithmic plots given in figure III-2 of Young et al. (1978). The lines drawn are linear least squares fitted to the data points. The data points from the Utah experiments all fit closely to the line, which indicates that the degradation rate may have followed closely psuedo-first order kinetics over the time period covered (282 days after Orange application to 1,150 days after Orange application). The slope of the line gives k_b^1 (Utah) for the Utah experiments, which is equal to -2.1×10^{-3} days^{-1}. Contrary to the low scatter of the Utah points, the four data points from the Florida

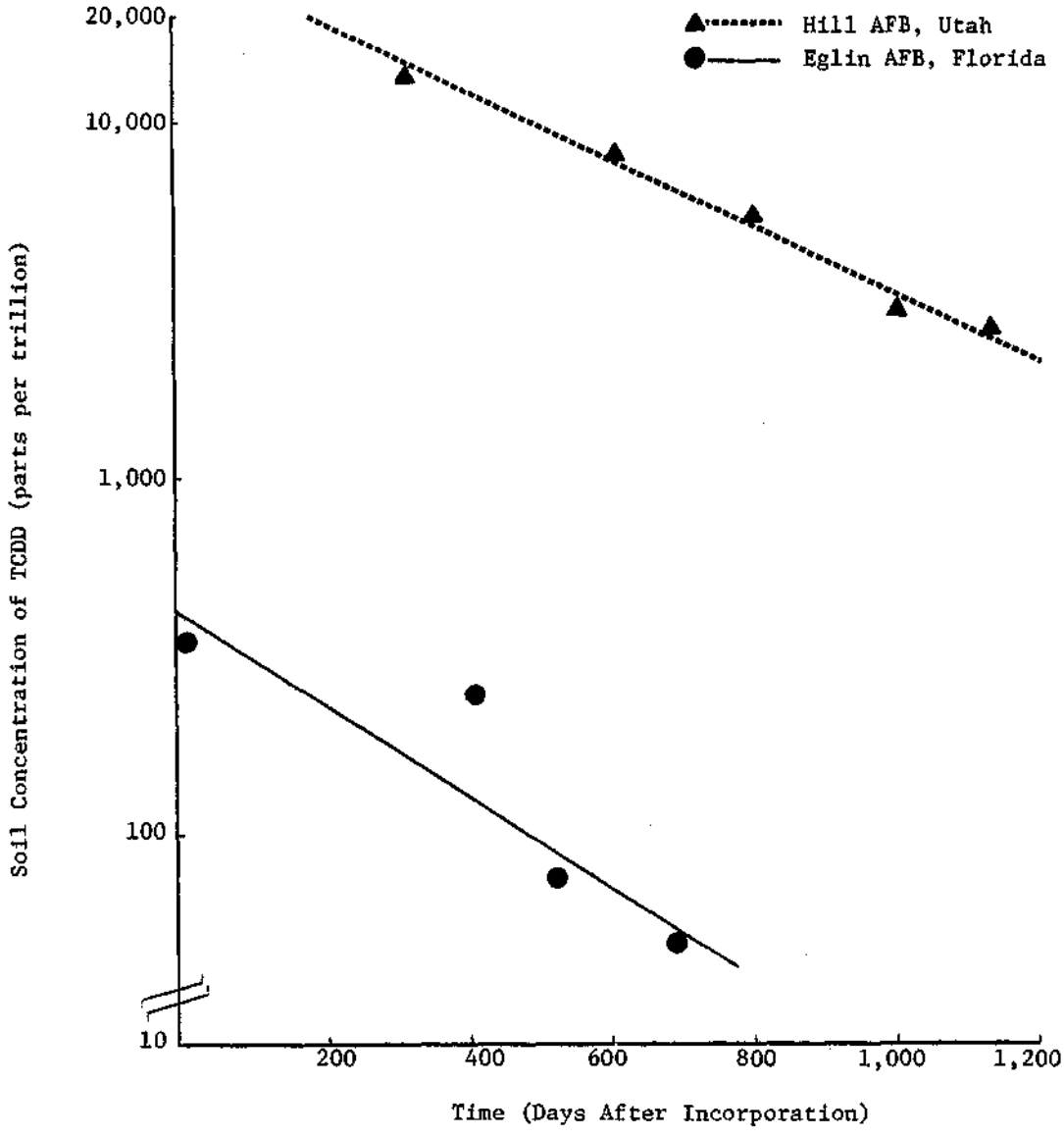

Figure 3-1. SEMI-LOGARITHMIC PLOT OF SOIL CONCENTRATIONS (PARTS PER TRILLION) OF TCDD IN HERBICIDE ORANGE BIODEGRADATION STUDIES AT EGLIN AFB, FLORIDA, AND HILL AFB, UTAH

(Figure III-2, Young et al., 1978)

experiment are widely scattered. Therefore, another linearly fitted line was drawn through the last three data points which reduced the scatter somewhat. The slope of the line based on three points is equal to -5.31×10^{-3} days^{-1}, which we assume to be equal to k_b^1 (Florida).

The scatter of the data points from the Florida experiment may have been due to the initial presence of a lag time during which cell acclimation could take place and which would not follow pseudo-first order kinetics until acclimation had occurred. Note that the first Florida data point is well below the line linearly fit to the last three data points. There is no apparent lag time from the Utah data, but in contrast to the first Florida data point at 5 days after Orange application, the first Utah data point is at 282 days after Orange application. Therefore, the lag time may have occurred prior to 282 days. However, Young et al. (1978) estimate that the initial concentration of TCDD in the Utah soil was 148 ppb based on calculations but not on actual measurement. If their calculations are accurate, the initial rate of TCDD degradation prior to 282 days would have been much greater instead of much slower than the apparently constant pseudo-first order rate after 282 days.

During the periods that the rate processes follow pseudo-first order kinetics, the half-lives can be calculated from the following equation derived from equation 2-13 by substituting 1/2 Co for C and rearranging:

$$t_{1/2} = \ln 2/k_b^1 \qquad (3\text{-}14)$$

Where

$t_{1/2}$ = half-life during time periods in which pseudo-first order kinetics are followed.

Substituting the values of k_b^1 (Utah) and k_b^1 (Florida) into equation 3-6 gives: $t_{1/2}$ (Utah) = 330 days and $t_{1/2}$ (Florida) = 130 days. The $t_{1/2}$ (Utah) value is the same as the one estimated by Young et al. (1978), but the $t_{1/2}$ (Florida) estimate is shorter because they based their estimate on a linear fit to all four data points.

The half-lives calculated for time intervals over which first order kinetics appear to be approximately followed are, of course, not valid for time intervals in which first order kinetics are not followed. For example, the time required to remove 50 percent of the applied TCDD from the Florida plot is obviously greater than the calculated half-life, assuming first order kinetics over the last three data points. However, if the estimated initial concentration of 148 ppb TCDD (Young et al., 1978) in the Utah plot is accurate, the time required to remove 50 percent of the applied TCDD was much less than the calculated half-life, assuming first order kinetics for the data points presented.

Based on the Air Force studies, it appears that TCDD may be removed from some soils by pseudo-first order biological processes after an initial period of acclimation. The half-life for pseudo-first order kinetics is independent

of the TCDD concentration. Therefore, even though TCDD concentrations in the Air Force studies were probably far higher than the concentrations which would have been found in Vietnamese soils, the estimated pseudo-first order half-lives for the Air Force study may serve as a useful estimate of pseudo-first order half-lives in Vietnamese soils with the following limitations: 1) the pseudo-first order half-lives should be dependent on the quantity and type of cell populations in the soil and temperature, which may be quite different in Vietnamese soil; and 2) initial non-first order degradation rates will depend upon initial TCDD concentrations in the soil.

3.2.8 Bioaccumulation, Bioconcentration, and Biomagnification by Aquatic Organisms

Several groups have determined the potential bioaccumulation (uptake), bioconcentration (ratio of tissue to water concentrations), and biomagnification (increase in tissue concentrations at succeedingly higher trophic levels) of TCDD by aquatic organisms in model ecosystems.

Matsumura and Benezet (1973) determined wet weight bioconcentration factors (ratio of TCDD concentrations in wet tissue to TCDD concentrations in water) for daphnia and ostracod, which are freshwater crustaceans, and for brine shrimp, mosquito larvae, and silverside fish. The bioconcentration factors for daphnia and ostracod were determined as follows: a thin film of ^{14}C-labeled TCDD was applied to the inner surface of a glass container and algae, which is a food source for daphnia and ostracod, was grown for 24 hours in the container. The algae was then transferred along with the culture medium to an aquarium containing the crustaceans for 4 to 7 days. The ^{14}C content of the water was determined by extracting with chloroform and counting the ^{14}C in the extract. The ^{14}C content in tissues was determined either by homogenizing and then extracting the TCDD from the tissues, or by tissue construction followed by measuring the evolved $^{14}CO_2$ in a trapping solution. In all cases, the ^{14}C content of the tissues and water were assumed to be still in the form of ^{14}C-TCDD but verification of the assumption was either not done or not discussed. The measured bioconcentration factors for daphnia and ostracod were, respectively, 2,198 and 107 g, based on reported water concentrations of 0.4 ppb and 2.6 ppb, respectively. However, the reported water concentrations were higher than the reported aqueous solubility of TCDD (0.2 ppb), so some of the measured water concentration was probably TCDD adsorbed to suspended particulate matter and not necessarily available for uptake. Bioconcentration factors based on the aqueous solubility of TCDD (0.2 ppb) would be 4,395 and 1,395 g for daphnia and ostracod, respectively. TCDD levels in the algae were not reported, so estimates of biomagnification potential cannot be made.

Matsumura and Benezet (1973) determined bioconcentration factors for brine shrimp, mosquito larvae, and silverside fish as follows. A thin film of ^{14}C-labeled TCDD was deposited on 1 g of sand and then added to an aquarium containing the test organisms. After 4 to 7 days, the tissue and water were analyzed for ^{14}C content as described previously. The ^{14}C content was again assumed to be entirely ^{14}C-labeled TCDD. The measured bioconcentration factor for the brine shrimp based on a TCDD concentration in water of 0.1 ppb was 1.57×10^3. The bioconcentration factors for the mosquito larvae and the

silverside fish were, respectively, 2.85×10^3 and 54, based on a water concentration (including food) of 1.3 ppb. Therefore, although the mosquito larvae serves as a food source for the fish, there does not appear to be any biomagnification of TCDD. However, the experiments were short-term (4 to 7 days).

Isensee and Jones (1975) determined bioconcentration factors on a dry weight basis for algae, snails, daphnids, and mosquito fish in model ecosystems. The aquatic organisms represented part of two food chains: algae --> snails, and daphnids (small freshwater crustaceans) --> mosquito fish. Thus, biomagnification potential was also determined. The experiments were run as follows. They applied ^{14}C-labeled TCDD to soil, placed the soil in tanks and filled the tanks with approximately 4 liters of water. One day later, approximately 100 daphnids, eight snails, and algae were placed in the tanks along with a few ml of old aquarium water which contained diatoms, protozoa, and rotifers. After 30 days, two mosquito fish were added to each tank. After 33 days the organisms were removed and analyzed for ^{14}C content. The tissues were dried and then combusted. The $^{14}CO_2$ that evolved during the combustion was trapped in solution and counted. The analysis of methanol extracts of the mosquito fish and snails by TLC indicated that approximately 87-93 percent of the ^{14}C was probably TCDD. The calculation of bioconcentration factors was based primarily on the assumption that all ^{14}C was ^{14}C-TCDD. The ^{14}C content of the water was determined by centrifuging the water to remove suspended sediment, evaporating the water, and analyzing the remaining residue by combustion (counting of evolved $^{14}CO_2$ in trapping solution). Final water concentrations for eight experiments ranged from 0.05 ppt to 239 ppt, which is approximately the aqueous solubility of TCDD. The bioconcentration factors for algae ranged from 2.0×10^3 to 1.9×10^4, with an average of 9.5×10^3. The bioconcentration factors for the snails ranged from 5.4×10^3 to 4.7×10^4, with an average of 2.2×10^4. The bioconcentration factors for the snails were slightly higher (approximately 2x) than those for the algae but no significant biomagnification was observed. The bioconcentration factors for the daphnids ranged from 1.8×10^4 to 4.8×10^4 with an average of 2.9×10^4. The bioconcentration factors for the mosquito fish ranged from 9.2×10^3 to 6.3×10^4 with an average of 2.8×10^4. Again, no significant biomagnification was observed. However, the mosquito fish were in the tanks for only 3 days.

Although the extrapolation from model ecosystems to the environment is uncertain, it appears that TCDD levels in aquatic organisms exposed to TCDD in water for several days will probably be significantly greater than the TCDD concentrations in the water. However, there is no evidence that significant biomagnification will occur during progression through different entropic levels.

Several studies have been performed to determine TCDD levels in aquatic organisms from water that has received direct 2,4,5-T application or that contains runoff from areas treated with 2,4,5-T. Shadoff et al. (1977) failed to detect TCDD (detection limit 10 ppt) in 10 samples of bass or one sample each of the viscera and eggs of catfish from a pond in Arkansas, which received rainfall and irrigation drainage from rice fields treated with 1.25 pounds per acre of 2,4,5-T for at least 18 years and whose water was continually reused for irrigation. They also failed to detect TCDD in

10 samples of catfish and walleyed pike from a Texas impoundment which received drainage from a large area treated with 0.5-4 pounds per acre and equivalent of 2,4,5-T for at least 20 years. Although the 2,4,5-T application per spraying was lighter in Arkansas and Texas than in Vietnam (0.5-4 pounds per acre compared to 13.2 pounds per acre in Vietnam), the number of repeated sprayings was generally greater and the duration of spraying was longer than in Vietnam. Also, the water in the pond in Arkansas was used repeatedly for irrigation of fields sprayed with 2,4,5-T 4 to 8 weeks previously.

Young (1975; Young et al., 1978) detected approximately 12 ppt TCDD in one body sample of mosquito fish and two viscera samples of sailfin fish taken from a stream adjacent to a 1-mile-square test area at Eglin AFB which had received approximately 160,000 lbs of 2,4,5-T (250 pounds per acre) from 1962 to 1970. They detected 4, 18, 4, and 85 ppt TCDD in pooled samples of skin, gonad, muscle, and gut, respectively, from sunfish taken from a pond on the test area. Analysis of the gut of bluegill indicated that a major food source was terrestrial insects. They failed to detect TCDD in numerous other fish species taken from the stream and pond. Bartleson, Harrison, and Morgan (1975; Esposito, 1980) detected an average concentration of 150 ppt TCDD in the composite bodies of 20 mosquito fish taken from a pond next to a herbicide loading area at Eglin AFB (Florida). They also detected between 150 ppt and 740 ppt TCDD in the liver and fat of 18 sunfish taken from the pond.

Dow (1978; Esposito, 1980) determined TCDD levels in fish from the Tittahanasee River, which receives treated wastes from a Dow plant. TCDD levels in catfish taken from various locations in the river and its tributaries ranged from 70 ppt to 230 ppt.

Baughman and Messelson (1973) detected between 18 ppt and 810 ppt average TCDD concentrations in fish and crustaceans caught by Vietnamese fishermen in 1970. The fish and crustaceans analyzed were caught in or near areas which received heavy sprayings of herbicide from 1967 through 1970. The specimens were frozen and analyzed approximately 2 1/2 years later. The tissues were homogenized and TCDD was extracted from the tissues with various organic solvents. The extracts were analyzed for TCDD by gas chromatography-mass spectroscopy. Carp and two species of catfish caught in the Dong Nai River a few miles northeast of Saigon contained average TCDD concentrations of 540, 810, and 520 ppt, respectively. Catfish and river prawn caught in the Saigon River a few miles northwest of Saigon contained 70 ppt and 42 ppt TCDD respectively. Croaker and prawn caught off the coast near the Rung Sat region southeast of Saigon contained an average 79 ppt and 18 ppt TCDD respectively. The TCDD concentrations given were calculated on a wet tissue basis.

3.2.9 Bioaccumulation, Partitioning, and Monitoring in Terrestrial Animals and Plants

Studies by Fanelli et al. (1980), the Air Force, and Young et al. (1978) on the levels of TCDD in animals collected from areas with high TCDD contamination indicate that the bioconcentration in terrestrial animals of TCDD from soil may be generally less than the concentration in aquatic organisms of TCDD from water. Fanelli et al. (1980) detected TCDD in field mice collected in a heavily contaminated area near Seveso, Italy. TCDD concentrations in the mice ranged from 0.07 ppb to 49 ppb and averaged 4.5 ppb, based on 14 samples.

TCDD concentrations in the upper 7 cm of soil collected from the same area ranged from 0.01 ppb to 12 ppb with an average of 3.5 ppb. Young et al. (1978) summarized various Air Force determinations of TCDD levels in wildlife and soil collected from 1972 to 1978 in a 3-kilometer testing area at Eglin AFB (Florida) which had received approximately 73,000 kg of 2,4,5-T contained in Orange and Purple between 1962 and 1970. Concentrations of TCDD in wildlife included beach mice (300-1,500 ppt in liver), hispid cotton rat (<10-210 ppt), meadowlark (100-1,020 ppt in liver), mourning dove (50 ppt in liver), Savannah sparrows (69 ppt in liver), and the six-lined racerunner lizard (360-430 ppt in muscle). TCDD levels in 54 soil samples collected from the same area ranged from <10 ppt to 1,500 ppt and averaged 165 ppt. Although it appears, based on the above studies, that bioconcentration factors for terrestrial animals are generally less than aquatic species, terrestrial animals were not necessarily confined to the test area (particularly the birds), contrary to the aquatic species which were confined to aquariums.

Several groups have tried to determine TCDD levels in cow milk and in beef fat of animals grazing on land which was sprayed with 2,4,5-T or accidently contaminated with TCDD. Fanelli et al. (1980b; Esposito, 1980) determined TCDD levels in milk in the Seveso, Italy area shortly after an industrial accident had released large quantities of TCDD to the environment. Milk from cows less than 1 km to over 5 km south and southeast of the plant was analyzed. Concentrations of TCDD in the milk ranged from 59 ppt to 7,919 ppt, depending primarily upon the distance of the grazing pasture from the plant. However, the release of TCDD in the Seveso area was several orders of magnitude greater than releases of TCDD to areas in Vietnam. Mahle et al. (1977) failed to detect TCDD to 1 ppt in milk from cows in Missouri, Arkansas, and Oklahoma that grazed on lands that typically were sprayed with 2 pounds per acre of 2,4,5-T annually. The application of 2,4,5-T in Vietnam per spraying was typically much heavier (13.4 pounds per acre), but was generally not repeated on an annual basis. Kocher et al. (1978), Meselson et al. (1978), and Solch et al. (1978), all in Esposito (1980), have detected TCDD in beef fat from cattle which grazed on lands treated with 2,4,5-T. TCDD levels ranged from 4 ppt to 70 ppt.

Studies by Isensee and Jones (1971) indicate that only small amounts of TCDD were bioaccumulated from soil by oats and soybeans. They applied ^{14}C-labeled TCDD to soil at 0.06 ppm and 0.10 ppm. Oats and soybeans grown to maturity in the soil contained less than 0.15 percent of the applied ^{14}C. Other studies indicated that translocation of TCDD applied to the leaves and other parts of oat and soybean plants was negligible. Approximately 94 percent of the applied TCDD remained on the soybean leaves 21 days after application.

Cocucci et al. (1979) studied the adsorption and translocation of TCDD in various garden vegetable plants and fruit trees grown in TCDD-contaminated soil in the Seveso, Italy area. The average TCDD levels in the aerial parts of carrot, potato, onion, and narcissus plants were generally somewhat lower than the TCDD levels in underground parts, but TCDD levels in both were generally comparable to TCDD levels in the surrounding soil. Studies on cherry, fig, pear, apricot, and peach trees indicated that TCDD levels were generally lower in fruits than in leaves and much lower in fruits than in twigs.

3.3 ENVIRONMENTAL FATE OF CACODYLIC ACID

3.3.1 Physical and Chemical Properties

Cacodylic acid is a colorless crystalline compound at 25°C (Midwest Research Institute, 1975). It is described as nonvolatile and has a very high aqueous solubility (NAS, 1974).

The chemical structure of cacodylic acid is given in table 2-1. The compound does not decompose in sunlight (NRC, 1974). It undergoes various biological transformations in soil, including reductive methylation to volatile dimethyl and trimethyl arsines, and oxidative cleavage of the carbon-arsenic bond to form CO_2 and arsenate (AsO_4^{3-}).

3.3.2 Biological Transformations, Transport and Persistence

Woolson and Kearney (1973) determined the persistence of cacodylic acid in three soils under both aerobic and anaerobic conditions. They applied ^{14}C-labeled cacodylic acid to the soils. After incubating for 24 days, the soils were analyzed for ^{14}C content and arsenic (As) content. The aerobic soils contained an average of approximately 65 percent of the As initially applied and 24 percent of the ^{14}C initially applied. The authors assume that the 35 percent loss of the As from the soil could have occurred only through the formation and subsequent evaporation of volatile organo-arsenic compounds. Therefore, 35 percent of the total 75 percent ^{14}C loss from aerobic soils can be accounted for by evaporation of volatile organo-arsenic compounds. The loss of the other 41 percent ^{14}C was assumed to be due to the oxidation of cacodylic acid to $^{14}CO_2$ and AsO_4^{-3}. After 24 days' incubation, the anaerobic soils contained an average of approximately 39 percent As and 39 percent ^{14}C. Therefore, all loss of ^{14}C from the anaerobic soils was assumed to be due to the formation and subsequent evaporation of volatile organo-arsenic compounds.

Woolson (1977), by gas chromatography-mass spectrometry, later identified volatile organo-arsenic compounds evolving from the biodegradation of cacodylic acid to be primarily dimethyl and trimethyl arsenic.

3.3.3 Bioconcentration of Cacodylic Acid

Isensee et al. (1973) determined the bioconcentration of ^{14}C-labeled cacodylic acid by various aquatic organisms from water in a model aquarium. Three fish, 30 daphnia, 100 snails, and algae were exposed to cacodylic acid for 3, 29, 32, and 32 days, respectively. The organisms exposed represented parts of two food chains: algae --> daphnids, and daphnids --> fish. The bioconcentration ratios for the algae and daphnids, based on the ^{14}C content of the water and wet tissues, were, respectively, 1,635 and 419. The bioconcentration ratios for daphnia and fish were, respectively, 1,658 and 21. Therefore, although aquatic organisms appear to bioconcentrate cacodylic acid and/or a metabolite of cacodylic acid, biomagnification does not appear to occur.

3.4 CONCLUSION

Phenoxy herbicides in water may undergo a number of photolytic changes to form chlorophenols, or through photoreduction may be dechlorinated to form phenoxyacetic acids. Under dry conditions, 2,4-D, 2,4,5-T, and picloram are readily photolyzed and substantial losses have been noted. Photolysis of TCDD is strongly dependent on the surrounding medium; rapid photolysis is noted of TCDD in Orange or organic solvents, as opposed to slower rates in aqueous suspensions or on soil. Photolysis of TCDD seems to produce less chlorinated and less toxic dioxins; continued irradiation by sunlight may result in decomposition of the dibenzo-p-dioxin structure.

2,4-D, 2,4,5-T, and picloram show little adsorption to soil, and they are all moderately soluble in water. However, leaching does not appear to be a primary mechanism for the environmental distribution of 2,4-D or 2,4,5-T. Picloram has a somewhat higher potential for vertical displacement or leaching in soils. TCDD has less aqueous solubility and high soil adsorption, and has a high soil or sediment/water partition coefficient, and thus has a relatively low potential for leaching.

The n-butyl esters of 2,4-D and 2,4,5-T are much more volatile than 2,4-D, 2,4,5-T, picloram, or the dimethylamine salts. TCDD is essentially non-volatile.

The primary difference between TCDD and other herbicides is in its environmental persistence. In both soils and water TCDD has a long environmental life, and there was fairly high level of bioconcentration observed in aquatic organisms. 2,4,5-T has a negligible half-life in the environment, while 2,4-D and picloram had slightly longer persistence. Cacodylic acid also has a relatively short environmental life; it is postulated to form organoarsenic compounds which are subsequently volatized.

Despite its high rate of bioconcentration, there seems little evidence of any biomagnification of TCDD in an aquatic environment. This appears to be true for 2,4-D, 2,4,5-T, picloram, and cacodylic acid, as well.

The following chapter looks at the metabolism in humans and animals of the herbicides under study.

CHAPTER 3.

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CHAPTER 4

METABOLISM, ENZYME INDUCTION, AND MECHANISM OF ACTION

In this chapter, several aspects of metabolism and for TCDD, receptor-mediated effects are considered. Patterns and rates of absorption, distribution, and excretion of the phenoxy acids and TCDD are described under Biodynamics. Sites and pathways for biotransformation of the phenoxy herbicides and TCDD are described under Biotransformation. Studies that evaluate the potency of TCDD in inducing enzymes are described in the section, Enzyme Induction; these inducible enzymes, in general, have not been shown to be responsible for the biotransformation of the herbicides that induce them. The mechanism of action of TCDD has been the subject of many studies, and these studies are discussed in the sections entitled Mechanisms of Action. For the remaining compounds, fewer studies have been published; and these studies are described by chemical without further subdivisions, for each subject category.

4.1 2,4-D

Dermal absorption of 2,4-D, as well as the pharmacokinetics of plasma clearance and excretion of oral doses, have been determined in human studies. Animal studies have investigated the biodynamics and biotransformation of 2,4-D. No studies were found on the ability of 2,4-D to induce any enzyme system or on its mechanism of toxicity. Esposito et al. (1980), Leng (1977), Loos (1975), and Young (1978) have reviewed literature on phenoxy acid metabolism.

4.1.1 Human Studies

4.1.1.1 Biodynamics

The rate of dermal absorption of 2,4-D has been measured in human volunteers. When [^{14}C]-2,4-D was administered intravenously to human volunteers, 100 percent of the dose was excreted in the urine during the subsequent five days. The biological half-life for excretion was 13 hours. [^{14}C]-2,4-D was then applied to the forearm, and excretion of radioactivity was monitored over the subsequent eight days. Using this method, 5.8 percent of the dose was absorbed and excreted. This rate was increased to 14.7 percent by occluding the application site with plastic film for 24 hours after dosing (Feldman and Maiback, 1974; Maiback and Feldman, 1974).

The kinetics of absorption and excretion of orally ingested 2,4-D have been studied in man. After six male volunteers each ingested a subtoxic dose of 5 mg 2,4-D per kg, urine and blood samples were collected and monitored for 2,4-D levels. From pharmacokinetic analysis of the data, the half-life of plasma clearance was determined to be 33 hours (Kohli et al., 1974). In a similar study, five men each ingested 5 mg 2,4-D per kg and urine and blood samples were collected and analyzed for 2,4-D. This dose was completely

absorbed from the gastrointestinal tract and produced no clinical symptoms. 2,4-D was cleared from the blood with a half-life of 11.6 hours, and both plasma clearance and urinary execretion followed first-order kinetics. (Sauerhoff et al., 1977; Sauerhoff et al., n.d.).

Blood and urine levels of 2,4-D in patients who intentionally ingested large quantities have been analyzed pharmacokinetically. In one case, after ingestion of a sublethal dose of 2,4-D estimated to have been 400 mg/kg, plasma clearance was calculated to be 16.7 hours (Young and Haley, 1977; Young and Haley, n.d.). In another case, an estimated 70 ml of a 10 percent 2,4-D preparation had been ingested. Initially, the half-time of clearance was 219 hours and the urinary pH was below 6.5. After alkaline diuresis was started, the pH increased to 7.5 and the half-life decreased to 42 hours. Continued therapy elevated the pH to 8.8 and, at this pH, the half-life of plasma clearance of 2,4-D was 5 hours (Park et al., 1977).

The amounts of 2,4-D excreted by workers occupationally exposed to 2,4-D has been used to estimate total doses absorbed by these workers. Aerial applicators excreted an average of 0.012 mg/kg body weight/day of 2,4-D after a 12-day exposure period; ground applicators excreted a mean of 0.013 mg/kg of 2,4-D over a 6-day period after a 1-day exposure period (Nash et al., in press: Nash et al., 1981). In another study (Shafik, et al., 1971), urine samples of workers were analyzed for 2,4-D levels. Levels ranged from 0.2-1.0 ppm for samples from farmers and spray operators, while no 2,4-D was detected in samples from herdsmen, farm laborers, and pesticide project officers.

These pharmacokinetic studies incidate that ingested 2,4-D is absorbed rapidly and completely by the gastrointestinal tract, but that it is not absorbed extensively after dermal exposure to a limited area of the skin; by these routes and by intravenous injection, 2,4-D is rapidly and completely excreted by the kidney, with a biological half-life for clearance of about one-half day. Only in the case of severe poisoning and resultant acidosis is this rate considerably slower; upon alkaline diuresis, clearance is stimulated to a higher rate than observed in non-treated cases after lower doses were given.

4.1.1.2 Biotransformation

In the pharmacokinetic studies described above, in which known quantities of 2,4-D were administered to volunteers, urine samples were analyzed for 2,4-D. Kohli et al. (1974) did not detect metabolites by gas chromatography. Sauerhoff et al. (1977) reported that 82 percent of the administered dose was excreted as the acid and 12 percent as conjugates.

4.1.2 Animal Studies

4.1.2.1 Biodynamics

The rate of absorption of 2,4-D by the rat lung has been investigated. In 1.4 minutes, 50 percent of intratracheally instilled doses of 2,4-D were

absorbed by the lung, indicating that alveolar transport was probably by diffusion (Burton et al., 1974). Retention of 2,4-D by cultured human embryonic lung cells was relatively low, compared to retention of other herbicides that are known to bioaccumulate and that are far less water-soluble than 2,4-D (Murakami and Fukami, 1978).

In various species, 2,4-D as the acid, salt or ester, has been shown to be transported rapidly to tissues. In the mouse, clearance rates from the body were higher for the butyl ester than the octyl ester of 2,4-D, and both were higher than the rate for clearance of the acid (Zielinski and Fishbein, 1967). Plasma clearance in the rat was in turn more rapid than in the pig, calf, and chicken, although the half-lives in the same species for the triethanolamine or sodium-potassium salts were the same as for the butyl ester. In the rat, these half-lives were 3-6 hours, and in the other species they were 7.5-12 hours (Erne, 1966). In another study in the rat, (Buslovich et al., 1973) the sodium salt was cleared far more rapidly than the diethylamine salt, although both rates fell substantially with time, perhaps due to renal toxicity from the high doses used (405-555 mg/kg). Shafik et al. (1971) also observed decreases in the rate and total amount of 2,4-D excreted in urine by rats, as the total dosage was increased.

2,4-D was distributed evenly among various tissues in several species, after acute or chronic exposure. The plasma levels were usually slightly higher than other tissues, followed by renal levels, and then hepatic and pulmonary levels, although all were within the same order of magnitude. 2,4-D was further distributed to the plasma fraction of whole blood primarily, and to the cytosol subfraction in cells (Erne, 1966; Khanna and Fang, 1966; Buslovich et al., 1973). 2,4-D showed high affinity binding to the albumin fraction of serum (Mason, 1975; Haque et al., 1975). 2,4-D was not accumulated in fat or muscle of sheep or cattle during a 28-day feeding experiment (Clark et al., 1975).

Although 2,4-D levels in the brain were lower than in other tissues, the distribution of 2,4-D to the brain has been investigated further in relation to 2,4-D's activity as a neurotoxin. At a high dose (250 mg/kg) which produced myotonia and lethargy, the levels of 2,4-D in the rat brain and cerebrospinal fluid increased by 11- and 39-fold, respectively, over levels after a slightly toxic dose of 100 mg/kg; after 500 mg/kg, these increases were 18- and 67-fold, respectively. In contrast, hepatic levels increased only five- and six-fold, after 250 and 500 mg/kg doses, respectively, over the levels produced by the 100 mg/kg dose. The authors suggested that toxic symptoms were associated with proportionately higher neural levels than hepatic or other tissue levels (Elo and Ylitalo, 1979, 1977). The mechanism of uptake by the brain was studied in the rabbit. 2,4-D was taken up by the organic anion system of the rabbit brain (choroid plexus) in vitro. Transport was energy-dependent and was inhibited by other anions (Pritchard, 1980).

In all species that were studied, 2,4-D was excreted almost exclusively by the kidney. At high doses or in animals that were maintained on low protein diets, in vitro renal clearance by goat kidney was decreased. At high doses, both plasma binding and renal tubular secretion mechanisms became saturated (Orber, 1980a; 1980b). 2,4-D was accumulated by the renal organic transport system in the rat, but in a different manner than the way other

compounds (para-aminohippurate) are handled by this system in vitro (Koschier et al., 1978). 2,4-D uptake by the renal transport system was greater in the rabbit than in the rat (Berndt and Koschier, 1973). A small amount, (3-6 percent) of an oral dose was excreted in the feces by rats, and 2,4-D was detected in nursing offspring, indicating that transfer via milk occurred (Fedorova and Belova, 1974).

4.1.2.2 Biotransformation

Hydrolysis of the ester of 2,4-D butyl ester by the rat and the pig was demonstrated by Erne (1966). In the pig, an average of 7 percent of the excreted 2,4-D was conjugated (i.e., released by acid hydrolysis). In other studies, no urinary metabolites were detected in any other species, including the rat (Khanna and Fang, 1966), the cow (Lisk et al., 1963), the sheep (Clark et al., 1964) or the goat (Orberg, 1980). 2,4-D metabolites were not detected in expired air of rats (Khanna and Fang, 1966).

4.2 2,4,5-T

The biodynamics of 2,4,5-T and formation of urinary metabolites have been studied in man and in a number of mammalian species. As with 2,4-D, 2,4,5-T has not been studied as an inducer of microsomal enzymes, and its mechanism of action has not been investigated.

4.2.1 Human Studies

4.2.1.1 Biodynamics

Dermal absorption of 2,4,5-T was estimated in man (Newton, 1978, cited in RPAR, 1979). A 144-inch denim cloth soaked with 40 ml of 2,4,5-T solution was placed on the upper thighs of four volunteers, wrapped in plastic, and kept in contact with the skin for 2 hours. The concentrations of 2,4,5-T used for the four volunteers were different. Urine samples over the subsequent 5 days were collected and analyzed for 2,4,5-T levels. Total urinary excretion levels were then estimated by extrapolating the data to the time-point when no further excretion occurred. The rate of absorption was not directly proportional to the concentration sprayed. The rates of absorption were 0.22, 0.42, 0.57, and 1.13 mg/sq. ft. per 1 hr. for spray concentrations of 2, 4, 16 and 32 lb. of 2,4,5-T acid per 100 gallons, respectively.

Three studies have investigated the pharmacokinetics of 2,4,5-T in man, after known doses were administered. In all three studies 2,4,5-T was administered orally to healthy male volunteers between 31-58 years old. In two of the studies, doses of 2-5 mg/kg were ingested. No adverse effects were noted in physical exams or clinical laboratory tests in one study (Gehring et al., 1973) and adverse effects were were not noted by the subjects in the second study (Kohli et al., 1974). In the third study, doses of 100-150 mg/kg were ingested but the subsequent conditions of the volunteers were not mentioned (Matsumura, 1970). In all three studies,

blood and urine samples were collected after ingestion and analyzed for 2,4,5-T. The data were then subjected to pharmacokinetic analysis.

All three investigations found that 2,4,5-T was rapidly and completely absorbed from the gastrointestinal tract. Plasma clearance was also rapid, with approximately 80 percent of the dose excreted in the urine within 4 days. The half-life for absorption was estimated at 0.75 hour; the plasma half-life for plasma clearance was 19-23 hours at low doses, and 11 hours at high doses. Plasma clearance followed first-order kinetics in all three experiments. All of the 2,4,5-T in the plasma was reversibly bound to protein (Gehring et al., 1973).

The amount of 2,4,5-T absorbed by workers from occupational exposure has been estimated from the total amounts excreted in the urine. The amounts excreted correlated with the occupation of the worker and the extent of protected clothing worn. One study reported that an average of 1 mg of 2,4,5-T was excreted over a 24-hour period by four workers who spryaed 2,4,5-T from tractors for 2-4 hours period. (Kolmodin-Hedman and Erne, 1980).

Lavy (1978) reported that excretion of 2,4,5-T was highest for workers involved in mixing 2,4,5-T formulation and lowest for flagmen involved in aerial spraying. After two exposures the total amounts excreted were 0.131 and 0.003 mg/kg body weight, respectively for these two groups of workers. No special precautions were made during this study to minimize exposure. Ramsey et al. (no date) estimated total excretion levels using several methods of pharmacokinetic analysis of urinary excretion data. The values reported ranged from 0.002 mg/kg for helicopter flagmen to 0.073 mg/kg for mixers. In another study, levels of 1.1- 3.6 ppm of 2,4,5-T were detected in the urine of spray operators while 2,4,5-T was not detected in the urine of farmers (Shafik et al., 1971)

4.2.1.2 Biotransformation

In pharmacokinetic studies described above, in which known doses of 2,4,5-T were administered to man, urine was analyzed for metabolites (Matsumura 1970, and Gehring et al., 1973). None were detected by gas chromatography and conjugates were not detected in ether extracts of acid-hydrolyzed samples.

4.2.2 Animal Studies

4.2.2.1 Biodynamics

2,4,5-T was absorbed rapidly by the rat lung. Of a tracheally instilled dose of 2,4,5-T in solution, 50 percent was absorbed in 1.7 minutes, a rate that implied that pulmonary absorption occurred by diffusion across a membrane (Burton et al., 1974). Cultured human lung cells were found to take up 2,4,5-T minimally, compared to other chemicals which are known to bio-accumulate in animals (Murakami and Fukami, 1978). The authors noted that 2,4,5-T was more water-soluble than the other persistent pesticides studied,

and concluded that uptake was high for relatively insoluble, bioaccumulated compounds, and low for soluble, non-persistent compounds. However, retention of herbicide by cultured cells is determined by influx and efflux, and these results may have reflected a difference in the efflux of these chemicals out of the cell, rather than their rate of uptake.

Plasma half-lives for 2,4,5-T were determined in several species. In the male rat, the half-life for a 100 mg/kg oral dose was 3 hours (Erne, 1966). In other studies, the half-life was dependent upon the dose, with half-lives of 4-5 hours for interavenous or oral doses in the order of 5 mg/kg, and 19-23 hours for 100 mg/kg doses (Sauerhoff et al., 1976; Piper et al., 1973). These results are not in agreement with the rates for human exposures. Compared to 2,4-D, 2,4,5-T was cleared at a slower rate by the mouse (Zielinski and Fishbein, 1967).

Pharmacokinetic analysis of the data on plasma clearance in the rat revealed that the nonlinear pattern of the data from high doses was substantially altered when a component for enterohepatic recycling was introduced into the pharmacokinetic model (Colburn, 1978). Contributions from both significant enterohepatic recycling and saturation of the renal transport mechanism were suggested as causes of the slower clearance at higher doses. Administration of ion exchange resins in cases of 2,4,5-T intoxication was recommended as a measure potentially to reduce the 2,4,5-T tissue and plasma levels, as well as the toxicity. Evidence that the renal mechanism is saturable has also been produced in the chicken (Erne and Sperber, 1974) and the rat (Hook et al., 1974; Shafik et al., 1971).

Other factors that have been shown to influence the clearance of 2,4,5-T are related to the species and age of the animals tested. Newborn rats showed clearance rates of 97 hours, compared to 3.4 hours in adults (Fang et al., 1973). The half-life for clearance of a 5 mg/kg oral dose in dogs was 77 hours (Piper et al., 1973), compared to 4 hours in the rat. Clearance of 2,4,5-T by cattle and sheep was rapid, however, and within the same order as observed in the rat (St. John et al., 1964; Clark and Palmer, 1971). Tissue distribution in cattle also followed patterns in other species, with high levels in the kidney and low levels in fat (Clark et al., 1975).

In the dog, the slow rate of 2,4,5-T clearance has been considered a likely explanation for the dog's higher sensitivity to the toxic effects of 2,4,5-T. In vitro studies have demonstrated that 2,4,5-T was higher for the adult rat than for the newborn rat or the dog (Hook et al., 1974), providing evidence that different rates of renal excretion were responsible for the differences in plasma clearance. Renal transport was also reduced in vitro by addition of plasma, suggesting that binding of 2,4,5-T to plasma protein decreases its availability for renal clearance in the dog in vivo, in addition to slower renal secretion into the urine (Hook et al., 1976). In vitro renal accumulation of 2,4,5-T in the rabbit was higher than in the rat (Berndt and Koschier, 1973). The rate of clearance in the rabbit has not been determined, but its toxicity is lower than in the rat (see chapter 6).

Renal tubular transport by the organic anion system is probably the mechanism of renal handling of 2,4,5-T (Koschier and Berndt, 1976). In vitro accumulation by this system was shown to be energy-dependent (Berndt and Koschier, 1973). At high doses, 2,4,5-T produced nephrotoxicity, which

reduced the rate of clearance by the perfused rat kidney (Koschier and Acara, 1979). 2,4,5-T was found to bind to renal microsomes and the binding was suggested to result in retention of high levels of 2,4,5-T that produce nephrotoxicity following administration of a large dose (Koschier et al., 1979). High-affinity 2,4,5-T binding to bovine serum albumin (probably at tryptophan residues) has been demonstrated (Haque et al., 1975; Mason, 1975) as well as binding to protein in human plasma (Gehring et al., 1973). The binding affinities for various species have not been compared, which would determine whether higher plasma binding in the dog may contribute to the lower renal clearance in this species.

Along with its high-affinity binding to renal tissue and plasma proteins, 2,4,5-T was distributed among various other tissues and then was cleared rapidly from these tissues. Following oral administration to rats, the kidney had from 10- to 100-fold higher levels than those of other tissues (except the stomach at early times after dosing); plasma levels were also substantially higher than other tissues, but lower than renal levels. Tissue clearance rates averaged 3.4 hours, following a low dose of 2,4,5-T (Fang et al., 1973). The same relative distribution of 2,4,5-T to the kidney, compared to other tissues, was observed in sheep (Clark and Palmer, 1971). 2,4,5-T did not accumulate in the fat of cattle during a 32-week exposure period (Clark and Palmer, 1971) or in fat or muscle of sheep or cattle during a 28-day exposure period (Clark et al., 1975).

2,4,5-T was not excreted in expired air by rats, while 3-14 percent of oral doses were excreted in feces at higher doses; transfer of 2,4,5-T to offspring via milk has been shown in the rat (Fang et al., 1973; Piper et al., 1973; Sauerhoff et al., 1976).

4.2.2.2 Biotransformation

Although at least 94 percent at intravenous dose was identified as unchanged 2,4,5-T in the urine in one study (Sauerhoff et al., 1976), as many as three minor metabolites have been isolated (but not identified) in the urine in other studies after oral administration, which were more likely to be detected following larger doses (Fang et al., 1973; Piper et al., 1973). In another study, 10-20 percent of excreted 2,4,5-T was in bound form (susceptible to acid hydrolysis) and N-(2,4,5-trichlorophenoxyacetyl) glycine were identified in the urine of rats given 50 mg/kg of 2,4,5-T orally (Grunow et al., 1971).

4.3 TCDD

A large number of studies have investigated the metabolism of TCDD and its biological effects on a cellular level. The only studies that used human tissues were studies that determined TCDD residue levels or enzyme induction in blood cells or in cell lines derived from human tissues. Animal studies have investigated the biodynamics, biotransformation, enzyme induction, and mechanism of action of TCDD. The role of receptors in the biological effects elicited by TCDD has been considered in some studies. The role of biotransformation in explaining species differences has also been considered in several recent studies presented in this section.

4.3.1 Human Studies

4.3.1.1 Biodynamics

Human tissue samples that have been analyzed for TCDD were all taken from people with exposure to TCDD from unknown sources, with unknown levels and durations of exposure and by unknown routes. The possible exception to this was the case of a woman who lived in the Seveso area (zone A) for 2 weeks. When the woman died of pancreatic carcinoma 3 months after the accident at the ICMESA plant, tissues removed at autopsy were analyzed for TCDD. Tissue levels were 1.84 ppb in fat, 1.04 ppb in pancreas, 0.15 ppb in liver, and 0.04-0.06 ppb in lung, kidney, and brain (Reggiani, 1979).

Gross (1980) described the results of TCDD analyses in human tissues from three sources. No TCDD was detected in 44 adipose and liver samples removed at autopsy in hospitals serving agricultural areas. These areas were in the Southern U.S., in principal rice-growing regions which were considered likely to use 2,4,5-T. The detection limit for 43 of the samples was 1-10 ppt. In 103 milk samples including 72 mothers in the northwestern U.S. where 2,4,5-T use was considered likely, no TCDD was detected (1 ppt, average limit of detection). Adipose tissue from 22 veterans were also analyzed. Ten samples have detectable levels of TCDD, but the levels were not reported and whether the positive samples were from Vietnam veterans was not reported.

Nisbet (1980) cited studies in which 5-16 ppt of TCDD was detected in 1 adipose sample (the donor was not described) and 11-31 ppt was detected in milk samples in 4 of 17 samples (3 samples were from women from Kansas and Texas, and 1 was from Italy). Without any information on exposure levels, these data cannot be used to assess the patterns of TCDD biodynamics in man, compared to animal data.

4.3.1.2 Enzyme Induction

Aryl hydrocarbon hydroxylase (AHH) activity in human cells has been induced by TCDD treatment in vitro. Niva et al., (1975) showed that AHH activity in human lymphoyctes and in human Chang liver cells responded to TCDD treatment, although the dose required to produce half-maximal induction in lymphocytes was higher than the doses required for half-maximal induction in cells from other species. Kouri et al., (1974) also reported responsiveness of lymphocyte AHH activity to TCDD. The induced levels were low (approximately 3 times the control levels).

4.3.2 Animal Studies

4.3.2.1 Biodynamics

TCDD clearance from plasma has been shown to be slow. In the 7-day period following intraperitoneal administration of [^{3}H]-TCDD to monkeys and rats, only 1 percent of the dose was excreted in the urine and 4-5 percent in

the feces. Radioactivity in the monkey was highest in tissues with high lipid content, including the skin and fat, and in muscle. In the rat, 40 percent of the radioactivity remained in the liver 1 week after dosing (Van Miller et al., 1976). Male rats retained an even higher proportion of a dose of TCDD in the liver than females. Half-lives for clearance of TCDD from the whole body were shorter for males (12 days) than females (15 days) (Fries and Marrow, 1975).

In another study in the rat, the half-life for body clearance of a single oral dose was calculated at 17 days. The liver retained 3, 4, and 1 percent (per gram of tissue) of the dose, 3, 7, and 21 days, respectively, after dosing, and fat levels with 53, 13, and 3 percent in the feces, urine, and expired air respectively (Piper et al., 1971; Piper et al., 1973). After repeated doses were administered orally to rats, the feces remained as the major route of excretion. Radioactivity in the liver and fat reached steady state levels at the same rate as for the whole body, with a half-life of 7 weeks (Rose et al., 1976). The validity of the pharmacokinetic constants in the rat has been challenged, however, because the constants were calculated assuming first-order kinetics for TCDD in the rat; the data have since been shown to be compatible with a zero-order kinetic model as well (Hiles and Bruce, 1976).

In the guinea pig, intraperitoneal administration of a single dose of TCDD resulted in high TCDD levels in the fat after 1 day (Gasiewicz and Neal, 1978; 1979). After 15 days, lower levels of TCDD were found in the fat, while the levels in the liver had increased 3 fold and increases occurred in the adrenal, kidney, and lung levels, during this period. These increases, the authors suggested, resulted from mobilization of fat stores. The liver contained 11 percent of the injected dose in this study, similar to the proportion of an intraperitoneal dose administered to the monkey (Van Miller et al., 1976) but in contrast to the level reported for the rat of 40 percent. In accordance with these findings, only the rat shows severe hepatotoxicity after TCDD treatment. Teitelbaum and Poland (1978) demonstrated that hepatic uptake of TCDD was increased by TCDD pretreatment and that the non-cytosol fractions showed enhanced capacity for TCDD binding in vitro, after the pretreatment.

Gasiewicz and Neal (1978; 1979) reported that only 5 and 1 percent of an intraperitoneal dose of TCDD was excreted in the feces and urine, respectively, by guinea pigs in 15 days. Excretion was linear for 23 days. Assuming that the rate continued to remain linear, the half-life for excretion by both routes was calculated at 30 days. Nolan et al. (1979) reported that only half of an oral dose of TCDD was absorbed by guinea pigs and that the fat, thymus, liver, and adrenals retained the highest levels 22 days after dosing. The half-life for clearance from these tissues was estimated to be between 22 and 43 days.

Recently, the hamster has been shown to be less sensitive than other species to the acute effects of TCDD (Olson et al., 1980). In this species, the highest tissue levels of TCDD, administered intraperitoneally or orally, were recovered in the liver, fat, and adrenals, during the 35-day period after administration (Olson et al., 1980). The urine and feces contained 35 and

50 percent respectively, of the dose and the half-lives for elimination by these two routes were 11 to 15 days for oral and injected doses. The authors concluded that the rapid excretion in the species contributed to its lower sensitivity to TCDD.

4.3.2.2 Biotransformation

Early studies reported that radioactivity in the rat liver remained as TCDD, which was not metabolized (Rose et al., 1976). TCDD radioactivity was located specifically in the microsomal fraction of the liver cells (Allen et al., 1975). In the mouse, similarly, TCDD was localized in the microsomal fraction of liver cells and remained intact, without undergoing biotransformation (Vinopal and Casida, 1973). Beatty (1977), Beatty et al. (1978), Beatty and Neal, (1976) produced indirect evidence that TCDD was metabolized, by demonstrating a correlation between elevated mixed function oxidase levels and decreased toxicity. Tulp and Hutzinger (1978) showed that a series of polychlorinated dioxins were all metabolized by hydroxylation at the 2,3,7, or 8-position, except for octachlorodibenzo-p-dioxin, which is chlorinated at each of these positions, blocking hydroxylation. Although TCDD was not included in this study, the authors pointed out that it would not be likely to be susceptible to hydroxylation, since all four sites are blocked.

Recently, several studies have examined bile samples for TCDD metabolites. Ramsey et al. (1979) collected 24-hour bile samples of rats after they received 2-6 oral doses of TCDD. Five metabolites were isolated by liquid chromatography. The metabolites were more polar than TCDD, based on their extraction into selective solvents, and included glucuronide conjugates, based on susceptibility to beta-glucuronidase. The rate of biliary excretion was comparable to the rate of fecal excretion. Matthews and Kato (1979) reported that 90 percent of the TCDD excreted in bile was in the form of metabolites. Poiger and Schlatter (1979) also cited in Donzel et al., 1980, also reported that rats that were administered TCDD orally, excreted the metabolized compound in bile at a rate that accounted for fecal excretion. Only unmetabolized TCDD was recovered from the liver. Bile samples, collected over 3-4 days, were subjected to various chromatographic, enzymatic, and extraction procedures. The isolated metabolites were not completely identified, but their chemical characteristics were consistent with water-soluble conjugates, were labile to glucuronidase-arylsulphatase digestion, and possibly contained phenolic hydroxyl groups. Olson et al. (1980) isolated one major metabolite and several minor metabolites, by high pressure-liquid chromatography, from the bile of hamsters after TCDD was administered intraperitoneally. These authors suggested that the faster excretion rate in this species reflected a faster rate of TCDD metabolism, which led to the formation of polar metabolites that were rapidly excreted in bile. Guenther et al. (1979) proposed that TCDD was metabolized to arene oxides by the cytochrome P-450 system, although the proposed, highly-reactive metabolite was not isolated.

4.3.2.3 Enzyme Induction

TCDD has been shown to induce various hepatic and extraphepatic enzymes. Table 4-1 lists enzymes that were shown to be induced by TCDD, as well as enzymes that did not show increased activity following TCDD treatment. In these studies, TCDD induction of hepatic microsomal enzymes involved protein synthesis. Inhibitors of protein synthesis, like actinomycin-D, prevented induction (Beatty and Neal, 1978; Niwa et al., 1975; Hook et al., 1975a; Lucier et al., 1975a), and addition of TCDD to the enzyme preparation in vitro did not stimulate enzyme activity (Lucier et al., 1975a). TCDD also produced increased levels of cytochrome P-450, P-448, and b_5 (Hook et al., 1975b; Lucier et al., 1973; Guenther and Nebert, 1978). The a, b, and c forms of cytochrome P-450 that were induced in rabbit liver were shown to correspond to specific enzyme activities (Johnson and Muller-Eberhard, 1977).

TCDD induced a spectrally distinct type of P-450 whose spectral characteristics resembled methylcholanthrene (MC)-induced cytochrome. TCDD also induced select enzymes, similar to the pattern of MC induction and in contrast to the non-specific pattern of phenobarbital (PB) induction (Poland and Glover, 1974; Aitio and Parkki, 1978; Pohl et al. 1976; Greig and DeMatteis, 1973). A combination of maximally inducing doses of 3-MC and TCDD did not produce additional enzyme induction, indicating that both inducers may act by the same mechanisms (Poland and Glover, 1974; Niwa et al., 1975).

TCDD appeared to induce a kinetically different glutathione S-transferase than either MC or PB induced (Baars et al., 1978). TCDD was found to induce the same aldehyde dehydrogenase that was produced by hepatoma cells, but was distinct from the PB-induced isozyme (Lindahl et al., 1978). The TCDD-induced increase in aryl hydrocarbon hydroxylase (AHH) activity, the enzyme that catalyzes the hydroxylation of benzo(a)pyrene to 3-hydroxy-benzo(a)pyrene, was higher than for most other enzymes studied. TCDD was about 30,000 times more potent at stimulating AHH activity than 3-MC, making it one of the most potent enzyme inducers known (Poland and Glover, 1974). Likewise, DT-Diaphorase induction by TCDD was greater and lasted longer than induction by a 200-fold greater dose (by weight) of 3-MC (Beatty and Neal, 1976).

TCDD-stimulated enzyme induction was remarkably long-lasting. A single dose of TCDD has resulted in enzyme activities that were still significantly elevated 35 days after 31 nanomoles of TCDD per kg was administered. In contrast, the effect of a single dose of 75 micromoles of 3-MC per kg, which produced the same magnitude of response on AHH activity, was no longer evident after 8 days (Poland and Glover, 1974).

Other enzymes remained elevated for a long time after TCDD treatment. Rat liver DT-diaphorase activity was substantially elevated 21 days after a single intraperitoneal dose of 25 ug TCDD per kg (Beatty and Neal, 1978); rat liver glucuronyltransferase was elevated 30 days after a dose of 5 ug/kg and 73 days after 25 ug/kg was given (Lucier et al., 1975a); biphenyl hydroxylase was significantly elevated 73 days after a 25 ug/kg dose (Hooke et al., 1975a); and aniline hydroxylase was elevated, 28 days after a 25 ug/kg dose; corresponding elevations in microsomal protein levels and cytochrome P-450 and b_5 were observed at this time point, as well (Lucier et al., 1973). Renal

TABLE 4-1: ENZYMES RESPONSIVE TO TCDD INDUCTION

Enzymes Tested:		Enzyme Source		
Inducible	Non-Inducible	Species	Tissue	Reference
AHH[a]		human	lymphocytes	Kouri et al, 1974
AHH		6 spp.	cell lines	Niwa et al, 1975
AHH		rat	hepatoma	Bradlaw et al, 1975
AHH	aminopyrine-DM[b] N cyt. C reductase	rat, mouse chicken	liver	Poland & Glover, 1974
AHH, GT[c], cyt.[d] P-450 and b_5, aniline-H[e]	aminopyrine-DM benzphetamine-DM ethylmorphine-DM	rat	liver	Lucier et al, 1973
AHH, cyt. P-450 UDP-GT Biphenyl-H[e]	(3 in above listing) AHH UDP-GT	rat guinea pig, rabbit rat guinea pig, rabbit guinea pig, rabbit	liver liver lung, kidney, gut lung, kidney, liver lung	Hook et al, 1975
AHH, cyt. C reductase UDP-GT	epoxide hydratase glutathione-S-T[f]	rat rat	liver, kidney, lung liver, kidney, lung	Aitio & Parkki, 1978
GT, AHH		rat	kidney	Fowler et al, 1977
Glutathione-S-T[f]		rat	liver	Baars et al, 1978
p-nitrophenol-GT	steroid-GT	rat	liver	Lucier et al, 1975
DT-diaphorase	 DT-diaphorase	rat guinea pig	8 tissues liver, others	Beatty & Neal, 1978
ALA[g]		chicken	embryo	Poland & Glover, 1973
	ALA	rat	liver	Woods, 1973

TABLE 4-1: ENZYMES RESPONSIVE TO TCDD INDUCTION (Continued)

Enzymes Tested:		Enzyme Source		
Inducible	Non-Inducible	Species	Tissue	Reference
AHH, cyt. P-448, 450 Acetanilide-4-H		rat rat	liver liver	Guenther & Nebert, 1978
Biphenyl-H, cyt. P-450	steroid-H	rat	liver	Hook et al, 1975
Ald. dehydrogenase		rat	liver	Lindahl et al, 1978
AHH, aniline-H 7-ethoxycoumarin deethylase		mouse mouse	skin skin	Pohl et al, 1976
Hexabarbital[h]		mouse, rat	liver	Greig & De Matteis, 1973
Zoxazolamine[h]		mouse, rat	liver	Greig, 1972
Cyt., AHH	p-aminophenol-GT steroid-GT p-aminophenol-GT	pregnant rat rat fetal rat	liver liver liver	Lucier et al, 1975
AHH		fetal rat	extra-hepatic	Berry et al, 1977
FAA[j], AHH	 FAA, AHH	rat (fetal, maternal) rat	liver placenta, adrenal	Berry et al, 1976
Cyt. P-450		rabbit	liver	Norman et al, 1978

a Aryl hydrocarbon hydroxylase

b DM = demethylation enzyme

c GT = glucuronyl transferase

d cyt. = cytochrome

e H = hydroxylase

f T = transferase

g ALA = delta-aminolevulinic acid

h (relevant metabolic enzymes for this compound)

j FAA = fluorenyl acetamide hydroxylation

benzpyrene hydroxylase and glucuronyl transferase activities were also substantially elevated 16 days after a single oral dose of 25 ug/kg (Fowler et al., 1977), indicating that hepatic sequestration of TCDD may not necessarily explain the long duration of this effect.

On day 5 of gestation, pregnant rats were administered 3 ug TCDD per kg; 73 days after exposure, the 56-day-old offspring had substantially elevated para-nitrophenol glucuronidase levels (Lucier et al., 1975b). This result, as well as results for other hepatic and non-hepatic enzymes in young rats exposed to TCDD only prior to birth, indicates that TCDD induction can be transmitted transplacentally. In the rabbit, TCDD transplacentally-induced hepatic cytochrome b but not cytochrome c, while both were induced in the adult (Norman et al., 1978). This difference in response probably reflects differences in the hepatic cytochrome system with age. Doses of TCDD that produced half-maximal induction in AHH activity varied between 0.4 and 1.2 nmole/kg for the chicken, rat, and several strains of mice. In the chicken, ALA synthetase was induced by a dose of 4.7×10^{-12} mole per egg (Poland and Glover, 1973). A single dose of 0.2 ug/kg to rats produced a significant induction of biphenyl hydroxylase activity (Hook et al., 1975a) and of aniline hydroxylase and AHH (Lucier et al., 1973).

In several experiments that compared the effects of TCDD induction in male and female rats, females were more sensitive than males, to low doses of TCDD which induced AHH, para-nitrophenol glucuronyltranferase, and biphenyl hydroxylase activities (Lucier et al., 1975a; Hook et al., 1975a; Lucier et al., 1973). TCDD induction was not limited to hepatic enzymes: skin, kidney, lymphocyte, and lung enzymes responded to TCDD induction (Berry et al., 1977; Aitio and Parkki, 1978; Fowler et al., 1977; Pohl et al., 1976); these enzymes in the testes as in the intestine were not induced (Aitio and Parkki, 1978). TCDD also has not been shown to induce enzymes in a variety of cell lines and primary cell cultures 48 hours after it was added to culture medium (Bradlaw et al.: 1975, 1976, 1980; Bradlaw and Casterline, 1979; Kouri et al., 1974; Niwa et al., 1975). These culture systems have been suggested as potential TCDD assay systems because extremely low levels of TCDD were able to elicit positive responses. The kinetics of enzyme induction in vitro and the relative responsiveness of cells from various strains of mice followed the same patterns as were observed in vitro (Niwa et al., 1975).

The strikingly low doses of TCDD that produce enzyme induction and the long-lasting inductive effect that TCDD elicits are analogous to the high potency of low doses of TCDD in producing toxicity and death and the delayed pattern of this toxicity. However, the wide range in sensitivity to TCDD toxicity among different species contrasts with the narrow range of doses that produce comparable enzyme induction in various species (Poland and Glover, 1974). In fact, the guinea pig, the species most sensitive to acute TCDD toxicity, showed no inductive response to TCDD at doses that elevated enzyme levels in the rat, a species that shows relatively low sensitivity to acute TCDD toxicity (Beatty and Neal, 1978). The extent of in vitro toxicity of various cell lines after TCDD treatment also did not correlate with sensitivity to AHH induction for the same cell lines (Niwa et al., 1975; Knutson and Poland, 1980) or to the sensitivity to TCDD in vivo (Beatty et al., 1975; Knutson and Poland, 1980). Although the possibility exists that

the toxic effects of TCDD on lipid metabolism and nutrient utilization may be derived from TCDD's effects on activities of specific enzymes, this relationship and the potential enzymes involved in this effect have not been demonstrated.

4.3.2.4 Mechanism of Toxicity

The remarkable potency of TCDD, its unusual temporal pattern of toxicity, and its severe dermal and metabolic effects have led to various studies on the mechanisms of TCDD toxicity. The relationship between enzyme inducibility and the toxicity of TCDD has been the subject of a series of elegant and thorough studies from several laboratories. Cell surface receptors for TCDD have been identified (Greenlee and Poland, 1979; Poland et al., 1979; Carlstedt-Duke, 1979; Carlstedt-Duke et al., 1979). This receptor has been shown to be a gene product of the Ah locus (Nebert et al., 1973; Okey et al., 1979; 1980; Nebert and Jensen, 1979). Binding of an enzyme inducer, including TCDD or 3-MC, to this receptor is a necessary requirement before enzyme induction can occur. However, receptor binding does not guarantee that enzyme induction will occur (Okey et al., 1980).

Nuclear transfer of the receptor-inducer complex has also been demonstrated (Okey et al., 1979; 1980; Greenlee and Poland, 1979). Strains of mice that show low responsiveness to AHH induction by TCDD (Poland et al., 1974; Kumaki et al., 1979) were also shown to have lower hepatic binding affinity for TCDD. A genetic mutation at the Ah locus has been suggested to have caused an alteration in the structure of the receptor that diminishes its binding affinity to TCDD (Poland and Glover, 1975). Enzyme induction results from changes that occur after TCDD binds to hepatic cytosol receptors and the complex is subsequently transported to the nucleus (Greenlee and Poland, 1979; Poland et al., 1976). The role of the Ah gene product in cytochrome P-450 induction is the subject of several reviews (Nebert and Jensen, 1979; Fox, 1979; Poland and Kende, 1976; Poland and Glover, 1978). These receptors may play a role in eliciting the toxic effects of TCDD, in addition to the inductive effects (Poland and Glover, 1973; Poland, 1979; Neal et al., 1979). Poland and Glover (1980) have demonstrated that strains of mice with high-affinity receptors for TCDD, and high sensitivity to TCDD enzyme induction, also showed greater sensitivity to toxic effects of TCDD, including thymic atrophy and cleft palate teratogenicity than strains with low affinity binding and low inductive responsiveness. The thymus, an organ that shows severe atrophy after TCDD is administered, was shown to contain a high affinity receptor for TCDD, relative to other tissues (Carlstedt-Duke, 1979).

Strains of mice that showed decreased AHH responsiveness also lost their susceptibility to the porphyrogenic effect of TCDD (Jones and Sweeney, 1980), both of which may require TCDD-receptor binding in order to be elicited. The porphyrogenic effects of TCDD are only elicited in animals with sufficient (normal) levels of iron. The mechanism by which iron deficiency protects against TCDD-induced liver toxicity and porphyria is unknown (Jones and Sweeney, 1979; Sweeney et al., 1979).

Toxicity and teratogenicity of TCDD have also been suggested to result from the binding of TCDD metabolites to protein or from an effect on thymidine utilization. In one study, TCDD metabolite binding to protein in vitro was highly favored over metabolite binding to DNA (Guenther et al., 1979). Cellular utilization of thymidine, measured in vitro as nuclear incorporation of thymidine, was stimulated by TCDD (Conaway and Matsumura, 1977). The relevance of these in vitro findings to mechanisms of enzyme induction and toxicity awaits further studies.

4.4 DIQUAT

The metabolism of diquat has been reviewed (Calderbank and Slade, 1976; Rose and Smith, 1977); diquat was absorbed by the lung. In the rat, the process of pulmonary absorption was biphasic and energy-dependent, probably related to uptake by alveolar membranes on the airway side (Charles et al., 1978). Diquat was poorly absorbed from the gastrointestinal tract. Only 10-20 percent of a 12 ug/kg oral dose was absorbed 6 hours after being administered to the dog (Bennett et al., 1976). Less than 20 percent of oral doses administered to rats was excreted in the urine, with the remainder recovered in feces. Since bile contained only 5 percent of the dose after 24 hours, and subcutaneous doses of diquat did not appear in feces, the fecal levels probably represented unabsorbed compound. About 70 percent of the oral dose of diquat was recovered as metabolites, which was attributed to degradation by gut microbes (Daniel and Gage, 1966).

Paraquat, a structural analog of diquat and a pulmonary toxin, has been shown to concentrate in the lung. Diquat, which does not produce toxicity in the lung, also does not concentrate in the lung. These results have been confirmed by experiments in the rat in vivo (Sharp et al., 1972; Kurisake and Sato, 1979) and in vitro (Rose et al., 1976; Rose et al., 1974; Abou-Donia et al., 1976) and in the mouse (Litchfield et al., 1973).

In the rat, diquat was distributed to the liver and cartilage at higher concentrations than other tissues, and did not reach the brain. Distribution to all tissues, as well as clearance from the tissues, was rapid. Diquat was cleared from the mouse in 24 hours after intravenous injection, primarily via feces. Only about 6 percent of the dose was recovered in the urine of rats in the 2-week period after a single oral dose was administered. In an 8-week feeding study, no evidence of tissue accumulation of diquat was produced (Litchfield et al., 1973). Less than 10 percent of the doses of diquat administered to the rat, rabbit, and guinea pig was excreted in the bile, while most of the remainder was recovered in urine. Only 1-5 percent of the doses given to rabbits was metabolized, to an unidentified compound (Hughes et al., 1973). Diquat metabolism to two unidentified metabolites has also been reported for the embryonic chicken (Leakey and Hemingway, 1975).

Free radical formation has been proposed as the reactive form of diquat in eliciting toxic effects. The potentiation of diquat's toxicity in the presence of oxygen (Kehrer et al., 1979) has been suggested to result from stimulation of free radical conversion by oxygen, while carbon monoxide inhibits free radical formation (Baldwin et al., 1974). This subject is

discussed further in chapter 6, Acute Toxicity. Diquat binding to melanin was demonstrated to be ionic in nature and was proposed to lead to diquat uptake in the eye, the target organ for diquat's chronic toxicity (Larsson et al., 1977).

4.5 DIURON AND MONURON

Diuron undergoes biotransformation in all species that have been studied. After oral ingestion of 38 mg/kg by a 39-year-old woman, diuron was recovered in the urine in the form of two metabolites. These metabolites were 1-(3,4-dichlorophenyl)-3-methylurea and 1-(3,4-dichlorophenyl)-urea. No unaltered diuron was recovered (Geldmacher, Mallenckrodt, and Schussler, 1971).

In the rat and the dog, diuron was metabolized and excreted in the urine and feces. Urinary metabolites were identified as N-(3,4-dichlorophenyl) urea (the prominent metabolite), N-3,4-dichlorophenyl)-N^1-methylurea, 3-4-dichloraniline, and 3,4-dichlorophenol. Unmetabolized diuron was also detected. Chronic administration did not lead to tissue accumulation (Hodge et al., 1967). In the cow, diuron was excreted in urine and to a lesser extent in feces, but not in milk. Urinary metabolites were identified as 3-(3,4-dichlorophenyl)-1-methylurea and 3-(3,4-dichlorophenyl)urea (Kalra and Chahal, 1979). Monuron is also metabolized prior to urinary excretion (Midwest Research Institute, 1975).

Diuron activity as an inducer of hepatic microsomal enzymes has been observed in the rat. Several diuron metabolites elicited this effect as well. The enzymes that were inducible were p-nitroanisole o-demethylase, aminopyrine N-demethylase, and o-p-nitrophenyl phenylphosphonothionate detoxification enzyme (Kenoshita and DuBois, 1970; Corthay et al., 1977). Diuron also produced type I spectral changes in cytochrome P-450 in vitro (Mailman and Hodgson, 1972). Monuron also induced hepatic enzymes in the rat. Midwest Research Institute (1975) and Geissbuhler et al., (1975) have reviewed the metabolism of monuron and diuron. Although little is known about the metabolism of monuron, the results of one rat study by Ernst (1969; cited in Midwest Research Institute, 1975) suggested that monuron underwent demethylation of both methyl groups and underwent hydroxylation at one of the ring carbon positions.

4.6 BROMACIL

Bromacil biotransformation has been demonstrated in man and in the rat, with 5-bromo-3-sec-butyl-6-hydroxymethyluracil as the principal metabolite in both species. 5-Bromouracil was not recovered in the urine of either species (Midwest Research Institute, 1975). Dairy cows that were administered bromacil by oral intubation for 4 days excreted the herbicide in milk, but not in urine or feces. Bromacil was not metabolized in vitro by rumen fluid or the supernatant fraction of homogenized liver (after 10,000 G centrifugation) (Gutenmann and Lisk, 1970).

4.7 PICLORAM

In the dog, 90 percent of an oral dose of picloram was absorbed and excreted within 48 hours. No metabolites were detected in urine. In cattle, 0.02 percent of picloram, administered by gavage, was excreted in milk. Three days after administration ceased, picloram was not retained in any tissues (Foy, 1976).

4.8 CACODYLIC ACID

Workers that applied cacodylic acid over an 11-week period were found to excrete arsenic in urine. Average arsenic levels ranged from 24 to 172 ug per 24 hours. Urine levels, but not blood levels, of arsenic correlated with the estimated exposure levels of the individual workers. The average exposure was estimated at 817 gm per man per week. The authors noted that some workers noticed the odor of garlic in areas they had treated with cacodylic acid and the possibility that workers were exposed to arsine gas was suggested (Wagner and Weswig, 1974).

Another study reported average levels of 0.56 ppm arsenic in the urine of forest workers that applied cacodylic acid over a 2-month exposure period and noted that urine levels fell quickly when exposure ended (Tarrant et al., 1972). Wagner and Weswig (1974) suggested that the lower urine levels found in their study reflected more careful handling of the herbicide by the workers in their study.

In these human studies, the source of arsenic was not identified. The possibility that the workers were exposed to other forms of arsenic, such as arsine gas, was alluded to in one study, but was not determined. Therefore, the urine levels may not have reflected absorption of cacodylic acid exclusively. Assuming that arsenic was derived from cacodylic acid, the differences in urine levels may have reflected different rates of metabolism, rather than different levels of exposure among the workers.

Hwang and Schanker (1973) studied the absorption of cacodylic acid by the small intestine of the rat, in vivo. The half-time for absorption of cacodylic acid was 201 minutes for concentrations between 1 and 100 mM. Cacodylic acid appeared to be absorbed by diffusion, based on the rates of absorption of other organic arsenical compounds and the correlation of these rates with chloroform-to-water partition coefficients, but not with molecular weights.

Stevens et al. (1977) studied the metabolism of cacodylic acid in the rat. The half-lives for absorption by inhalation and oral routes were 2.2 and 248 minutes, respectively. The latter half-time was close to the value of 209 minutes reported by Hwang and Schanker (1973). After intravenous administration, three kinetic components were observed for plasma clearance, with half-times of 0.01, 0.22, and 3.42 hours. After all doses were administered by three routes, the half-time for clearance of cacodylic acid from whole blood was 76-92 days. Cacodylic acid was cleared from all other tissues rapidly. Cacodylic acid also cleared from tissues of cattle and chickens

after exposure terminated, although whole blood levels were not determined (Boggen, 1968; Peoples, 1963: both cited in Midwest Research Institute, 1975). The tissue distribution of [^{14}C]- cacodylic acid in the rat was the same as for [^{74}As]- cacodylic acid (Stevens et al., 1977), which the authors concluded was an indication that cacodylic acid was probably not metabolized to inorganic arsenic. Further observation that only trace amounts of [^{14}C]-CO_2 were recovered in expired air following [^{14}C]- cacodylic acid administration indicated that demethylation was a minor metabolic pathway. Cacodylic acid was excreted primarily in urine. When administered 1 day prior to parturition, cacodylic acid readily crossed the placenta.

The extent of degradation of cacodylic acid to inorganic arsenic by mammalian species is unknown. Indirect evidence suggests that exposure to inorganic arsenic that is derived from cacodylic acid, following absorption of the organic compound, is limited. First, cacodylic acid is far more toxic than inorganic arsenic. The oral LD_{50} for cacodylic acid is approximately 830 mg/kg, compared to 75 mg/kg for sodium arsenite and 15 mg/kg for arsenic trioxide (Palm, 1968).

In addition, dimethylated arsenicals have been recovered from human urine samples, sometimes in large amounts relative to the levels of inorganic arsenic in the samples (Yamauchi and Yakamura, 1979; Braman and Foreback, 1973; Braman, 1975). The source of the dimethylated arsenicals was unknown in each of these studies and could have resulted from methylation of arsenic, absorbed as inorganic or monomethylated arsenic, or from excretion of absorbed cacodylic acid that did not undergo biotransformation. However, the observed excretion of methylated arsenic compounds argues against biotransformation of all absorbed cacodylic acid to arsenic.

4.9 SUMMARY AND CONCLUSIONS

Both 2,4-D and 2,4,5-T are rapidly and completely absorbed from the gastrointestinal tract of man although dermal absorption in man is limited. Absorption from the lung is rapid in animals. Both phenoxy acids are cleared rapidly from blood. The half-life plasma clearance in man ranges from 12 to 23 hours. Almost all of the dose is excreted by the kidney.

Differences in the rate of excretion among different species correlate with the relative toxicity of phenoxy acids in the same species, with a relatively low rate of excretion, and high toxicity in the dog. At high doses, saturation of the renal transport mechanism has been observed, along with nephrotoxicity, both leading to a decrease in clearance rates (which theoretically would increase apparent toxicity). The clearance rate of 2,4-D is also very low in humans when the urinary pH is low. Indirect evidence for enterohepatic recycling of 2,4,5-T based on kinetic modeling has been put forth and may provide a means of removing phenoxy acids in cases of acute poisoning.

After phenoxy acids are administered, tissue levels are highest for the plasma and kidney; both compounds are excreted in milk and are distributed to fetal tissues (see chapter 8), indicating the potential for embryo-toxic

effects. Neither compound is sequestered in fat or other tissues, indicating that they are unlikely to produce cumulative toxicity. 2,4-D is preferentially distributed to the brain following high doses (250 mg/kg), but not after lower doses (100 mg/kg) that produce no apparent neurotoxicity.

Few studies have succeeded in isolating metabolites of phenoxyacids. In human urine 12 percent of 2,4-D was recovered as conjugates in one study; in the rat and pig, a few studies have recovered up to 20 percent of doses of 2,4-D and 2,4,5-T as metabolites.

TCDD is cleared slowly from the body, with a half-life of 2-3 weeks in animals. TCDD is not sequestered in fat, although the liver retains a large portion of the administered dose. TCDD is excreted primarily in feces, which is the result of biliary excretion.

Biotransformation to water-soluble, polar conjugates has been demonstrated recently. Once these metabolites are formed, they are rapidly excreted in bile. Metabolites have not been detected in liver or fat. TCDD induces various hepatic and extrahepatic enzymes. Extraordinarily low doses of TCDD are capable of eliciting induction and enzyme levels remain elevated for extended periods of time, in some cases for several months. The pattern of TCDD induction more closely resembles methylcholanthrene induction than phenobarbital induction. Transplacental induction by TCDD has been demonstrated.

The mechanism of toxicity of TCDD remains unknown. TCDD binds to cytosol receptors; this binding which is thought to elicit its enzyme-inductive effects and separately may mediate its toxic effects; a role of enzyme induction in manifesting toxic effects has not been demonstrated. Three possible mechanisms for the differences in susceptibility among species can be proposed from the data that is currently available on TCDD metabolism and receptor studies:

(1) The rate of metabolism of TCDD to less toxic metabolites that are rapidly excreted in bile will influence the extent of toxicity for each species. The strongest evidence for this mechanism are the studies in the hamster, by Gasiewicz and Neal (1979) and Olson et al., (1980) which indicate that the rates of metabolism and excretion for this species are higher, and the toxicity is lower than for other species.

(2) The presence of high-affinity receptors is essential for enzyme induction by TCDD and differences in the amounts or affinity of these receptors may explain the differences in susceptibility of different species, and of different tissues. Segregation of sensitivity of toxic effects with enzyme induction and the receptor product of the Ah locus, reported by Poland and Glover (1980), supports this hypothesis.

(3) Differences in the distribution of TCDD to different organs may affect the degree of toxicity in that organ. In the rat, a species that shows hepatotoxicity from TCDD, 40 percent of a dose of TCDD

was sequestered in the liver, compared to 10 percent in the monkey and guinea pig, two species that are not susceptible to hepatotoxicity (Van Miller et al., 1976; Gasiewicz and Neal, 1979).

Diquat is absorbed by the lung, but is poorly absorbed from the gut. Diquat is not retained by the lung and is cleared from the body rapidly. Diquat is not bioaccumulated in fat after subacute exposure.

Diquat binding to melanin has been demonstrated in vitro and may potentially lead to diquat accumulation in the eye and the resultant production of cataracts after chronic exposure. The formation of diquat free radicals has been proposed as the active form of diquat in producing toxic effects, although under conditions of normal oxygen levels little evidence exists to support this theory.

Diuron is extensively metabolized in man and in other animal species. The kidney is the major route of diuron excretion. Diuron has been shown to induce several microsomal enzymes.

Bromacil is metabolized in man and in the rat, while picloram is not metabolized and is excreted rapidly.

Arsenic has been detected in the urine of forest workers exposed to cacodylic acid, although the possibility of concomitant exposure to arsine gas, which could also result in detectable levels of inorganic arsenic in humans, was suggested. In animals, indirect evidence, based on similar tissue distribution of radioactivity after either [^{14}C]-labeled or [^{74}As]-labeled cacodylic acid were given, suggests that cacodylic acid is not metabolized to inorganic arsenic. Cacodylic acid is rapidly absorbed from the lung and is absorbed from the gastrointestinal tract. Cacodylic acid is retained in whole blood. Excretion is primarily by the kidney and cacodylic acid crosses the placenta late in gestation.

CHAPTER 4.

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CHAPTER 5

HUMAN EXPOSURE TO TCDD FROM INDUSTRIAL AND MILITARY USES

TCDD (2,3,7,8-tetrachlorodibenzo-para-dioxin) is formed as an unintentional byproduct during the manufacture of 2,4,5-trichlorophenol (TCP), when certain reaction conditions are met. TCP is a precursor in the manufacture of 2,4,5-T, and TCDD is introduced into preparations of 2,4,5-T during this precursor step.

TCDD is formed when two molecules of 2,4,5-trichlorophenol undergo a condensation reaction. Several conditions favor the formation of dioxins, including heat, pressure, photostimulation, and catalytic action. No definitive study has determined the temperature required to form TCDD from TCP (Esposito et al., 1980). Excessive temperature during the manufacture of 2,4,5-T is generally considered to be a major factor responsible for the presence of TCDD in 2,4,5-T.

At reaction temperatures above 225°C, the reaction of 1,2,4,5-tetrachloro benzene and ethylene glycol to form trichlorophenol becomes exothermic (May, 1973) and unless the temperature is reduced an explosion can ensue. Seven explosions in factories that were manufacturing trichlorophenol have been reported and are listed in table 5-1. An eighth incident, which occurred in France in 1956 and involved acute exposure during an operation to steam-clean trichlorophenol processing equipment, is also listed and described with the seven explosions. Large quantities of TCDD have been released in these accidents.

Occupational exposure to TCDD has occurred in factories when TCDD was formed in excessive amounts because the reaction temperatures were high enough to favor dioxin formation, but not high enough to become exothermic and produce an explosion. These incidents are listed in table 5-2. Two additional incidents of human exposure to TCDD are considered in this section and listed in table 5-3. In one incident, TCDD-contaminated salvage oil was sprayed on horse arenas in Missouri in 1971, resulting in human and animal exposures. In the second incident, three laboratory workers in England were exposed while they were heating trichlorophenol or potassium trichlorophenate, or handling the products.

Military exposure to TCDD resulted from the use of herbicides that contained contaminated 2,4,5-T. In this chapter, information on the exposure levels of TCDD from explosions and industrial exposures are summarized. The human effects of TCDD exposure reported after industrial accidents, occupational exposures, and military exposure are then compared.

5.1 INDUSTRIAL EXPLOSIONS

Exposure conditions and symptoms reported for the 8 accidents listed in table 5-1 are described in this section.

TABLE 5-1. INDUSTRIAL EXPLOSIONS

Table 5-1a. References on Industrial Explosions That Involved TCDD

YEAR	LOCATION	REFERENCES
1949	Monsanto Nitro, WV	Suskind (1978); VA (1980) Zack and Suskind (1980)
1953	BASF Ludwigshafen, W. Germany	Goldmann (1972); Goldmann (1973) Thiess and Frentzel-Beyme (1977)
1956	Rhone Poulenc Grenoble, France	Dugois et al. (1958)
1962	Italy	Joint NIESH/IARC Working Group (1978) Young et al. (1978)
1963	Philips-Duphar Amsterdam, Netherlands	Dalderup (1974); Berlin et al. (1976) Rawls and Sullivan (1976); Hay (1977)
1966	Rhone Poulenc Grenoble, France	Dugois et al. (1968) Rawls and Sullivan (1976)
1968	Coalite & Chemicals Products Bolsover, Derbyshire, U.K.	Jensen and Walker (1972) Jensen et al. (1972; May (1973)
1976	ICMESA Seveso, Italy	Commoner and Scott (1976; Hay (1976) Rawls and Sullivan (1976); Fara (1977) Gianotti (1977); Walsh (1977) Bonaccorsi, et al. (1978); Greim (1978) Laporte (1978); Reggiani (1978) Homberger, et al. (1979) Malizia, et al. (1979) Pocchiari, et al. (1979) Reggiani (1979); Strik (1979) Reggiani (1980); VA (1980); VA (1981)

Table 5-1b. References Which Review Industrial Exposures to TCDD

Forth (1977)
IARC (1977)
IARC (Joint NIESH/IARC-1978)
Hay (1979)
Moses and Moore (1979)
Esposito et al. (1980)
Hay (1977)
Firestone (1978)
Young et al. (1978)
Malizia et al. (1979)
Crow (1980)
Suskind (1980)

TABLE 5-2. INDUSTRIAL EXPOSURES

Table 5-2a. References on Industrial Exposures to TCDD

YEAR	LOCATION	REFERENCES
1949	Nordheim, Westfallen, West Germany	Baader & Bauer (1951)
1951-52	2 factories in Middle Rhein, W. Germany	Bauer et al. (1961)
1954	Boehringer, Ingelheim	Kimmig & Schulz (1957)
	Hamburg, West Germany	Kimmig & Schulz (1957) Schulz (1957); Bauer et al. (1961)
1956	Diamond Alkali Newark, N.J.	Bleiberg et al. (1964) Poland et al. (1971)
1964	U.S.S.R.	IARC (1977)
1964	Dow Chemical (2,4,5-trichlorophenol production) Midland, Michigan	Firestone (1978) Cook et al. (1980) Rowe (1980)
1965-9	Spolana Czechoslovakia	Jirasek et al. (1973) Jirasek et al. (1974) Pazderova et al. (1974) Jirasek et al. (1976) Pazderova-Vejlupkova et al. (1980)
1970	Japan	Miura et al. (1974)
1972	U.S.S.R.	Zelikov and Danilov (1974)
1950-1975	Dow Chemical (2,4,5-T production) Midland, Michigan	Kramer (1974)* Ott et al. (1980) Townsend et al. (1980)*
1955 - 1977	Monsanto Nitro, West Virginia	VA (1980)

*unpublished reports

Table 5-2b Other Industrial Exposures[1]

YEAR	LOCATION	CHEMICAL MANUFACTURED	NUMBER OF WORKERS EXPOSED
1952-3	Boehringer West Germany	Trichlorophenol	37
1953-1971	Rhone Poulenc Grenoble, France	Trichlorophenol	59
1956	Hooker U.S.A.	Trichlorophenol	---
1960	Diamond Shamrock U.S.A.	Trichlorophenol	---
1970 (?)	Bayer West Germany	Trichlorophenol	5
1973	Linz Nitrogen Works Austria	Trichlorophenol	50
1974	Bayer Verdingen, West Germany	Trichlorophenol	5
1975[2]	Thompson-Hayward Kansas City, Kansas	Trichlorophenol	--
1979	Vertek Chemical Plant Jacksonville, Arkansas	2,4,5-T	190

[1]These incidents were listed first in Hay (1977) and later in subsequent lists; no reports that provide exposure levels or symptoms have been identified; the 1979 incident was mentioned in Suskind, 1980.

[2]This incident was listed as an explosion by Hay (1977) and as an occupational overexposure in subsequent lists by Young et al. (1978) and Esposito et al. (1980).

Table 5-3. REFERENCES ON OTHER HUMAN EXPOSURES TO TCDD

YEAR	LOCATION	POPULATION DESCRIBED	REFERENCES
1971	Missouri	Farm residents	Carter et al. (1975) Case (1976) Beale et al. (1977) Kimbrough et al. (1977) Crow (1980)
1970	England	3 laboratory workers	Oliver (1975)
1967-1970	Vietnam	Vietnamese residents	NRC (1974)
		Vietnamese refugees	Tung et al. (1971) Rose and Rose (1972) Tung et al. (1980)
		Vietnam veterans	Bogen (1979) Halprin (1980) Stellman & Stellman (1980) VA (1980)
		Vietnam military	Harmon (1971) Allen (1980)

5.1.1 Exposure Conditions

Several variables in the eight accidents that are relevant for a comparison of the data on health effects from these incidents are considered below. These variables include:

- Dosage
- Duration and routes of exposure
- Concomitant exposure
- Latency period
- Unexposed controls.

5.1.1.1 Dosage

The types and severity of health effects observed after TCDD exposure are presumably related to the doses of TCDD received. Unfortunately, almost no information on exposure levels following explosions or during occupational exposure has been published and only a very limited comparison of dosage levels and resultant health effects can be made.

In two early accidents, TCDD was not known to be a contaminant released during the accidents or a potential health hazard. In 1949, when the Nitro, West Virginia accident occurred, TCDD had not been identified as a potential contaminant of the manufacture of trichlorophenol. Only after TCDD was identified following similar explosions elsewhere was the etiologic role of TCDD in the Nitro incident assumed. An incident in 1956 in a French factory manufacturing 2,4,5-T resulted in numerous cases of chloracne. The report that described these cases (Dugois et al., 1958) also mentioned that the causative agent could not be identified at the time, although any measures taken to identify the cause were not mentioned. A new manufacturing procedure, begun 2 years before the incident, introduced steam into a process that had been performed at colder temperatures. This steam process was stopped following the accident and other precautions were instituted to prevent worker exposure to released vapors. Subsequently, no new cases were reported.

After the 1953 accident at the BASF factory, an extensive search for the agent responsible for the workers' health effects was made (Goldmann, 1972; 1973). Symptoms of chloracne that resembled the condition observed in the workers were reproduced in rabbits treated with crude extracts of the TCP distillate. None of the chemicals known to be present in the distillate were acnegenic when tested individually. A series of chemicals that were considered potential contaminants were synthesized, and several of these compounds, including TCDD and several chlorinated dibenzofurans, produced potent acnegenic responses. Analysis of the distillate for its dioxin content led to confirmation of the presence of TCDD. The concentration of TCDD the workers encountered remained unknown.

TCDD levels were measured after three explosions. After the 1963 explosion in Amsterdam, a soot-like substance was released into the factory.

Analysis of this substance revealed that it contained 1,000 ppm TCDD. Estimates of the total amount of TCDD released into the factory hall ranged from 30 to 200 g (Crow, 1980). After the 1968 explosion in England, levels of up to 40 mg of TCDD per m^2 were detected on wall surfaces (Crow, 1980). These levels are considered to correspond roughly to the amounts released in the Amsterdam factory. Three years after the explosion in England, two workers who worked with one of the few salvaged pieces of equipment came down with severe cases of chloracne within 3-4 weeks after they were exposed. TCDD could not be detected on the apparatus by either chemical or biological assays (May, 1973). Assays of the sebum discharged from the skin of one of the affected men 1 year after exposure did not contain measurable amounts of TCDD by the gas-liquid chromatographic method used, which had a lower detection limit of 0.1 ppm; the sebum flow rate was normal (Jensen et al., 1972).

After the explosion in Italy in 1976, a cloud that contained TCDD was released over the surrounding countryside. Extensive measurements of TCDD were made of the affected areas, which resulted in establishing three zones of contamination: zones A, B, and R had areas with up to 270, 44, and 15 ug of TCDD per m^2, respectively. Mean values for various parts of zone A were 15-29 ug/m^2 and for zone B were 4 ug/m^2. Soil samples from zones A, B, and R contained greater than 10, 0.1-10, and less than 10 ug TCDD per kg, respectively (Reggiani, 1978). In the previous accidents, vapors released during the explosions were contained in limited areas of the factories. In Seveso, vapors were released into the environment, diluting the TCDD concentration substantially.

Only one estimate of human TCDD body burdens based on measured tissue levels of TCDD was found in the published literature. A woman who lived in Seveso's zone A for 2 weeks (between the accident and the time when the evacuation was ordered) died in October 1976 of pancreatic cancer. This death was not attributed to TCDD exposure. Tissues removed at autopsy were analyzed for TCDD; the levels found led to an estimate of 0.5 mg of TCDD per kg body weight absorbed at the time of the incident, assuming a half-life of 30 days for TCDD (Reggiani, in VA, 1981). Information to compare relative exposures is inadequate. Even when the information on TCDD levels in the vicinity several months or years after the accident is available, only two attempts to measure it in people have been recorded. One attempt was successful, but was dependent on several pharmacokinetic assumptions and was based on measurements from one person at only one point in time. The actual levels workers were exposed to, the length of the exposure period, or the amounts absorbed remain unknown.

5.1.1.2 Duration and Routes of Exposure

The eight industrial accidents listed in table 5-1a are being considered together because they resulted in acute exposure of workers (or residents, in the Seveso incident) to TCDD during a short period of time. However, inadequate decontamination measures or lack of knowledge regarding the presence of TCDD and its high order of toxicity led, in most cases, to continued exposures after the explosions occurred. No specific decontamination procedures were described after the Nitro accident. Extensive efforts were made to decontaminate the BASF factory after the explosion, and manufacturing of other products

was eventually resumed. Two years after the decontaminated facility was reopened, the first new case of illness occurred. The facility was closed again and in 1969 the autoclave and contaminated equipment were demolished (Goldmann, 1972). Decontamination measures in the English facility were also inadequate, and 79 cases of chloracne were reported in workers who were not present during the explosion but were exposed only to the "decontaminated" facility. Consequently, the contaminated equipment was dismantled and buried (May, 1973). Despite extensive measures taken to decontaminate the Amsterdam facility, residual TCDD remained and the factory was dismantled and parts were encased in concrete and sunk in the Atlantic Ocean (Berlin, 1976; Dalderup, 1974). Decontamination efforts in zone A in Seveso have not been completed and the factory that released TCDD is not being used, even though TCDD was not released inside the facility.

Cases of chloracne occurred in people who were never in the factories where the explosions took place. People whose only exposure to TCDD was from contact with affected Nitro workers developed chloracne; in one case a man who drove a truck that had been parked near the factory developed chloracne (Suskind, 1978). The health effects of Nitro workers who were not present when the explosion occurred was the subject of a separate investigation, which is described in the next section. After the BASF and British explosions, family members of workers who never entered the facility developed chloracne (Goldmann, 1972; Jensen et al., 1972). Workers involved only in the cleanup operation in Amsterdam contracted chloracne (Rawls and Sullivan, 1976). The Seveso incident resulted in high exposures of zone A residents over a 2-week period (before evacuation was ordered), and low, continuous exposures of residents of zones B and R, based on TCDD analyses of soil samples. Residents of one zone ate food from other zones and worked and traveled between the zones, complicating exposure estimates for these people (Bonaccorsi et al., 1978). After the explosion in France in 1956, chloracne was reported only in workers (Dugois et al., 1958); however, not knowing that TCDD was the causative agent may have prevented investigators from relating other cases among family members to the factory incident. In conclusion, exposure to TCDD probably was not limited to acute exposure during the explosions, but in many cases involved long-term exposures to low levels of TCDD that were not removed during the decontamination process, and in some cases involved indirect exposure of people who were never in contact with the contaminated factories.

The routes of exposure of TCDD are speculative. TCDD exposure by the workers was probably by pulmonary and dermal routes. The Seveso residents were probably exposed by the oral route, in addition, since food supplies were contaminated. Children who played outdoors had potential for much higher inhalation and dermal exposures than adults who remained inside.

5.1.1.3 Concomitant Exposures

Chemicals other than TCDD were present in the factories or were released during the explosions. The types and amounts of chemicals released are subject to speculation, as no results of quantitative or qualitative analyses of vapors during explosions were made. However, trichlorophenol was likely to be present in most cases and the early skin burns observed within the first two days of the Seveso accident have been attributed to trichlorophenol

(Walsh, 1977). Sodium hydroxide, sodium chlorophenate, other phenates, and ethylene glycol were probably present in Stack emissions of the ICMESA plant (Melvin, personal communication).

The phase of the trichlorophenol reaction that was taking place when the accidents occurred probably affected the types and amounts of polychlorinated chemicals that were released. In the 1963 accident in Amsterdam, the accident occurred during the beginning of the reaction when mainly tetrachlorobenzene was likely to be present and polychlorinated dibenzofurans were likely to be produced. The accidents in 1968 in the United Kingdom and in 1976 in Seveso occurred near the end of the reaction when mainly trichlorophenol was likely to be present, which produces TCDD (Joint NIEHS/IARC Working Group, 1978).

Some of the symptoms that were not observed in all accidents may have been the result of concomitant exposure to chemicals found only in specific incidents. However, different levels and durations of TCDD exposure may also be responsible for effects seen only after certain accidents. Some symptoms were not mentioned in descriptions of patients from some of the accidents because they were associated with conditions that probably were not suspected and could not be diagnosed without performing specific tests. For example, diagnosis of porphyria cutanea tarda requires that urine samples be analyzed for porphyrin content. This analysis is not routine and other symptoms of porphyria cutanea tarda, such as hirsutism and hyperpigmentation, may have gone unnoticed if porphyria was not suspected.

5.1.1.4 Latency Period

In comparing the consequences of these industrial accidents on human health, the duration of time that has elapsed between the accident and examination of patients is significant. Hepatotoxicity, measured by liver enzyme tests, was observed in the United Kingdom and Seveso workers, in tests performed several days or weeks after the accidents, but in both groups of patients these effects were transient and were not observed in subsequent tests (May, 1973; Reggiani, 1978).

The onset of early symptoms of chloracne, including redness and itching, was evident several days after exposure. Comedones that are characteristic of chloracne, on the other hand, may take 2 months to develop, but in severe cases such as occurred at the Nitro plant, lesions were still evident 28 years later (Crow, 1980). The results are complicated even when the time interval between the accident and examination are known, because exposure may have continued during an unknown part of this interval and the duration of sympto in some cases correlates to the severity of exposure, an unknown variable. The time between exposure and the onset of symptoms other than chloracne usually was not mentioned in reports of the accidents and was understandably more difficult to identify, as in the case of asthenia.

5.1.1.5 Unexposed Controls

Most reports and studies of workers exposed to TCDD are descriptive. They usually have not included data from control groups, as a basis for

comparison of experimental data. Two exceptions are the control group of Italian children who were compared to the TCDD-exposed children in Seveso for immunologic characteristics (Pocchiari et al., 1979), and several control groups in Germany used to evaluate the incidence of mortality in the BASF workers (Thiess and Frentzel-Beyme, 1977).

5.1.2 Symptoms Experienced After Exposure

Table 5-4 lists some of the symptoms reported after the industrial explosions. Except for Seveso data, all symptoms were observed in workers.

5.1.2.1 Chloracne

Chloracne occurred in exposed people after all eight accidents and is considered characteristic of TCDD exposure. The clinical symptoms that developed in all of these workers appear to have been similar in nature, varying primarily in severity and duration. These symptoms have been described clearly in most reports and have been the subject of several reviews (Birmingham, 1964; Schulz, 1968; Crow, 1970; Braun, 1970; Taylor, 1974; Crow, 1978a; Crow, 1978b; Taylor, 1979).

After some accidents, chloracne was severe, resulting in lesions still apparent many years after exposure ended. After the Nitro accident, 14 of the 122 people originally affected still had skin lesions 28 years later (Crow, 1980). The most severe cases involved workers considered most likely to have received high exposure to TCDD. After the explosion in Amsterdam, about 50 cases of chloracne developed. Thirteen years later, 10 cases remained (Hay, 1977).

Seventy-nine workers in the factory in England developed chloracne; however, only seven cases remained 4 years later. Chloracne did not develop in any of the 13 men who survived the original explosion (May, 1973). The lower doses of TCDD likely to have been encountered in the decontaminated British factory probably explains the lower severity, compared to workers in the Nitro and Amsterdam accidents.

Most of the 134 cases of chloracne from the Seveso accident occurred in children; however, only school-aged children, and not adults, were systematically screened for chloracne (Bonaccorsi et al., 1978). Children were not directly exposed in any of the other incidents but sometimes developed chloracne after contact with an exposed parent (Goldmann, 1972; Jensen et al., 1972). The Seveso data suggest that children may have a greater sensitivity to the acnegenic effects of TCDD than adults. The potential differences in exposure, between children (who were likely to have played outside) and adults, may have contributed to the differences in symptoms between children and adults.

Chloracne has appeared in many cases without any other apparent health effects. Half of the 42 chloracne cases from the BASF accidents had no other symptoms (Goldmann, 1972), and only two or three of the 44 workers in the

TABLE 5-4: HEALTH EFFECTS FROM INDUSTRIAL ACCIDENTS INVOLVING TCDD

Year	Location	No. Affected	Organ Systems Affected:										
			Dermal	Renal	Hepatic	Neuro.	Asthenia	Blood	GI	Lipid Metab.	Immuno.	CV	Pulm.
1949	Nitro, W.Va.	228	+		+	+	+	+		+			+
1953	BASF, W. Ger.	55	+	+	+	+	+					+	+
1956	Grenoble, Fr.	17	+		+		+						
1963	Amsterdam, Neth.	44	+		-		+		+				
1968	Bolsover, Derbyshire U.K.	79	+		-		+	+	-				
1976	Seveso, Italy	134	+		+	+	+	+	+		+	-	

Code: (+) An effect was observed;

(-) An effect was not observed in patients examined (or questioned) about the effect;

( ) Patients were not examined or questioned, regarding any effects in the indicated organ system.

Amsterdam accident (Crow, 1980) and the 79 workers in England (May, 1973; Hay, 1977) who developed chloracne had any other adverse effects; these effects were limited to asthenia. These trends have led to the conclusion that chloracne is the hallmark of TCDD poisoning (Crow, 1980). After most accidents, only patients that developed chloracne were examined further for other symptoms.

5.1.2.2 Porphyria Cutanea Tarda

This condition results from a disturbance in the capacity to break down hemoglobin, which leads to high levels of porphyrins in the liver and urine. Like chloracne, it is a relatively unusual condition which is not frequently encountered in other occupational situations; some cases have been shown to be inherited, rather than acquired.

Porphyria cutanea tarda was observed after the Nitro incident, and symptoms of this condition, including hirsutism and hyperpigmentation, were observed along with urine and liver changes (Suskind, 1978). After the Seveso accident, several cases of increased porphyria excretion and hyperpigmentation were observed; chromatographic examination of urine samples for levels of porphyrins excreted revealed patterns resembling mild chronic hepatic porphyria (Strik, 1979). These effects were observed only in a small portion of the exposed people in Seveso and were transient.

Porphyria cutanea tarda was not mentioned in the accounts from the other accidents. High doses of TCDD or repeated exposures may be required before this condition is observed. The possibility that concomitant exposures may have produced this condition in the Nitro and Seveso patients cannot be ruled out.

5.1.2.3 Hepatotoxicity

Aside from the liver function tests mentioned above, which showed transient effects immediately after exposure in workers exposed in England (May, 1973) and Seveso (Reggiani, 1978), hepatotoxicity was reported after other accidents. Hepatomegaly was observed in Nitro workers (Suskind, 1978) and Seveso residents (Bonaccorsi et al., 1978); hepatitis occurred in four of the 42 affected workers in the BASF factory (Goldmann, 1973) and hepatic disorders were mentioned (but not described) as a consequence of the accident in France in 1956 (Dugois, 1958).

Unlike chloracne, hepatotoxicity has not been reported consistently after TCDD accidents. When it is reported, it is absent in a large proportion of the people that develop chloracne. Furthermore, the different manifestations of hepatotoxicity, including hepatitis, hepatomegaly, and transient abnormal liver function tests may have resulted from different causes or as secondary or compensatory effects of TCDD exposure. It is not clear whether parameters of hepatic structure or function were even examined in the incidents that do not mention them.

5.1.2.4 Neurological Effects

Neurological effects were reported after every accident. These effects always included asthenia and in particular, fatigue. The incidence of fatigue varied. The frequency of this complaint was low among the affected workers in the Amsterdam and United Kingdom accidents (Crow, 1980; May, 1973; Hay, 1977), but was one of the few symptoms other than chloracne that was mentioned.

Other neurologic symptoms that were described after the Nitro, BASF, French, and Seveso accidents included headaches, sleep disturbances, irritability, and confusion (Suskind, 1978; Goldmann, 1972; Dugois et al., 1958; Walsh, 1977). The subjective nature of these symptoms decreases the reliability of identifying their frequency or severity. Peripheral neural damage involving the sensory organs and polyneuritis were mentioned in the Nitro and BASF workers. Pain in the extremities was mentioned by Nitro workers (Suskind, 1978) and encephalomyelitis occurred in some BASF workers.

Seven of the 42 BASF workers with chloracne developed problems related to the central nervous system (Goldmann, 1972; 1973). Delayed peripheral nerve conduction was observed by one examiner, but not by another examiner who studied the same population from Seveso (see VA; 1981). Seveso workers were examined for neurological function; 4 percent (8 subjects) were diagnosed as having polyneuropathy of peripheral nerve fibers. Three of these people were hospitalized and diagnosed as having polyneuropathy of the lower extremities. Other common causes of polyneuropathy were ruled out in these patients (Pocchiari et al., 1979).

5.1.2.5 Other Effects

Gastrointestinal effects were observed after the Seveso accident (Bonaccorsi et al., 1978) and the accident in France in 1956, although no other details were provided from the latter incident (Dugois et al., 1958). The delay in evacuation following the Seveso accident may have led to ingestion of TCDD-contaminated food. Oral exposure was unlikely after the other incidents and may explain why gastrointestinal effects occurred only after the Seveso incident.

Effects on the kidneys, heart, lung, and spleen each were reported in only the BASF workers (Goldmann, 1972; 1973) and, therefore, do not appear to be consistent effects of acute TCDD exposure. Different types of blood effects were reported for three of the accidents and were transient in one case (Suskind, 1978; May, 1973).

Reproductive effects were examined after the incidents in Amsterdam (Rawls and Sullivan, 1976) and Seveso (Homberger et al., 1979; Rehder et al., 1978; Reggiani, 1978), but no effects were demonstrated. Data on reproductive effects from the Seveso incident are considered in another section of this report.

Immune function was evaluated after only the Seveso incident (Reggiani, in VA, 1981; Homberger et al., 1979). In vitro tests produced evidence of decreased immune competence, although the affected children at Seveso did not

experience a higher incidence or severity of childhood diseases than children from other parts of Italy.

5.1.2.6 Mortality

No deaths were attributed directly to TCDD exposure immediately after the accidents.

Long-term mortality studies are being carried out on people exposed to or affected by the accidents at Nitro, BASF, Amsterdam, the United Kingdom, and Seveso. The incidence of death from cancers and from cardiovascular causes was not elevated in a study of the Nitro workers (Zack and Suskind, 1980). Of the 17 deaths among the BASF workers that occurred in the 24-year period after the accident, six were attributed to cancers, including four gastrointestinal cancers. The first death was caused by pancreatic necrosis and involved the first worker who entered the "decontaminated" facility; he was not present when the accident occurred (Thiess and Frentzel-Beyme, 1977).

A higher-than-expected incidence of death from myocardial infarction has been observed in the Amsterdam workers (Zack and Suskind, 1980; Joint NIEHS/IARC Working Group, 1978). Only one death has occurred in the workers from England, and was caused by coronary thrombosis (Joint NIEHS/IARC Working Group, 1978). The Seveso data on mortality rates indicate that the accident did not produce an observable increase in the death rate (Homberger et al., 1979).

The long latency period expected for cancers to develop requires that mortality studies should be conducted on a long-term basis; IARC is coordinating efforts in this area. In general, the number of deaths among the approximately 500 people affected in these incidents is very small. Trends have emerged in the data that exist today to indicate that TCDD exposure may increase the likelihood of death from cancer or cardiovascular reasons, but these trends were found in only one of the groups of workers and were not duplicated in any other.

5.1.3 Conclusions

In conclusion, chloracne and asthenia are consistently reported in the accidents described above. The incidence of asthenia is low compared to the incidence of chloracne. The subjective nature of asthenia complicates evaluating its significance as a primary effect of TCDD or a secondary effect of patients suffering from the discomfort and disfigurement of chloracne and the fears associated with exposure to toxic agents.

Other neurological disorders and hepatic disorders were commonly observed after these accidents. They were usually studied in chloracne patients and were more likely to occur in groups of workers with incidences of asthenia, although the specific symptoms involving these organ systems varied after the accidents. Peripheral neuritis and hepatomegaly were the most commonly encountered of these conditions.

Porphyria cutanea tarda occurred in the Nitro workers, who in general suffered more types of effects and more severe effects than workers from most of the other accidents, except for the BASF workers. These workers may have been exposed to the highest level of TCDD, as was suggested above.

5.2 OCCUPATIONAL EXPOSURE THAT DID NOT INVOLVE EXPLOSIONS

Twenty incidents other than explosions have been reported that resulted in exposure of workers to TCDD. These incidents are listed in table 5-2.

Nine incidents, listed in part 5-2b of the table, were listed in a table by Hay (1977) and have appeared on several subsequent lists of accidental exposures, but no other information on the circumstances of exposure or clinical manifestations of TCDD exposure have been reported and the incidents are not described in this section. The incident in 1975 in Kansas was described by Hay as an explosion, but was listed as an industrial exposure in more recent lists by Young et al. (1978) and Esposito et al. (1980). Three incidences of industrial exposure occurred in West Germany: in Westfallen in 1949, in Middle Rhein in 1951-52, and in Hamburg in 1954 (table 5-2).

5.2.1 Exposure Conditions

TCDD was identified as the likely cause of chloracne in several incidents, although the work environment was not sampled in any of the factories to determine actual levels of contamination to which workers were exposed. Workers in Westfallen who developed symptoms in 1949 were working with pentachlorophenol. Trichlorophenol was used only briefly in this facility and at the time was not considered to be the source of the intoxications (Baader and Bauer, 1951).

The relationship of TCDD to adverse health effects was determined for several incidents. TCDD was identified in the byproduct material of the Hamburg factory and one of the investigators confirmed that it was acnegenic after he applied it on his own skin and developed lesions resembling those seen in the workers. Other materials, including the purified trichlorophenol preparation and its trichlorophenol precursor, were not acnegenic in animal studies (Bauer et al, 1961). TCDD was identified on walls and in some of the products manufactured at the Czechoslovakian factory. The air was not analyzed and no quantitative analysis of any samples was reported (Pazderova-Vejlupkova et al., 1980).

Levels of TCDD were determined in the workplace for products made at several factories. Diamond Alkali had produced 2,4,5-T with a TCDD contamination level of 10-25 mg/kg at the time chloracne was identified among workers at this plant. Manufacturing procedures were changed to decrease this contamination level, and 7 years later a reexamination of the workers revealed marked improvement in their clinical picture as well as a reduction in the TCDD level to 1 mg/kg (Poland et al., 1971). At the Dow Chemical facility, air sampling revealed 2,4,5-T concentrations up to 0.8 mg/m^3. The TCDD levels were not measured, but between 1966 and 1972 the TCDD levels were required to be no higher than 1 ppm (Ott et al., 1980).

5.2.1.1 Duration and Routes of Exposure

In general, the appearance of symptoms of chloracne in workers has been interpreted as an indication that toxic chemicals were being released, and usually has resulted in changes in manufacturing conditions, improvements in industrial hygiene, and the elimination of new cases of chloracne. However, the intervals between the start of exposure, the appearance of symptoms, and the decrease or elimination of exposure have not been identified in most of the accounts, precluding any estimates of latency periods between exposure and symptoms, or threshold doses, or time periods below which symptoms do not occur. The health of Dow workers 6 years after chloracne was first diagnosed was reported, but no data were presented on their health status when the problem was first diagnosed (Firestone, 1978; Rowe, 1980).

Unlike the incidents involving accidents, the occupational exposures offered a reasonable risk for oral exposure in cases where food was stored or consumed in the workplace. The relative importance of exposure by various routes of exposure was seldom mentioned in reports and is not known.

5.2.1.2 Concomitant Exposures

Other than trichlorophenol, 2,4,5-T, and TCDD, chemicals to which workers were exposed were rarely mentioned in the reports discussed in this section. In two cases, however, concomitant exposures were mentioned and in both of these cases certain adverse health effects observed in the workers could be attributed to these chemicals. Seven of 10 workers exposed to pentachlorophenol in the Westfallen factory in 1949 developed bronchitis. This symptom was not reported in the other incidents (except for one case in the Hamburg workers) and may have been caused by pentachlorophenol (Baader and Bauer, 1951).

A mortality study of the Monsanto workers revealed a ninefold higher incidence of bladder cancer than expected. However, exposure to parabenobithenol (PAB), a known bladder carcinogen, was confirmed in at least 80 percent of these cases (Gaffey in VA, 1980). The impact of concomitant exposure to other chemicals on the health of workers exposed to TCDD is impossible to evaluate, as many of these people worked in the chemical industry for several decades (see VA 1981) and were exposed to a wide variety of chemicals.

5.2.1.3 Unexposed Controls

Most reports provided descriptions of health of workers, without any comparison to control groups. Control groups were used in evaluating the results of the second study of Diamond Alkali workers (Poland et al., 1971) and in the Czechoslovakian study to establish an expected range of values for the metabolic tests performed on the workers (Pazderova-Vejlupkova et al., 1980).

5.2.2 Symptoms Experienced After Exposure

Table 5-5 lists organ systems that were affected in workers who were occupationally exposed to TCDD. Symptoms discussed in this section are related to:

- Chloracné
- Porphyria cutanea tarda
- Hepatotoxicity
- Neurological effects
- Other effects.

5.2.2.1 Chloracne

As observed after the industrial accidents, almost every industrial exposure in table 5-2 has included cases of chloracne and, in fact, the appearance of chloracne has usually initiated the suspicion of TCDD exposure. Frequently, other clinical symptoms are reported only for patients that developed chloracne. Severe cases of chloracne occurred from the early exposures, including ten cases in 1949, nine of which remained 1 1/2 years after exposure ended. Seventeen workers were involved in the pentachlorophenol operation, but the skin conditions of the seven men who were not examined were not stated (Baader and Bauer, 1951).

Twenty-nine workers in the Diamond Alkali plant were diagnosed with chloracne, although the total number of workers exposed to potential TCDD-contaminated operations was not reported (Bleiberg et al., 1964). Seven years later, 18 percent of 73 workers at this facility had chloracne (Poland et al., 1971). In 1964, chloracne was reported in 69 of 83 workers in the USSR (IARC, 1977) and in 49 of 60 workers at Dow in the U.S. (Cook et al., 1980).

Other incidents of chloracne involving 78 workers in Czechoslovakia (Jirasek et al., 1973), 14 in Japan (Miura et al., 1974), and one in 1972 in the USSR (Zelikov and Danilov, 1974) were reported without giving the total numbers exposed. In these last two incidents, chloracne was the only clinical symptom observed. However, a 20-year study of Dow workers involved in the manufacture of 2,4,5-T revealed no chloracne or any other health effects among these workers (Kramer, 1974; Ott et al., 1980; Townsend et al., 1980).

5.2.2.2 Porphyria Cutanea Tarda

Porphyria cutanea tarda was observed in workers from two factories. Among 78 Czechoslovakian workers examined, 76 had chloracne and 11 had hepatic lesions and disorders of porphyrin metabolism; one of the 11 did not have chloracne (Jirasek et al., 1973; 1974; 1976; Pazderova et al., 1974; 1980). Eleven of the 29 Diamond Alkali workers examined had porphyria cutanea tarda (Bleiberg et al., 1964). In the study 7 years later, none of the 73 workers had porphyria (13 had chloracne) and the authors of this report (Poland et al., 1971) concluded that chloracne and porphyria cutanea tarda were two independent disease entities in these workers.

TABLE 5-5: HEALTH EFFECTS OF OCCUPATIONAL EXPOSURE TO TCDD

			Organ Systems Affected:										
Year	Location	No. Affected	Dermal	Renal	Hepatic	Neuro.	Asthenia	Blood	GI	Lipid Metab.	Immuno	CV	Pulm.
1949	Nordheim,W.Ger.	17	+			+							+
1952	Middle Rhein, W. Ger.	60	+				+						
1954	Boehringer Hamburg, W. Ger.	31	+		+	+	+		+			+	+
1956	Diamond Alkali Newark, N.J.	29	+		+	+	+	+	+	+			
1964	USSR	128	+		+		+		+				
1964	Dow Chemical Midland, Mich.	60			-		+						
1965-69	Spolana Czeckoslovakia	78	+		+	+	+						
1970	Japan	14	+		-					+			
1972	USSR	1	+					-	-				

Code: (+) An effect was observed;

(-) An effect was not observed in patients examined (or questioned) about the effect;

( ) Patients were not examined or questioned, regarding any effects in the indicated organ system.

The Japanese workers did not have porphyria cutanea tarda (Miura et al., 1974) and no other mention of porphyrin analyses or symptoms of this disease were mentioned in the accounts of the other industrial exposures. Hyperpigmentation occurred in all of the workers in Westfallen (Baader and Bauer, 1951), and was observed in the Hamburg workers (Kimmig and Schulz, 1957) and in both studies of the Diamond Alkali workers (Bleiberg et al., 1964; Poland et al., 1971), it was not observed in the 1972 Soviet exposure (Zelikov and Danilov, 1974). Descriptions of this symptom were inadequate to classify it as a manifestation of chloracne or porphyria.

5.2.2.3 Hepatotoxicity

Liver biopsies from Hamburg workers revealed hepatic disorders in a few cases, which involved fatty infiltration of iron deposits. Liver impairment was observed in some patients with severe chloracne who were exposed in 1964 in the Russian factory (IARC, 1977). Liver enlargement and abnormal liver function tests were observed in the Czechoslovakian workers (Jirasek, 1974; Pazderova-Vejlupkova et al., 1980), although the Dow workers had normal liver function tests (Firestone, 1978).

Hepatotoxicity observed after the industrial accidents is manifested in different ways. Reports rarely mention whether a patient was examined for a palpable liver and liver function tests appear not to have been done in other workers.

5.2.2.4 Neurological Effects

Neurological disorders were observed in workers from all of the early industrial exposures (the first seven are listed in table 5-5). Asthenia was observed in the Middle Rhein and Hamburg workers listed in table 5-2 (Bauer et al., 1961), in the Russian workers exposed in 1964 (IARC, 1977), in the Czechoslovakian workers (Pazderova-Vejlupkova et al., 1980) and in one Dow worker who experienced depression (Firestone, 1978). The Diamond Alkali workers were administered the Minnesota Multiphasic Personality Inventory (Poland et al., 1971). Scores for one part of the test were found to correlate with the severity of chloracne of the workers. Headaches, fatigue, sweating, dizziness, and sleep disturbances were the most common asthenic symptoms reported. Weakness of the lower extremities was reported in the same groups of workers that experienced asthenia. Joint pain, peripheral neuropathy or loss of sensory function was also reported for each of these groups of workers.

5.2.2.5 Other Effects

Gastrointestinal problems were common among the same groups of workers that experienced asthenic symptoms. Abdominal pains were reported in 30 percent of the Diamond Alkali workers (Poland et al., 1971) and in some of the Hamburg workers (Bauer et al., 1961; Kimmig and Schulz, 1957) and in the Russian workers exposed in 1964 (IARC, 1977). Nausea, vomiting, and diarrhea

in one group of workers (Poland, 1971), and decreased appetite and weight loss in another (Bauer et al., 1961), were reported. One of the Dow workers continued to experience difficulty in swallowing 6 years after TCDD exposure had ceased (Firestone, 1978). Increased serum cholesterol levels were observed in 10 percent of the Diamond Alkali workers (Poland et al., 1971) and in the Japanese workers (Miura et al., 1974); there is no evidence that serum cholesterol was analyzed in other workers. No abnormal hematology results were observed for the Russian workers exposed in 1972 (IARC, 1977), although 10 percent of the Diamond Alkali workers had decreased white blood cell counts (Poland et al., 1971).

The only cardiovascular problems reported were in the Hamburg workers and included orthostatic hypotension (Bauer et al., 1961).

Chronic bronchitis observed in West German workers might have been attributable to pentachlorophenol exposure, as mentioned above.

5.2.2.6 Mortality

A mortality study of workers suspected of industrial exposure was performed on workers at Monsanto who were employed between 1949 and 1969, the year Monsanto ceased production of trichlorophenol (Gaffey, in VA, 1980). The study included an identification of the causes of the 164 deaths that occurred among a total of 885 workers. Higher than normal incidences of deaths from bladder cancer, lung cancer, and arteriosclerotic heart disease were reported. The ninefold increase over the expected number of deaths from bladder cancer was attributed to exposure of these workers to paramenobitherol, a bladder carcinogen. The other two causes of death were increased both in TCDD-exposed workers and in all other workers as well, and was attributed to exposure to the industrial environment, with no increased risk attributed to TCDD exposure. Mortality studies were also performed on Dow workers involved in the 1964 outbreak of chloracne (Ott et al., 1980) and in 2,4,5-T production (Cook et al., 1980). Neither study identified an increased mortality with industrial exposure; however, only 4 and 11 deaths, respectively, have occurred in the two study populations.

5.2.3 Conclusions

In general, the signs of toxicity in workers exposed to TCDD were similar whether or not the exposure involved an explosion. Chloracne was by far the most common condition recognized in workers. Earlier incidents of occupational exposure, which occurred prior to 1970 or accidents before 1960, in general produced more symptoms, higher frequencies of affected workers among those exposed, and worse cases of chloracne than reported for later incidents. If workers experienced only one condition, it is was almost always chloracne (partly biased by the way the studies were conducted); if they had two, the second one was asthenia.

These trends are general impressions; as described in the foregoing discussion, inadequate descriptions of worker health, lack of analyses, and

lack of reporting results in quantitative terms precludes making strong statements regarding these trends. These trends also suggest that workers involved in the earlier incidents were exposed to higher TCDD levels than other workers. A logical hypothesis that emerges from this soft evidence is that hepatic and neurological effects require a higher threshold dose than chloracne.

Porphyria cutanea tarda requires specialized tests to detect; it may also require high or extended TCDD exposure, or may have a slightly different etiology than the other symptoms, or a different time course to explain its relative infrequency. Animal studies have produced evidence that porphyria cutanea tarda results from chronic exposure and not acute exposure to TCDD. Since no decontamination followed the Nitro accident, these workers probably were exposed over a long period of time; these were the only workers who had symptoms of porphyria after an industrial explosion. Human data are in agreement with animal studies in this instance.

Two human health effects which were common after industrial exposure but not after the explosions were gastrointestinal disorders and elevated serum cholesterol levels.

The actual extent of the consequences of TCDD accidents and occupational exposures on human health cannot yet be stated with confidence because: 1) most reports did not identify the number of workers who were exposed but not affected; 2) the descriptions of the health status of workers who did not develop chloracne were not provided; and 3) the incidence and severity of health effects were rarely compared to appropriate control groups.

5.3 HUMAN EXPOSURE TO TCDD FROM INDUSTRIAL WASTE DISPOSAL, AND LABORATORY EXPOSURE

Two incidents are considered in this section and are listed in tables 5-3 and 5-6. One incident considered in this section involved human exposure to TCDD in industrial waste that was combined with salvage oil and sprayed on three horse arenas in Missouri to control dust. The second incident involved three scientists in two government laboratories in England. Two men had heated trichlorophenol (or its potassium salt, in one case) to synthesize TCDD for use as an analysis standard, and the third handled one of the samples after it was diluted. Despite their awareness of potential hazards and precautions to avoid personal exposure, they developed chloracne and other health effects.

5.3.1 Exposure Conditions

The dosage, route, and duration of exposure, and concomitant exposures and control groups for the incidents involving TCDD exposure at the Missouri horse arenas and the British laboratory, are described in this section.

TABLE 5-6: HEALTH EFFECTS OF HUMAN EXPOSURE TO TCDD

Location	Organ Systems Affected:										
	Dermal	Renal	Hepatic	Neuro.	Asthenia	Blood	GI	Lipid Metab.	Immuno	CV	Plum.
United Kingdom[a]	+		-	+	+	-	+	+			
Missouri[b]	+	+	-	+	+	-	+				
Vietnam Veterans[c]	+			+	+		+			+	+
Vietnam Veterans[d]	+		+	+	+		+		+		
Vietnam Veterans[e]	+			+	+		+				
Vietnam Refugees[f]	+			+	+		+				

Code: (+) An effect was observed;

(-) An effect was not observed in patients examined (or questioned) about the effect;

( ) Patients were not examined or questioned, regarding any effects in the indicated organ system.

Reference a - United Kingdom, Oliver, 1975
Reference b - Missouri, Beale et al., 1977
Reference c - Vietnam Veterans, VA, 1980
Reference d - Vietnam Veterans, Bogen, 1979
Reference e - Stellman and Stellman, 1980
Reference f - Rose and Rose, 1972

5.3.1.1 Dosage

Investigation into the cause of a high incidence of animal mortality and human illness at three horse arenas in Missouri led to the identification of levels of 31.8-33.0 ug of TCDD per gram soil at the farm where human illness occurred (Carter et al., 1975). The date of sample collection and analyses was not reported. The source of TCDD was traced to an industrial hexachlorophene producer in Missouri who disposed of distillate residues from its trichlorophenol production with the same company that sprayed the arenas. The waste residue that remained in the storage tank at the industrial site in 1974 was found to contain 306-456 ug of TCDD per gram residue.

Estimates of the potential amounts of TCDD synthesized by the British scientists, or of amounts of trichlorophenol or potassium trichlorophenate initially used to synthesize TCDD, were not reported. One of the workers handled only a diluted sample of TCDD. This worker developed symptoms of asthenia, but not chloracne. The other two men worked with the concentrated lots of TCDD they synthesized, and both developed chloracne (Oliver, 1975).

5.3.1.2 Route and Duration of Exposure

Human illness from the Missouri incident occurred in four children and in the mother of two of the children (Carter et al., 1975). These children played in the contaminated arena. The child most severely affected played in the arena daily between the time of spraying and recognition of symptoms. The major exposure was probably dermal. The arena was sprayed on May 26, 1971 and hemorrhagic cystitis was diagnosed in one child on August 21, 1971. The time of appearance of other symptoms in the patients, including skin lesions, was not stated.

Since the laboratory workers took precautions to avoid contact by skin or inhalation, their routes of absorption are unknown. Although symptoms did not develop in two of the three scientists until two years after the day they synthesized or handled TCDD, no intervening instances of potential exposure could be identified (Oliver, 1975).

5.3.1.3 Concomitant Exposures and Controls

The chemical compositions of the salvage oils sprayed on the arenas were not reported. Potentiating or synergistic effects of combinations of hydrocarbon compounds, including the effects of solvents on the rate of dermal absorption of TCDD, are presently only matters for speculation.

The laboratory workers were also exposed to many other chemicals. This factor was considered when a control group was established to compare the results of serum cholesterol values. This group was comprised of laboratory workers who were exposed to the same chemicals as the patients, except that they had no known exposure to TCDD (Oliver, 1975).

5.3.2 Symptoms Experienced After Exposure

Symptoms reported in the children that played in the sprayed horse arenas (and one parent) and in the British scientist are described here, under the categories:

- Chloracne
- Porphyria cutanea tarda
- Hepatotoxicity
- Neurological effects
- Other effects.

5.3.2.1 Chloracne

The original reports of the health effects of people exposed to the TCDD-contaminated horse arenas did not mention the presence of chloracne. Later communications by an interested dermatologist with the physicians who examined the patients revealed that the 6-year-old girl who was diagnosed as having hemorrhagic cystitis, as well as her mother and 10-year-old sister, had skin conditions which included blackheads and were consistent with chloracne. The inquiring dermatologist concluded that the conditions were probably mild and went unnoticed by clinicians who were inexperienced in diagnosing chloracne or did not expect to see this condition (Crow, 1980). Twin 2-year-old boys from the second arena that was sprayed also developed skin conditions consistent with chloracne (Crow, 1980). No accounts of this Missouri incident ever clarified the number of people who resided on the farms that were sprayed or the proportion of exposed people who were affected. As observed in the Seveso incident, children were more affected than adults, but their exposure, was likely to be higher from play than adult exposure.

One of the three British laboratory workers developed chloracne, with no other symptoms. The other two workers were from a second laboratory. Only one of these two workers developed chloracne. Other symptoms that developed in the workers from the second laboratory were very similar (Oliver, 1975).

5.3.2.2 Porphyria Cutanea Tarda

There is no indication that urinary porphyrins were measured after the Missouri incident. This analysis was performed after the laboratory workers contracted chloracne, but no evidence of porphyria was produced. Two of the workers developed hirsutism and the third developed an unusual pigmentation which was attributed to chloracne (Oliver, 1975).

5.3.2.3 Hepatotoxicity

Liver function tests performed on the 6-year-old girl from Missouri and on the three laboratory workers were all negative (Beale et al., 1977; Oliver,

1975). Hepatomegaly or other evidence of hepatotoxicity were not mentioned in accounts of either incident.

5.3.2.4 Neurological Effects

The mother and sister of the affected child complained of intermittent headaches at the time hemorrhagic cystitis was diagnosed in the younger child (Carter el al., 1975). No other evidence of neurological effects was produced at that time (within 4 months of the onset of exposure) or at a 5-year followup examination which included a detailed neurological examination of the younger child (Beale et al., 1977).

Two of the British laboratory workers experienced several symptoms of asthenia, including, in particular, fatigue and irritability. Both reported experiencing transient visual problems. One worker reported having headaches and difficulty in muscular and mental coordination and the other worker experienced neurologic pain in one thigh. These symptoms emerged 2 years after the workers were exposed to TCDD (Oliver, 1975).

5.3.2.5 Other Effects

Hemorrhagic cystitis in the 6-year-old girl from the sprayed Missouri farm was accompanied by hematuria and proteinuria (Beale et al., 1977). These symptoms were resolved 1 week after they were recognized, although hemorrhagic areas of the bladder were still demonstrable by cystoscopy 3 months later.

The mother and sister complained of abdominal pains and diarrhea. Two of the laboratory workers also experienced abdominal pains and flatulence (Oliver, 1975).

One worker reported having lost about 6.5 kg in weight but experienced no loss in appetite. The second experienced a loss in appetite (but no weight loss) as well as diarrhea and indigestion.

Three years after the exposure took place, all three workers had elevated serum cholesterol values (above 300 mg/100 ml).

5.3.2.6 Conclusions

These two incidents reaffirm that chloracne is a likely consequence of TCDD exposure. They also demonstrate that chloracne may not be an obvious effect and can be overlooked by the examining physician. Furthermore, neurological symptoms can occur in workers with or without chloracne. The latency period for symptoms to develop can be on the order of 2 years.

Gastrointestinal problems and elevated serum cholesterol levels were common results of these incidents, as well as of other occupational exposures described in the previous section. Hypercholesterolemia remained 3 years after exposure in all three laboratory workers, while only residual signs of hirsutism in one worker and indigestion and flatulence in the second worker remained.

The level of TCDD in the soil in Missouri arenas also provides a comparison of exposure levels to levels from other incidents, although the source that reported this level (Carter et al., 1975) did not indicate whether this level was present shortly after the spraying when toxicity was recognized, or several years later, when the industrial storage tank was assayed for TCDD.

5.4 HUMAN EXPOSURE TO TCDD FROM MILITARY USE OF HERBICIDES

This section addresses five populations whose alleged exposure to TCDD are thought to be related to military use of herbicides in Vietnam.

5.4.1 Exposure Conditions

Health effects have been reported which the reporters suggested were associated with military use of chemicals in South Vietnam. Reports of health effects, listed in table 5-3, include conditions submitted as claims by Vietnam veterans (VA, 1980), symptoms observed by a physician who conducted a 10-month study of 78 Vietnam veterans (Bogen, 1970), the results of a nationwide study conducted by mail on 535 Vietnam veterans (Stellman and Stellman, 1980), a survey of 98 Vietnam refugees in Hanoi (Rose and Rose, 1972), and a survey of health effects of Vietnamese by the National Academy of Sciences (NRC, 1974).

5.4.1.1 Dosage

An estimate of 0.080 ug of TCDD per kg of topsoil in South Vietnam has been set forth based on the amount of TCDD in herbicides used in Vietnam, the size of the areas sprayed and the rates of spraying (Westing, 1978). Using the same methods of estimation, these authors estimated that contamination of the Missouri horse arenas was 110,000 ug TCDD/kg, compared to 32,000 ug TCDD/kg actually measured by Carter et al. (1975) and 5.9 ug TCDD/kg in Seveso soil.

Estimates from another source were based on surface area (Reggiani, 1978); for South Vietnam an estimated .083 g/ha of TCDD was applied, compared to 5,900 g/ha in Missouri and 5.6 and 0.013 g/ha in zones A and B, respectively, in Seveso.

A committee organized in 1971 to evaluate the effects of herbicide use in Vietnam set forth as one of its goals quantitative analyses of Vietnamese soil for herbicide residue levels, but security problems in postwar South Vietnam precluded obtaining any Vietnamese soil for analysis (NRC, 1974).

5.4.1.2 Route and Duration of Exposure

The major routes of herbicide exposure of U.S. servicemen in South Vietnam were dermal and inhalation, assuming they consumed U.S. military food rations. Vietnamese citizens were also exposed by ingestion when their food sources were sprayed.

The refugees whose symptoms were described by Rose and Rose (1972) all experienced alleged exposure to spray missions. They were requested to report the effects of the last spray mission they experienced; 95 percent had experienced at least two spray missions and 60 percent had experienced at least three missions. The survey was conducted between 1970 and 1971. All three of the reports of symptoms of Vietnam veterans in table 5-3 were published between 1979 and 1980, at least 10 years after herbicide use in Vietnam ceased.

5.4.1.3 Concomitant Exposures

The health effects of herbicides used in war are difficult to distinguish from those caused by other elements of war. The skin rashes and eye problems that were reported by Vietnamese refugees immediately after some spray missions have been attributed to CS spray missions, for example (Rose and Rose, 1972).

Pentachlorophenol, a wood preservative used in Vietnam, is produced by a process that is likely to introduce dioxins and chlorinated dibenzofuran contaminants, but not TCDD. Symptoms from these contaminants are likely to be indistinguishable from those of dioxin (Moses and Moore, 1979).

5.4.1.4 Controls

The complaints submitted by Vietnam veterans or by Vietnamese refugees have not been compared to symptoms reported by other veterans or refugees not known to be exposed to TCDD. Only one systematic examination of people known to have been in Vietnam between 1966 and 1969 (the period of heavy use of defoliants) has been conducted by physicians and reported (Bogen, 1979). No details of study design or methods were reported, however, and no clinical biochemical analyses or standardized procedures were described which would provide objective parameters for comparison. Furthermore, no control group was employed in the study and the study population was self-selected.

5.4.2 Symptoms Experienced After Exposure

Symptoms observed in Vietnamese or Vietnam veterans and suspected to be related to military use of herbicides are listed in table 5-6.

5.4.2.1 Chloracne

Skin rashes or conditions were reported in each study. However, no study included a description of skin conditions that would allow comparisons to be made with chloracne. Among the Vietnamese refugees, 16 percent reported prolonged skin conditions that involved pustules, scabs, or eczema.

In a book prepared by the U.S. Army (Harmon, 1971), skin diseases encountered in the military in Vietnam were described. Chloracne was not

mentioned. A 1-year field study conducted in South Vietnam in 1966-67 and reported by Harman showed cystic acne to be the major dermatologic disease which resulted in medical evacuation. This condition occurred primarily in soldiers who previously had acne and experienced a worsening of the condition about 6 weeks after they arrived in Vietnam. Allen (1977) reported about 7 percent of the U.S. military hospital admissions in Vietnam from 1965-1972 were for skin diseases. About 10 percent of cases examined in a U.S. Army dermatology clinic between 1970-1971 were cases of acne.

A recent report (Halprin, 1980) has suggested that chloracne has been uncommon among Vietnam veterans, even when examinations were conducted by dermatologists familiar with this condition and specifically seeking evidence for it.

5.4.2.2 Porphyria Cutanea Tarda and Hepatotoxicity

Little evidence for these conditions has been reported. Hepatitis and jaundice were reported by 10 percent and 5 percent, respectively, of Vietnam veterans that filed claims (VA, 1980).

5.4.2.3 Neurologic Effects

Veterans and refugees have reported fatigue, dizziness, and other symptoms of asthenia (VA, 1980; Bogen, 1979; Rose and Rose, 1972; Stellman and Stellman, 1980). Peripheral neuritis, joint pain, or numbness and sensory problems were also commonly reported (VA, 1980; Bogen, 1979; Stellman and Stellman, 1980).

5.4.2.4 Other Effects

Gastrointestinal problems were reported by most groups and usually involved diarrhea, vomiting, and abdominal pains. Increased susceptibility to infections (Bogen, 1979), and pulmonary and cardiovascular problems (VA, 1980) were each reported by one group.

Reproductive problems were reported in four of the reports and involved child deaths, miscarriages, or birth defects (Tung et al., 1971; Rose and Rose, 1972; Stellman and Stellman, 1980; NRC, 1974). However, the incidents were not documented, nor compared to expected rates for control groups. The types of birth defects and proportions of each were also not presented.

A high incidence of carcinogenicity was also claimed, but the type of cancers and expected rates were not presented.

5.4.3 Conclusions

The information on human health effects from exposure to TCDD in Vietnam suffers from lack of any systematic approach to collecting and documenting

data by health professionals. Furthermore, exposure levels in Vietnam are unknown and the groups described do not seem to constitute those likely to have had higher than usual exposure to herbicides in particular (as would be expected of military personnel involved in spray missions, for example).

The symptoms reported were non-specific and are logically associated with many components expected to be present in a war zone. A comparison of symptoms presented in the cited reports to those of control groups would be needed to establish higher than expected frequencies of adverse effects.

CHAPTER 5.

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CHAPTER 6

ACUTE TOXICITY

This chapter describes the effects of an acute (i.e., single) exposure of herbicide or TCDD in humans and animals. The first section of this chapter describes factors related to mortality produced by these compounds. For each compound, the potency of an acute dose is described in terms of its lethality, expressed as LD_{50} values, where these values are available. The LD_{50} is the dose that is lethal to 50 percent of the treated animals. For inhalation studies, potency has been expressed in terms of the LC_{50}, the concentration of vapor which produced 50 percent mortality in the exposed animals. The pattern of toxicity in terms of the target organs affected by the compounds and the causes of death, are also discussed in the first section of this chapter.

The second part of this chapter describes the acute effects observed for each organ system. Each section in this part describes the specific effects for a particular organ system. Within an organ system, these effects are organized by the chemicals that produced them. The only compounds that are described under each organ system are those which have been reported to cause acute effects in that system.

6.1 MORTALITY

Acute toxicity is usually expressed in terms of LD_{50} values, or the single dose that produces death in 50 percent of treated animals. These values are presented in this chapter for the herbicides under study, along with information on the latency period between treatment and death, the organs most severely affected, and the possible cause of death. Detailed descriptions of toxicological manifestations are provided in the annotations of the cited literature.

6.1.1 2,4-D

Both 2,4-D and 2,4,5-T have been frequently described as moderately toxic (Edson, 1960; Way, 1969; Dalgaard-Mikkelsen and Poulsen, 1962; Council for Agricultural Science and Technology, 1978). This conclusion is based on the LD_{50} values for these chemicals, which range between 300 and 1,000 mg/kg for a single oral dose to various species (see tables 6-1 and 6-2). Comprehensive reviews have been published on the acute toxicity of 2,4-D and 2,4,5-T (Rowe and Hymas, 1954; Dalgaard-Mikkelsen and Poulsen, 1962; IARC, 1977; Young et al., 1978; Gehring and Betso, 1978; NRC, 1974; President's Science Advisory Committee, 1971). References on the acute toxicity of 2,4-D are listed in table 6-3.

6.1.1.1 LD_{50}

The oral LD_{50} values for 2,4-D have been calculated for various species and are listed in table 6-1 according to the salt or ester of 2,4-D that was

Table 6-1. LD_{50} Values (in mg/kg) for 2,4-D

Species (Sex)	Chemical Form of 2,4-D Tested:					
	Acid	Sodium Salt	Alkanol-amine Salt	Isopropyl Ester	Mixed Butyl Ester	mono-to tri-propylene glycol butyl ether esters
Monkey		>214[a]				
Dog	100[d]					
Cat F					820[h]	
Rat M	375[a]					
F		805[a]			620[a]	570[a]
M,F		666[c] 2,000[i]		700[a]	1,500[i] 900[h]	
Mouse M	368[a]			541[a]		
F					713[a]	
M,F		375[c]			380[h]	
Guinea Pig M	469[a]	551[a]		550[a]		
F					848[a]	
M,F		1,000[c]				
Rabbit F					424[a]	
M,F		800[c]				
Chicken M,F	541[a]		380-765[a]	1,420[a]	2,000[a]	
Cow			150-188[e]		500-2,000	
Sheep		860[f]				
Pig			100-500[g]			

[a] Rowe and Hymas, 1954

[b] Bjorn & Northern, 1948

[c] Hill & Carlisle, 1947

[d] Drill and Hiratzka, 1953

[e] McLennan, 1974

[f] Shavgulidze et al, 1976

[g] Bjorklund and Erne, 1966

[h] Konstantinova, 1974

[i] Shillinger, 1960 (cited in Young, 1978)

Table 6-2. LD_{50} Values (in mg/kg) for 2,4,5-T

Species (Sex)	Chemical Form Tested:			
	Acid	Isopropyl Ester	Butyl Ester	Amyl Ester
Dog	100[a]			
Rat M	500			
F			481	750
M,F		495		
Mouse M	389; 674[b] 778[b]			
F		551	940	
Guinea Pig M	381			
F		449	750	
Rabbit M			712	
Chicken M,F	310			

[a] from Drill and Hiratzka (1953)

[b] from Roll (1971), for NMRI mice; all other values from Rowe and Hymas (1954)

Table 6-3. References on the Acute Toxicity of 2,4-D

Species	Route	Organs Affected or Studied: skin	liver	kidney	neural	muscle	blood	heart	lungs	gut	body wt.	Reference
Monkey	oral				x	x		x	x	x		Hill & Carlisle, 1947
Rat	oral						x					Hill & Carlisle, 1947
	oral		x				x					Szocs et al, 1970
	in vitro		x									Olson et al, 1974
	in vitro		x									Abo-Khatwa & Hollingsworth, 1974
Mouse	oral				x	x	x	x	x	x		Hill & Carlisle, 1947
	oral					x			x	x		Bucher, 1946
Rabbit	oral				x	x		x	x	x		Hill & Carlisle, 1947
	oral					x			x	x		Bucher, 1946
Guinea Pig	oral				x	x		x	x	x		Hill & Carlisle, 1947
Dog	oral					x			x	x		Bucher, 1946
	oral					x			x	x	x	Drill & Hiratzka, 1953
Sheep	oral			x		x						Shavgulidze et al, 1976
Pig			x	x	x	x			x	x		Bjorklund & Erne, 1966

tested. These data suggest that the pig and the dog show higher sensitivity to acute doses of 2,4-D than the other species examined, while the chicken shows the lowest sensitivity. A slower rate of metabolism of 2,4-D has been offered as a possible explanation of the higher sensitivity of the pig and dog (Bjorklund and Erne, 1966).

Disregarding which salt or ester was administered, the LD_{50} values for the rat, guinea pig, and mouse suggest that males are more sensitive to 2,4-D than females. The rabbit LD_{50} values suggest that female rabbits are more sensitive than males. However, 2,4-D has never been administered in the same chemical form to separate groups of males and females of the same species to validate this trend. The acid form appears to be the most toxic of the different chemical forms of 2,4-D. The LD_{50} values obtained for the other chemical forms from different laboratories show wider differences than those obtained from the same laboratory. Baker et al. (1953) orally administered 500 mg of 2,4-D butyl ester per kg to two dogs and observed no clinical effects or histopathology, 4 or 82 days after dosing. Whether the lack of sensitivity in this study compared to those in table 6-1 reflects differences in effects of the acid and butyl esters or other factors, such as type of dog used, cannot be resolved from the limited data for this species.

Most of the LD_{50} values for 2,4-D were published prior to 1967. It is not known whether manufacturing operations have changed since that time, altering the levels or types of contaminants in the preparations. The LD_{50} values obtained for rats by Russian and Czechoslovakian investigators are substantially higher than those obtained by investigators who used preparations manufactured in the U.S. The reasons for these discrepancies in LD_{50} values are unknown. For other species, LD_{50} values which were obtained from different laboratories varied by an order of two.

LD_{50} values were similar for doses of 2,4-D administered by gavage, by intubation, or intraperitoneally to the rat (Rowe and Hymas, 1954; Hill and Carlisle, 1947) and the guinea pig; subcutaneously to the mouse; or intravenously to the rabbit (Hill and Carlisle, 1947; Rowe and Hymas, 1954; Bucher, 1946; Young et al., 1978; Guseva, 1956).

The latency period between the time that 2,4-D was administered and death occurred is on the order of 2-9 days (Shavgulidze et al., 1976; Drill and Hiratzka, 1953) for most species. In some cases, death occurs rapidly after 2,4-D is administered; in these cases, ventricular fibrillation has been the cause of death.

6.1.1.2 Cause of Death and Target Organs

Lethal doses of 2,4-D produced the same symptoms in man as observed in other mammalian species. Neurotoxicity is the predominant effect of 2,4-D. Symptoms include stiffness of arms and legs, incoordination, lethargy, anorexia, stupor, and coma. Myotonia has been observed in man, as well as in the dog (Drill and Hiratzka, 1953), rabbit (Hill and Carlisle, 1947), and mouse (Bucher, 1946). Decreased body temperatures were observed in sheep (Shavgulidze et al., 1976) and mice (Bucher, 1946), but not cattle (McLennan, 1974) after a lethal dose of 2,4-D. Congestion of the viscera and renal

swelling, localized to the proximal convoluted tubules, has been observed in rats and guinea pigs. Gastrointestinal disorders have been observed in the pig, mouse, dog, and monkey (Bjorklund and Erne, 1966; Bucher, 1946) indicating that 2,4-D is an irritant to the gastrointestinal tract and is likely to induce vomiting in man (Young et al., 1978).

In the dog (Drill and Hiratzka, 1953) and pig (Bjorklund and Erne, 1966), death has been attributed to hepatic congestion and to pneumonia. In the mouse, rabbit, guinea pig, and rat, ventricular fibrillation has been the cause of death (Hill and Carlisle, 1947). Paralysis of respiratory muscles may have been a contributing cause of death in mice (Bucher, 1946) and sheep (Shavgulidze et al., 1976). Increased heart rates were noted in cows that ingested lethal or near lethal doses of 2,4-D (McLennan, 1974).

6.1.2 2,4,5-T

The potency of 2,4,5-T in producing lethal effects and the patterns of toxicity it produces are described in this section. Most of the data were published before 1970, when the dioxin contaminant of 2,4,5-T was first recognized.

6.1.2.1 LD_{50}

The LD_{50} values for 2,4,5-T for various species are listed in table 6-2, according to the chemical form that was administered. Except for the LD_{50} in the dog and two of the values for the mouse, all of the values were obtained from the same laboratory. As was observed for 2,4-D, the acid form of 2,4,5-T appears to be more toxic than the salts or esters. As with 2,4-D, of the species tested, the dog is most sensitive to 2,4,5-T. Male mice appear to be more sensitive to the lethal effect of 2,4-D than female mice. The paucity of data prevents any further analysis of differences in sensitivity between males and females for other species or differences in toxicity among various esters.

The validity of the LD_{50} values in table 6-2 has not been challenged or confirmed since they were published in 1953-1954 except for the data by Roll, who administered 2,4,5-T with less than 0.1 ppm TCDD. TCDD was not recognized as a contaminant of 2,4,5-T at the time the other studies were published, and there is no way to evaluate the contribution of potential TCDD contamination to the acute lethal toxicity of 2,4,5-T. The species differences that are observed for acute toxicity of TCDD are not apparent in table 6-2, but the LD_{50} values for 2,4,5-T may generally be lower than those for 2,4,5-T with no detectable TCDD present. The data by Roll suggest this trend. Differences in sensitivity among different strains of mice may also have contributed to the differences in mouse LD_{50} values in table 6-2, although the strain used by Rowe and Hymas (1954) was not stated. The effect of the route of administration of 2,4,5-T on its toxicity does not appear to have been studied.

6.1.2.2 Cause of Death and Target Organs

No cases of deaths from 2,4,5-T alone have been reported in humans. One case of suicide from a mixture of 20 percent 2,4-D and 40 percent 2,4,5-T was reported (Verhulst and Crotty, 1968). Symptoms typical of the neurotoxic effects of 2,4-D were described, along with vomiting, fever, hyperventilation, low blood pressure, and sinus tachycardia. Death occurred about 46 hours after the ingestion and was attributed to cardiac standstill. This report also mentioned another instance of hallucinations that occurred after inhalation of 2,4,5-T, but no other information was given.

References of the acute toxicity of 2,4,5-T in mammalian species are listed in table 6-4. High doses of 2,4,5-T have produced anorexia, ataxia, and mild to moderate stiffness in the hind legs in dogs (Drill and Hiratzka, 1953). No marked effects were observed in these dogs and one dog that died from 2,4,5-T exhibited no symptoms. Histopathologic changes in these animals were considered nonspecific and pneumonia was suggested as the cause of death. Rowe and Hymas (1954) described the effects of all of the phenoxy acids they tested (including 2,4-D, 2,4,5-T, Silvex, and 2-methyl-4-chlorophenoxyacetic acid) together. These effects included anorexia, weight loss, depression, tenseness, muscular weakness, and histological evidence of stomach irritation, minor kidney and liver injury, and occasional cases of congestion of the lungs. The cause of death was not reported.

Amounts of 2,4-D and 2,4,5-T that produce toxicity in cattle vary depending on the chemical form administered. A mixture of equal amounts of esters of 2,4-D and 2,4,5-T was administered to cows. A single dose of 1,000 mg/kg (33 percent 2,4,5-T and 35 percent 2,4-D) had no toxic effect. A single oral dose of 100 mg 2,4,5-T (amine salt)/kg administered to two pigs produced anorexia, vomiting, diarrhea, and locomotive disturbances. The same dose of 2,4-D (amine) produced diarrhea, stilted gait, and depression in two pigs, while 100 mg 2,4-D (ester)/kg was ineffective. After dosing, blood levels of 2,4,5-T peaked at 250 mg/ml, while 2,4-D (amine) levels reached 230 mg/ml; blood levels after 2,4-D (ester) was administered never exceeded 40 mg/ml. The investigators attributed the differences in toxicity among the compounds to the differences in circulating phenoxy acid levels.

Reports of accidental ingestion of lethal doses of 2,4,5-T by animals are rare. A case of fatal 2,4,5-T poisoning of a dog was surmised after levels of 2,000 ppm 2,4,5-T were measured in the stomach at autopsy. The likely cause of death was not stated by the veterinarian, who examined the dog two days after its death. Autopsy findings included hemorrhages of internal organs; evidence of vomiting was also reported (Leyland, 1975).

6.1.3 TCDD

6.1.3.1 LD_{50}

TCDD is often referred to as the most toxic man-made chemical known. This conclusion is based on the observation that the oral LD_{50} for TCDD in the

Table 6-4. References on the Acute Toxicity of 2,4,5-T

Species	Route	Organs Affected or Studied: skin	liver	kidney	neuro	musc.	blood	heart	lungs	gut	body wt.	Reference
Rat	oral			x								Stroo et al, 1979
	oral									x		Manis & Kim, 1979
	oral			(x)[a]								Sjoeden et al, 1977
	SC			x								Koschier & Berndt, 1976
	in vitro											Koschier & Berndt, 1977
	in vitro		x									Abo-Khatwa & Hollingsworth, 1974
	in vitro		x									Olson et al, 1974
Mouse	oral									x		Madge, 1977
	in vitro									x		Guthrie et al, 1974
Dog	oral					x			x	x	x	Drill & Hiratzka, 1953
	oral			x			x			x		Leyland, 1975

[a] measured indirectly

guinea pig is 0.6-2.1 ug/kg, one of the lowest LD_{50} values obtained in any species for any compound, except for several high molecular weight animal toxicants. In general, the oral LD_{50} values for TCDD for all species are low, although the values for various species vary by several magnitudes.

Table 6-5 lists the LD_{50} values for TCDD administered by several routes to several species. In some cases LD_{50} values have not been determined, but ineffective or marginally effective doses as well as doses lethal to over half of the treated animals are known. These values are given as the range of values that the LD_{50} falls within.

Few comparisons of TCDD either by different routes or in different sexes have been published. The LD_{50} values for rabbits were of the same magnitude after oral, intraperitoneal, and dermal exposures to TCDD (Moore, 1978). In one instance when the effects of TCDD were compared between groups of male animals and female animals, male rats were more sensitive than females (Schwetz et al., 1973). However, Moore (1978) reported the opposite trend in other species.

The reasons for the wide differences in LD_{50} doses for various species is at present unknown. As discussed in Chapter 4, differences in the amounts of TCDD-binding receptors present in different species (as differences in affinities of these receptors for TCDD) and differences in the rates of TCDD metabolism and excretion probably contribute to these differences in toxicity among species. The low sensitivity of the hamster has been shown to correlate with rapid biotransformation and excretion of TCDD in this species, compared to other species.

An understanding of the mechanisms of toxicity and of the variability of TCDD tissue distribution among different species will be required to understand this species variability. A high correlation has been observed between the extent of TCDD binding to receptors in a given strain of mouse and its potency as an inducer of hepatic mixed function oxidases in the same strain. The relationship between TCDD toxicity and enzyme induction has recently been suggested (Poland and Glover, 1980). The rate of binding of TCDD differs among several strains of mice. Only strains with high rates of binding showed sensitivity to thymic atrophy and cleft palate effects, and only these strains showed enzyme induction responses to TCDD.

6.1.3.2 Cause of Death and Target Organs

References on the acute toxicity of TCDD in mammalian species are listed in table 6-6. Death from TCDD usually occurs at least 1 week after an acute dose is administered and sometimes up to 7 weeks later. This latency period is very long, compared to the latency periods for other compounds. When higher doses of TCDD are administered, the time until death is not shortened appreciably, although the proportion of treated animals that die increases (Greig et al., 1973; Moore, 1978). This latency period indicates that TCDD is probably toxic at the cellular level, rather than causing death rapidly by impairing the physiologic function of an organ system such as the nervous system (Poland and Kende, 1976; McConnell et al., 1978a; 1978b).

Table 6-5. LD_{50} Values (in ug/kg for TCDD)

Species (Sex)	Route	LD_{50}	Reference
Monkey (F)	oral	<70	McConnell et al., 1978
Rat - Spartan (M)	oral	22	Schwetz et al., 1973
(F)	oral	45	Schwetz et al., 1973
Rat - Portion (F)	oral	190[a]	Greig et al., 1974
(F)	oral	125-500[b]	Greig et al., 1974
Mouse - C57BL	oral	126	Jones and Greig, 1975
	oral	114	Vos et al., 1974
	oral	>130	Schwetz et
(M)	oral	<150	Goldstein et al., 1973
(M)	ip	120	Vinopal and Casida, 1973
Guinea Pig (M)	oral	0.6-2.1	Schwetz et al., 1973
		<3	McConnell et al., 1973
Guinea Pig (M,F)	oral	1157	Olson et al., 1978b
(M,F)	ip	>3000	Olson et al., 1980
Rabbit (M,F)	oral	115	Schwetz et al., 1980
(M,F)	dermal	275	Schwetz et al., 1973
(M,F)	ip	252-500	Schwetz et al., 1973
Dog (M)	oral	30-300	Schwetz et al., 1973
(F)	oral	>100	Schwetz et al., 1973
Frog	oral	1,000	Vos et al, 1974

[a] in DMSO

[b] in arachis oil

Table 6-6. References on the Acute Toxicity of TCDD

Species	Route	Organs Affected or Studied: skin	liver	kidney	neural	lymph.	blood	heart	lungs	gut	body wt.	Reference
Monkey	oral		x	x								Seefeld et al., 1979
	oral	x	x	x		x	x				x	McConnell et al., 1978a
Rat	oral		x				x			x	x	Greig et al., 1973
	oral		x			x					x	Harris et al., 1973
	oral		x					x			x	Schwetz et al., 1973
	oral, ip		x							x		Manis & Apap, 1979
	oral, ip									x		Manis & Kim, 1979a
	oral									x		Madge, 1977
	oral, ip									x		Manis & Kim, 1979b
	oral		x			x						Bastomsky, 1977
	oral		x			x					x	Van Logten et al., 1980
	oral		x									Thunbert et al., 1979
	oral		x		x		x				x	Cunningham & Williams, 1972
	oral		x								x	Courtney et al., 1978
	oral		x									Jones, 1975
	oral		x									Guiney et al., 1978
	oral		x									Jones & Butler, 1974
	oral		x									Fowler et al., 1973
	oral		x									Hamada & Peterson, 1978
	oral		x									Peterson et al., 1979
	oral, ip		x	x								Greig et al., 1974
	oral, ip			x								Pegg et al., 1976
	ip			x								Hook et al., 1978
	ip		x				x					Buu-Hoi et al., 1977
	ip		x			x	x	x	x		x	Buu-Hoi et al., 1972a
Mouse	oral		x			x	x				x	McConnell et al., 1978b
	oral		x	x		x						Harris et al., 1973
	oral					x	x					Vos et al., 1974
	oral						x				x	Drill & Hiratzka, 1953
	oral										x	Luster et al., 1978
	oral		x									Jones & Greig, 1975
Guinea Pig	oral		x				x			x	x	Greig et al., 1973
	oral			x		x					x	McConnell et al., 1978b
	oral		x			x					x	Harris et al., 1973
	oral											Schwetz et al., 1973
Rabbit	oral, ip		x					x			x	Schwetz et al., 1973
	dermal, eye	x	x					x			x	Schwetz et al., 1973
Dog	oral		x					x			x	Schwetz et al., 1973
Hamster	oral, ip					x				x	x	Olson et al., 1980

The actual cause of death in TCDD-poisoned animals is often difficult to identify. Animals become emaciated and all fat reserves are depleted, even though food intake is not appreciably depressed (McConnell et al., 1978a; Harris et al., 1973) and absorption through the gut is normal (Greig et al., 1973). Death has been attributed to severe hepatic lesions observed in the rat, although this effect is often absent in other species such as the mouse and the guinea pig. In the hamster, oral doses showed higher toxicity than intraperitoneal doses, and ileitis and peritonitis were observed only after oral doses (Olson et al, 1980). The intestinal lesions were moderate to severe, and may have been responsible for the increased lethality of oral doses.

Severe thymic atrophy from lethal doses of TCDD has been observed consistently among different species (Harris et al., 1973; Schwetz et al., 1973; Vos et al., 1974; Olson et al., 1980). In the guinea pig and mouse, the change in thymus weight is the most sensitive indicator of TCDD exposure (Harris et al., 1973). The corresponding loss in immune function is more difficult to demonstrate and seems to be significant primarily in animals that were treated with TCDD before they reached adulthood. Deaths from pulmonary infections have been reported for TCDD-treated rats, but rats bred under sterile conditions also suffer the lethal effects of TCDD (Greig et al., 1973), indicating that the lethal effects of TCDD are not dependent on an active immune system. Hydropericardium, ascites, and subcutaneous edema occur in chickens that were fed toxic fat containing TCDD and other dioxins, and death has been attributed to pulmonary edema. Edema occurs in other species including rats, pigs, dogs, and monkeys, but to a limited extent (Firestone, 1973; Vos, 1978; Huff et al., 1980).

6.1.4 Diquat

The toxic effects of diquat are described in several reviews (Manzo et al., 1979; Witschi, 1977; Smith and Heath, 1976; Conning et al., 1969).

6.1.4.1 LD_{50}

LD_{50} values for diquat have been reported for oral and subcutaneous doses (Clark and Hurst, 1970). The oral LD_{50} values range from 30 mg/kg in cattle to 200-400 mg/kg in the hen. Values for the rat (231 mg/kg), the mouse (125 mg/kg), the guinea pig (100 mg/kg), and the dog (100-200 mg/kg) were all similar, indicating little variation among species. However, most of these values are based on results from groups of only three animals per species. Only groups of rabbits, rats, and mice were large enough in this study to produce reliable LD_{50} values. In another study, an acute oral dose of 100 mg/kg was lethal to one of two treated monkeys, while a dose of 400 mg/kg was lethal to both monkeys that received it (Cobb and Grimshaw, 1979). For both the dibromide and dichloride salts of diquat, subcutaneous LD_{50} values were 10-11 mg/kg in male and female rats. Acute inhalation and dermal exposures to diquat have been reported that did not elicit any toxicity. Exposure to 23 mg of diquat aerosol per liter air for 15-30 minutes caused no adverse effects in male rats (Gage, 1968). In rabbits, a dermal dose of 400 mg diquat/kg produced no toxicity (Clark and Hurst, 1970).

Several studies have demonstrated that animals exposed to high oxygen levels show high susceptibility to the acute toxicity of diquat. In one study, 20 mg diquat/kg produced 50 percent mortality in rats, while 10 mg/kg was not lethal. Half of the deaths occurred within 2 days of treatment. Other rats, exposed to 85 percent oxygen, were more susceptible to the lethal effects of diquat: a dose of 10 mg/kg produced 50 percent mortality, with a mean latency period to death of 41 hours; 20 mg/kg produced 100 percent mortality, with a mean latency period of 17 hours. Lesions observed in diquat-treated rats in 85 percent oxygen, but not in air, were type II cellular damage and lung edema. The authors implied that increased oxygen levels may facilitate generation of superoxide anions by diquat, which in turn may induce pulmonary toxicity (Pratt et al., 1980).

In another study, rats exposed to 100 percent oxygen showed reduced latency periods between intravenous administration of diquat and death, compared to rats in room air. Oxygen exposure also induced respiratory distress in diquat-treated rats, a symptom which was not observed in rats treated with diquat and exposed to room air. Since tissue distribution was not altered by high oxygen exposure, the authors concluded that increased oxygen facilitated diquat oxidation that might lead to the production of toxic oxygen radicals (Kehrer et al., 1979).

6.1.4.2 Cause of Death and Target Organs

Cases of human intoxication after exposure to diquat have been reported. One fatal case of diquat poisoning has been reported (Okonek and Hofmann, 1975). A 43-year-old woman intentionally ingested two unknown doses of diquat, 2 days apart. After the second dose, the woman was admitted to the hospital with severe ulceration of the mouth, throat, and esophagus, and anuria, and was in shock. Extracorporeal dialysis was performed twice, but was not effective in removing a significant amount of diquat and the patient died 46 hours after admission, of protracted cardiovascular collapse. Symptoms reported in cases of nonlethal diquat poisoning included ulcerations of the mouth and pharynx (Oreopoulos and McEvoy, 1969) after ingestion; high fever, cough, jaundice, and skin rash in one case of exposure by inhalation (Wood et al., 1970); and fever, dizziness, headaches, eye pain, and temporary disturbances in liver and kidney function tests in another case of inhalation exposure (Weirich, 1969). Direct contact with concentrated formulation of diquat and paraquat mixtures have been reported to cause ocular damage (Cant and Lewis, 1968) and nail damage (Sammon and Johnston, 1969).

The toxicity of diquat has been evaluated in several animal species. Clark and Hurst (1970) reported that the pattern of diquat poisoning was similar in all species they studied. One day after an oral dose was administered, animals became lethargic, developed some respiratory difficulty, and died within 2 weeks. Histopathological changes were limited to the gastrointestinal tract. Additional symptoms observed after subcutaneous exposure were pupillary dilation within several hours of the injection, which remained until death (within 2 weeks), and distended abdomens with bile-stained contents in the digestive tract. Reduction of lymphocytes and in the sizes of the spleen and thymus were observed several days after treatment. Injection

of high doses (four to five times the LD_{50}) produced lethargy within minutes, labored respiration within 1 hour, and muscle twitches, convulsions, and death within several hours.

Symptoms of diquat poisoning in the monkey include diarrhea and lethargy, and, in severe cases, coma. In addition, histopathological changes in the gastrointestinal tract and kidney occur in this species (Cobb and Grimshaw, 1979). In cattle, dehydration, rapid respiration and pulse, unsteady gait, and coma were observed prior to death; all were attributed to diquat poisoning. Histopathological changes involved the gastrointestinal tract, heart, liver, brain, and lungs (Thomas and Amor, 1968). The cause of death in diquat poisoning of animals remains unknown.

6.1.5 Cacodylic Acid

Peoples et al. (1979) reported the effects observed in 34 cases of accidental exposure of workers to organoarsenical herbicides. All cases involved exposure to cacodylic acid, sodium cacodylate, methanearsonic acid, its salts, or combinations of these compounds. The systemic effects of cacodylic acid and its salt involved gastrointestinal irritation. Direct contact of the eyes or skin with the herbicides resulted in mild injuries. None of the clinical symptoms persisted. The authors suggested that all of the accidents could have been avoided by proper use and maintenance of the applicator equipment.

Stevens et al. (1979; Stevens et al., 1976) studied the acute inhalation toxicity of cacodylic acid in the rat and mouse. Mortality was higher after exposure to a low concentration than after a high concentration in male rats, and was low in both male rats and in mice, precluding calculation of LC_{50} (the concentration causing 50 percent mortality) values. Two of 10 male rats and no mice died after exposure to particulate concentrations of cacodylic acid of 4.1 mg/liter air for 2 hours, while none of the rats and one mouse exposed to a higher concentration of 6.9 mg/liter died during the subsequent 2 weeks. The LC_{50} for female rats was calculated at 3.9 mg/liter, after a 2-hour inhalation exposure. Symptoms observed in rats and mice from inhalation exposure were respiratory distress, rhinorrhea, porphyrin-like encrustation of the eyes, erythemia, and histopathological changes of the lung and gastrointestinal tract. Cacodylic acid also elicited a weak respiratory irritant response.

LD_{50} values after intraperitoneal injection of cacodylic acid were 720 and 520 mg/kg in male and female rats, respectively, and 520 and 600 mg/kg in male and female mice, respectively. The intravenous LD_{50} in female rats was 470 mg/kg. Symptoms observed after cacodylic acid injections included loss of the righting reflex, rigidity, decreased body temperature, decreased thymic size, and dark adrenals and livers, in addition to the symptoms that resulted from inhalation exposure (Stevens et al., 1979).

LD_{50} values for cacodylic acid have been published in several reviews. Oral LD_{50} values for the rat were reported by Weakley (1977) to range from 600-3,200 mg/kg, by Woolson (1976) to range from 700-3,200 mg/kg, by Peoples

(1975) to range from 1,200-1,600 mg/kg, by Dietz and Moore (1978) to be about 700 mg/kg, and by Palm (1968) to be 830 mg/kg. Details of the methods used to determined these LD_{50}s or reasons for the wide variation in reported values were not presented in these review articles. Dietz and Moore (1978) reported that the dermal toxicity was far less than the oral toxicity of cacodylic acid in the rat. Woolson (1976) reported that the oral and intravenous LD_{50} in mice were 184 and 316 mg/kg and the subcutaneous LD_{50} in the dog was 1,000 mg/kg. Midwest Research Institute (1975) also reviewed the acute toxicity data for laboratory and domestic species.

6.1.6 Picloram

The LD_{50} values for picloram have been determined in several species. Based on the number of deaths that occurred within 14 days of an oral dose of picloram, the LD_{50} values were calculated to be 8,200 mg/kg in the female rat, 2,000-4,000 mg/kg in the female mouse, about 3,000 mg/kg in the female guinea pig, about 2,000 mg/kg in the rabbit, and about 6,000 mg/kg in the male chicken. Single oral doses of 650 mg/kg to sheep and 488 mg/kg to cattle produced no ill effects (Lynn, 1965). Another study also found that single oral doses of 720 mg/kg to sheep or 540 mg/kg to calves produced no ill effects (Jackson, 1966, cited in McCollister and Leng, 1969).

Dermal application of 4,000 mg/kg to rabbits for 24 hours produced local edema and pigmentation, but no other effect. An undiluted preparation and a 10 percent slurry in water were both slightly irritating to the rabbit eye, producing conjunctivitis that lasted 2 days (Lynn, 1965). Adverse symptoms and cause of death after fatal doses of picloram were not described. No cases of human poisoning from picloram have been reported.

6.1.7 Monuron

The toxicological data on monuron have been reviewed (IARC, 1976; Midwest Research Institute, 1975). The acute toxicity of monuron was studied in the rat. The oral LD_{50} for the rat was 1,480 mg/kg and the mean time to death was 27 hours (Boyd and Dobos, 1969).

Symptoms observed 1 to 8 hours after monuron treatment were primarily neural disorders, including ataxia, drowsiness, hyporeflexia, pallor, piloerection, exophthalmos, and tachypnea. After 24 hours, irritability, hyperreflexia, and diarrhea were observed. Prior to death, anorexia, dyspnea, hypothermia, loss of body weight, epistaxis, oliguria, and prostration occurred. Deaths were attributed to respiratory or cardiorespiratory failure. Histopathologic changes included gastric ulcers, gastroenteritis, hepatic necrosis, renal lesions, pneumonitis, a stress reaction in lymphatic tissues, and congestion in the brain, heart, and lung (Boyd and Dobos, 1969).

Rats fed low protein diets were more susceptible to the lethal effects of monuron than rats fed normal diets. This susceptibility seemed to be associated with their inability to tolerate weight loss from monuron in addition to the weight loss from the protein deficient diet (Boyd and Dobos, 1969).

6.1.8 Diuron

The LD_{50} for diuron was calculated at 1,017 mg/kg in the rat after a single oral dose. The average time to death was 24 hours. Gastritis, enteritis, dehydration of the cecum, congestion of the brain and lungs, and a yellowish color of the kidney resulted from diuron administration. Hypoflexia, hypothermia, loss of body weight, drowsiness, and bradypnea were also observed. Irritability and hyperreflexia occurred in survivors a day after treatment. Death was caused by respiratory failure. Histopathological changes were observed in the gastrointestinal tract, adrenals, thymus, spleen, kidney, and liver. Protein deficient rats showed higher susceptibility to diuron (with LD_{50} as low as 437 mg/kg) than rats fed standard diets (Boyd and Krupa, 1970). Diuron in vitro has been shown to reduce transport of glucose across the mouse intestine (Guthrie et al., 1974).

6.1.9 Dalapon

Dalapon has been reported to cause contact dermatitis. Seven forest workers were diagnosed with this condition, having known contact with this herbicide and having developed a rash that cleared rapidly. Dermatitis, which was observed in 21 other workers, was suspected to be caused by dalapon or sodium chlorate, although exposure to either chemical could not be confirmed. In response to a questionnaire on exposure to dalapon, workers indicated having frequently experienced such subjective symptoms as nausea, skin lesions, anorexia, and pain in the throat. The results of clinical chemistry tests were found to be similar for a group of dalapon sprayers and a control group (Matsushita et al., 1975).

Two animal studies considered the acute toxicity of dalapon. The acute oral LD_{50} of dalapon in the rat was 7,570 mg/kg in females and 9,300 mg/kg in males; values for female mice, female guinea pigs, female rabbits, and chickens were 4,600, 3,860, 3,860, and 5,660 mg/kg, respectively. Death occurred within one day of treatment. No gross pathological changes were observed and the only histopathological finding was an accumulation of fluid and gas in the gastrointestinal tract. When dalapon, as dry powder, was applied to the eye of a rabbit, it produced conjunctivitis and corneal injury within an hour; these lesions healed in several days (Paynter et al., 1960). Norris obtained lower LD_{50} values for dalapon, which were 5,660 mg/kg in female rats, 2,830-4,930 mg/kg in female guinea pigs, and 2,830-2,140 in female rabbits (published by Kenaga, 1974).

6.1.10 Bromacil

Sherman and Kaplan (1975) reported an LD_{50} value for male rats of 5,200 mg/kg. A dermal dose of 5,000 mg/kg produced no clinical signs or gross pathology in rabbits and the LC_{50} was greater than 4.8 mg/liter for the rat. Toxicological data on bromacil have been reviewed (Midwest Research Institute, 1975).

6.1.11 Agent White

Dermal application of a 5 percent solution of Agent White, a mixture of 10.2 percent picloram (triisopropanolamine salt) and 39.6 percent of 2,4-D (triisopropanolamine salt), produced no skin irritation of sensitization in man (Weimer et al., 1970).

The oral LD_{50} for Herbicide White in rats was 3,080 mg/kg. In comparison, 5,000 mg/kg of an 11.6 percent preparation of the potassium salt of picloram produced no deaths. Exposure of rats to air bubbled through the mixture produced no toxicities, following a 7-hour exposure. A dose of 2,000 mg/kg of Agent White administered dermally to rabbits produced slight hyperemia and slight necrosis, but no systemic toxicity (Weimer et al., 1970; Lynn, 1965). Single ocular applications of 0.01 ml of Agent White to rabbits caused blepharitis, iritis, and conjunctivitis; higher doses (0.05-0.2 ml) produced corneal opacity as well. These lesions cleared within 28 days (Weimer et al., 1970).

6.2 DERMAL LESIONS

None of the herbicides considered in this report produce significant dermal lesions, other than the local irritation described in section 6.1. TCDD, a potential contaminant of the herbicide 2,4,5-T, produces chloracne. Chloracne occurred in workers after every reported instance of occupational exposure to TCDD during the manufacture of trichlorophenol and after other industrial exposures that released TCDD (see chapter 5). TCDD has been shown to be responsible for chloracne: an investigator applied a 0.01 percent solution to his forearm and developed lesions characteristic of chloracne (Bauer et al., 1961). Mild dermatitis developed in 2 days, followed by comedones several days later.

The results of experiments in which TCDD was applied to the skin of human volunteers were presented in testimony in the Environmental Protection Agency is 2,4,5-T/Silvex Cancellation Hearing (Rowe, 1980). A 1 percent suspension of TCDD in alcohol chloroform was applied to the backs of 10 subjects on alternate days for 1 month. This protocol resulted in the application of a cumulative dose of 7,500 ug of TCDD. Eight of the 10 subjects developed chloracne, which lasted four to seven months. No other adverse health effects were noted from urinalysis or chemical tests monitoring blood, liver, and kidney function. A cumulative dose of 16 ug applied in the same manner was ineffective; no intermediate doses were tested.

TCDD applied to the rabbit ear produces local lesions resembling human chloracne. Inflammation and hyperkeratosis were produced after 0.01-0.005 percent TCDD in polyglycol was brushed on a rabbit ear (Kimmig and Schulz, 1957a; 1957b). Dermal doses above 0.002 percent, however, resulted in death from liver lesions before dermal lesions appeared (Kimmig and Schulz, 1957b). The rabbit ear bioassay was used to identify the presence of TCDD, through its acnegenic potential, after industrial accidents. A dermal dose of 0.3 ug of TCDD in acetone, applied in three applications over three days, was sufficient to cause hyperkeratosis in 14 days; higher doses produced keratinous masses in

the follicular epithelium that resembled comedones, and caused a reduction in the number of sebaceous cells that could be observed by histology (Jones and Kizek, 1962).

In agreement with these results, another laboratory (Rowe, 1980) reported that a dose of 0.2 ug of TCDD did not cause acne in the rabbit ear bioassay, 0.5 ug caused a marginal effect, and 4-8 ug caused a severe acnegenic response. From these results, man appears to be much less sensitive than the rabbit to the acnegenic effects of dermally applied TCDD.

In man, lesions usually do not occur in the follicles of beard hair, possibly because beard hair facilitates drainage of sebum and keratinaceous debris (Greig, 1979). The only strain of rodent that develops chloracne after TCDD exposure is a hairless strain of mouse. Acne-like lesions also develop on the lips of monkeys, along with hyperkeratosis of glands of the eyelids after oral administration of TCDD. Facial alopecia, blepharitis, and loss of fingernails and eyelashes also occur in monkeys after acute exposure (McConnell et al., 1978a). After horses were exposed to salvage oil which contained TCDD as well as many other chemicals, hyperkeratosis developed. This condition involved hair loss and the development of a thick layer of keratin covering the epidermis. Ulcerative dermatitis and hair loss were also observed in dogs, cats, and mice that were exposed to the sprayed salvage oil (Case and Coffman, 1973).

In man, as in the monkey and rabbit, histological appearance of skin lesions progresses from dilated hair follicles filled with keratin to the disappearance of sebaceous glands and the replacement of follicles with keratin (VA, 1980). Blepharitis and conjunctivitis have been observed in cases of TCDD-induced chloracne in man (Moore, 1978) and in the monkey (McConnell et al., 1978a). Delayed conjunctival chemosis has resulted from the direct application of 2 mg of TCDD to the conjunctival sac of the rabbit eye. A latency period of 13-22 days preceded the appearance of symptoms (Van Miller et al., 1976).

The mechanism of development of chloracne after TCDD exposure is unknown. Observations that lipid metabolism is disturbed by TCDD, that oil accumulates on the skin prior to the development of chloracne, and that certain fatty acids can cause follicular keratosis has led to the suggestion that fatty acid metabolism may play a role in the etiology of chloracne caused by TCDD (Vos, 1978). Vitamin A deficiency also can cause hyperkeratosis, but use of this vitamin as a therapeutic agent has not been very effective against TCDD-caused chloracne.

6.3 PULMONARY LESIONS

Pulmonary lesions are rare in animals exposed to the herbicides in this study. The effects of diquat treatment on the lung have been studied in detail. Diquat does not produce significant pulmonary toxicity. Acute intratracheally instilled and subcutaneously injected doses of diquat caused decreased body weights in rats, but only temporary decreases in vital capacity and static lung compliance 24 hours after administration (Lam et al., 1979).

Pulmonary lesions, including type II alveolar cell damage and edema, have been induced in diquat-treated rats by exposure to 85 percent oxygen but were not present in rats exposed to ambient oxygen and treated with the same dose of diquat (Pratt et al., 1980).

Various studies have focused on the biochemical changes that diquat elicits in the lung in attempts to identify the mechanism of action of paraquat, a structural analogue of diquat that produces lethal pulmonary lesions including edema and fibrosis. Intermediary metabolites of high energy, tricarboxylic acid cycle, and glycolytic pathways in the lung were decreased, and pulmonary levels of adenosine triphosphate (ATP) and adenosine monophosphate were increased, following an intraperitoneal dose of 50 mg diquat/kg to mice. Microsomal adenosine triphosphatase activity was not altered after diquat treatment. The authors suggested that increased ATP synthesis provided energy to repair cellular damage, although they did not describe any damage in diquat-treated lungs (Hawkins, 1980; Hawkins et al., 1979). Diquat was also shown to inhibit acetylcholinesterase in the rat lung (Brown and Maling, 1975). Whether this inhibition occurs at the neuromuscular junction, where it would be expected to cause paralysis, was not evaluated. At 10^{-5} M, diquat produced a significant stimulation of CO_2 production from glucose in slices of rat lungs, with a maximum effect observed at 10^{-4} M. This effect was also observed in lung slices from rats that were treated intravenously with diquat. Intravenous doses of paraquat were less potent than diquat in producing this effect (Rose et al., 1976). Both compounds also produced rapid decreases in pulmonary NADPH/NADP ratios that were sustained for 24 hours following an intravenous dose of 140-156 uM/kg of herbicide. Diquat produced damage to type I alveolar cells, while paraquat damaged type II cells in this experiment (Witschi et al., 1977).

Lung lesions were evaluated in rats after a lethal intraperitoneal dose of diquat was administered. No increase in water content (indicative of edema) or in thymidine incorporation into lung DNA (indicative of lung fibrosis) was detected. The mortality rate was 70 percent over 14 days, with 20 percent of the deaths occurring within three days. Diuresis and dehydration were the only effects of diquat that were mentioned (Smith and Rose, 1977). The effects of diquat on alveolar macrophage viability were comparable to those of paraquat, producing irreversible damage after 30 minutes at concentrations of 10^{-6} M/liter. Diquat was 10 times more toxic than paraquat to fibroblasts in vitro, indicating that in vitro toxicities of these compounds do not reflect their in vivo toxicities (Styles, 1974).

The distribution of paraquat to the lung has been shown to be four times higher than that of diquat; this difference in distribution between the two compounds probably explains the difference in pulmonary toxicities. This difference in distribution may also explain the ability of paraquat, but not diquat, to produce large, dose-dependent increases in albumin content of the lung (a measure of edema), following subcutaneous administration to rats (Shu et al., 1979).

6.4 HEPATOTOXICITY

6.4.1 2,4-D

One report considers the effects of 2,4-D on serum and hepatic liver enzymes (Szocs et al., 1970). A single oral dose of 625 mg/kg to the rat produced decreases in serum and liver aldolase activities; a 30 percent decrease in serum cholinesterase; increased serum and liver glutamic oxaloacetic transaminase and acid phosphatase levels; and fluctuations in the serum and liver glutamic pyruvic transaminase, alkaline phosphatase, succinic dehydrogenase, and catalase activities. These changes were monitored for two days after treatment. Centrilobular fat deposits were observed in the liver at this time.

6.4.2 TCDD

Several types of hepatotoxic effects have been described for TCDD. Structural alterations, including changes observed by gross or histopathological examination of the liver, are described first. Necrosis, fatty degenerative changes, cellular infiltration, multinucleation of parenchymal cells, and increased size of the liver are the types of changes considered in this section as structural alterations. Changes in serum enzymes that are released from the liver and are used to evaluate liver function in some situations are described next. Finally, changes in the structure of bile ducts and the rate of biliary excretion of exogenously added compounds are described. Distribution of TCDD to the liver and biliary excretion of TCDD are described in chapter 4.

6.4.2.1 Structural Alterations

Changes in the histological appearance of the liver after TCDD treatment are common. The types of changes that occur and the severity of these changes show wide variability among species. In the rabbit, death occurred 8-20 days after a large dose (0.05-0.1 mg/kg) of TCDD was administered; death was attributed to hepatic lesions (Kimmig and Schultz, 1957a; 1957b). Lesions in the rat have been observed that were severe and potentially contributed to death (Huff et al., 1980). Reproducible hepatic changes were observed in the mouse, but were usually not severe enough to cause death; in the monkey and guinea pig, hepatic changes were minimal or absent in animals that died of TCDD poisoning (McConnell et al., 1978a; 1978b).

After acute doses of TCDD are given, the types of hepatic changes observed vary among different species. In the rabbit, necrosis and diffuse fatty degenerative changes have been reported (Kimmig and Schultz, 1957a; 1957b). Centrilobular necrosis occurs in the dog, rabbit, rat, and mouse (Schwetz et al., 1973). Both extensive (Jones and Greig, 1975) and single-cell necrosis (McConnell et al., 1978b) have been reported in the mouse, while the lesion in the rat is usually focal or scattered (Vos et al., 1974; van Logten et al., 1980; Greig et al., 1973; Jones and Greig, 1975).

Cellular infiltration has been observed in livers of both rats and mice after TCDD treatment (Greig et al., 1973; Vos et al., 1974; McConnell et al., 1978b; Jones and Butler, 1974; Jones and Greig, 1975). Accumulation of lipids occurs in parenchymal cells of the mouse after TCDD poisoning, but not in the rat or monkey. Lipid accumulation has also been observed in mice that were starved (Jones and Greig, 1975; Jones and Butler, 1974; McConnell et al., 1978a; 1978b; Vos et al., 1974). Multinucleated parenchymal cells have been observed in the monkey (McConnell et al., 1978a) and rat. These multinucleated cells result from the fusion of parenchymal cell membranes (Jones and Butler, 1974; Jones, 1975-1254). Proliferation of the rough and smooth endoplasmic reticulum in rat hepatocytes has been demonstrated and is probably related to increased synthesis of microsomal proteins, induced by TCDD (Fowler et al., 1973).

Increased liver weight relative to body weight has been reported for rats and mice after a single dose of at least 50 ug TCDD/kg (McConnell et al., 1978a; 1978b; Greig et al., 1973). Removal of the adrenal or pituitary gland does not block this increase in liver weight in the rat (van Logten et al., 1980). In the mouse, the increase in liver weight was shown to correlate with increased hepatic levels of lipids, esterified fatty acids, and cholesterol, at the same time that liver protein and water contents were decreased and DNA content was unchanged (Jones and Greig, 1975). TCDD also has been shown to have no effect on DNA synthesis of hepatocytes after partial hepatectomy (Greig et al., 1974) or on mitochondrial respiration of hepatocytes (Courtney et al., 1978).

6.4.2.2 Serum Enzyme Activity

The evaluation of liver function by measuring serum enzymes that are assumed to be released from the liver as a response to toxic agents is dubious in TCDD poisoning. TCDD has a direct inductive effect on hepatic enzymes, and increased serum levels of these enzymes may not necessarily reflect altered liver function. Altered serum enzyme activities were reported after a massive dose of TCDD was administered to the rat (Buu-Hoi et al., 1972b), and were correlated with histological changes in the liver (Buu-Hoi et al., 1972a). Histological and tissue weight changes in the liver have been reported in the absence of changes in serum enzyme levels (Greig et al., 1973). Changes in serum levels in humans have been transient in individual workers who were free of any other symptoms (see chapter 5). In other workers these changes have been associated with altered lipid metabolism and cardiovascular disorders (Walker and Martin, 1979).

6.4.2.3 Biliary Excretion

Bile duct proliferation has been observed in the mouse, but not in the rat (Jones and Greig, 1975; McConnell et al., 1978b; Vos et al., 1974). Hyperplasia and thickening of the bile duct has been observed in the rat from a single dose of 10-25 mg TCDD/kg. Other hepatic changes caused by this dose were less obvious than the effect on the bile duct (Croft et al., 1977).

A single dose of TCDD produced a long-lasting decrease in biliary excretion of ouabain (Young and Peterson, 1976). This effect is thought to be mediated by TCDD at the surface membrane of the hepatocyte (Peterson et al., 1979), and is reversed by treatment with repeated doses of pregnenolone 16 alpha-carbonitrile or spironolactone (Hamada and Peterson, 1978). Acute TCDD treatment also depressed biliary excretion of biphenyls in the rat (Guiney et al., 1978), of indocyanine green in the monkey prior to death (Seefeld et al., 1979), and in the rat (Hwang, 1973), and of thyroxine in the rat (Bastomsky, 1977).

6.4.3 Diquat

Minimal liver changes involving focal necrosis and centrilobular fat droplets were observed in monkeys that were administered lethal doses of diquat. Serum glutamic oxaloacetic and pyruvic transaminase levels were also elevated (Cobb and Grimshaw, 1979). Liver changes attributable to diquat were not observed in laboratory species (Thomas and Amos, 1968); Clark and Hurst, 1970). Liver necrosis was not observed in rats that were administered lethal amounts of diquat, unless the rats were also deficient in selenium. Selenium is an essential constituent of the enzyme glutathione peroxidase. This enzyme catalyzes reactions that break down hydrogen peroxide and polyunsaturated fatty acid hydroperoxides, compounds that promote lipid peroxidation. Rats maintained on a selenium-deficient diet have enhanced potential for lipid peroxidation. When diquat was administered to these rats, survival was very low compared to survival of diquat-treated rats with normal selenium levels. Both lipid peroxidation (measured by determining ethane production in expired air) and liver damage were severe in the selenium-deficient rats after diquat treatment. Liver damage involved histopathologic evidence of necrosis and elevated serum glutamic pyruvic transaminase levels. Pretreatment with selenium 6-10 hours prior to diquat administration protected the rats from both the lethal and hepatic effects of diquat. However, selenium did not act by increasing glutathione peroxidase activity, as in several tissues this activity was not increased in selenium-pretreated rats (Burk et al., 1980; Burk et al., 1979). These results indicate that diquat can cause liver damage, probably through its ability to generate superoxide anions by lipid peroxidation. However, these processes do not seem to occur in diquat poisoning unless a situation such as selenium deficiency also exists which predisposes the animals to enhanced lipid peroxidation.

Studies of the biochemical mechanism of diquat have focused on its action in subcellular hepatic preparations. Diquat does not penetrate liver mitochondrial membranes. The liberation of hydrogen peroxide, when diquat free radicals are oxidized by molecular oxygen, is considered to be important in plants but not in animals. The free radical, released by the microsome, has been suggested as the active component in animal tissues where cyclic reduction and reoxidation of diquat may lead to aromatic hydroxylation and an increase in thiobarbituric acid-reacting components of phospholipids (Gage, 1968). Another report also demonstrated diquat's ability to inhibit microsomal oxidation (Kreiger et al., 1973). Kopaczyk-Locke (1973) suggested that diquat acts at the level of liver mitochondria by stimulating citric acid cycle dehydrogenase and uncoupling oxidative phosphorylation.

6.5 NEUROTOXICITY

Human and animal exposure to 2,4-D, 2,4,5-T, and TCDD that resulted in the development of various neuropathologic symptoms are discussed in this section. The majority of the cases involve exposure to 2,4-D herbicides; human exposure resulted mainly from inadvertent or accidental contact while spraying gardens or farm fields. Exposure in these cases was via cutaneous absorption and respiration. Several experiments that exposed rats, cats, and dogs to 2,4-D are also discussed. One experiment described in this section examines the neurologic effect of 2,4,5-T in rats, and one report describes the neurological effects on humans following exposure to TCDD. In addition, brief references are made to numerous industrial accidents, involving primarily 2,4-D and TCDD, that caused neuropathologic disorders in exposed humans.

6.5.1 2,4-D

Several clinical studies describe the neurologic symptoms resulting from acute exposure to 2,4-D. These reports indicate that exposure occurred during mixing or spraying liquid 2,4-D herbicides. The liquid chemical was splashed onto the skin, or was deposited onto the skin or inhaled as a mist.

Three humans exhibited pronounced neurologic disorders following cutaneous exposure to 2,4-D (Goldstein et al., 1959). Initial symptoms developed within two days of the exposure and included numbness in the fingers and toes, muscle aches and fatigue, tetany of the limb muscles, and muscular ataxia causing difficulty in walking. Clinical examination revealed partial or total hyporeflexia, absence of joint sensation, and fasciculations of the arm and leg muscles. Similar symptoms and clinical results were found in a 39-year-old farmer who had been exposed to 2,4-D while spraying. In addition, this individual exhibited paresthesia of the extremities; myokymia of facial, trunk and leg muscles; and loss of manual dexterity (Berkley et al., 1963). All of these neuromuscular disorders were apparent in a 21-year-old male exposed to 2,4-D while mixing herbicides (Wallis et al., 1970). Ulnar nerve conduction velocities in exposed individuals were measured (Goldstein et al., 1959; Wallis et al., 1970). All patients exhibiting the neurologic symptoms of 2,4-D poisoning had decreased conduction velocities. Electromyographic examinations, nerve block tests, and nerve biopsies indicated that 2,4-D inhibits the normal functioning and may even damage peripheral nerves (Wallis et al., 1970). The authors of the above studies all concluded that although the etiology was not known, 2,4-D was responsible for the peripheral neuropathy observed in their patients.

In addition to peripheral neuropathy, 2,4-D exposure may also cause neurologic abnormalities in the central nervous system. Kontak et al. (1973) recorded the electroencephalographic (EEG) patterns of 17 farmers who had been spraying 2,4-D herbicide. More than half of these farmers displayed aberrations in the spontaneous electrical activity of the cerebral cortex and reticular formation. No irregularities were evident in the EEG of another patient exposed to 2,4-D (Berkley et al., 1963). Since only one individual was examined, however, this result should not be compared directly to the findings of Kontak et al. (1973). Although the study of Kontak et al. (1973) was not entirely conclusive, the findings indicate that 2,4-D may affect the normal functioning of the brain.

Table 6-7 provides a brief overview of accidental exposures to 2,4-D, showing route of exposure, number of people examined, dose if known, and the main types of neuromuscular disorders that were evident in the individuals. The details of each accident, along with other health effects caused by the exposure, are described in the annotated bibliography. As seen in table 6-7, only two fatalities occurred. No clinical data were obtained prior to one of the deaths (Nielson et al., 1965), but the other fatality was preceded by hyporeflexia and ataxia (Seabury, 1963). Histological examination of brain tissue from one of the fatalities revealed degenerative changes in the ganglionic cells of the pons (Nielsen et al., 1965).

It is apparent that cutaneous, respiratory, or oral exposure to 2,4-D in humans produces a characteristic syndrome of neuromuscular disorders. The syndrome consists of hypesthesia and myotonia in the muscles of the extremities, hyporeflexia, and general muscular weakness leading to ataxia. These symptoms can appear singly or together, but usually at least one becomes evident within a few days of exposure. According to the reports, exposure to 2,4-D should not be fatal unless large quantities are absorbed cutaneously or the chemical is ingested. The exposed individual should regain neuromuscular control within a period of several months or a year (Wallis et al., 1970; Monarca and Di Vito, 1961).

The myotonic symptoms of 2,4-D poisoning in humans have been demonstrated to occur in experimental animals. Administration of various doses of 2,4-D produced myotonia in skeletal muscles of the rat (Danon, 1979; Eberstein and Goodgold, 1979; Ranish et al., 1977), dog (Drill and Hiratzka, 1953), and rabbit (Hill and Carlisle, 1947). Although the mechanism is not completely known, 2,4-D apparently increases the resting membrane potential in association with reducing chloride conductance (De Reuck et al., 1979). 2,4-D has produced the same neuromuscular disorders in humans as in animals. Pigs, calves, rats, and mice displayed symptoms of asthenia, lethargy, and ataxia when various 2,4-D compounds were administered intraperitoneally and orally (Hill and Carlisle, 1947; Bjorklund and Erne, 1966).

Experiments have been conducted investigating the effect of 2,4-D on EEG patterns of the rat, cat, and dog. Several studies demonstrated that 2,4-D causes irregularities in the EEG of anesthetized and non-anesthetized animals (Desi et al. 1962 and 1962b; Desi and Sos, 1962a). The irregularities found were primarily a decrease in spontaneous cerebral electrical activity and a reduction or complete loss of desynchronization in the cerebral cortex and reticular formation. In addition, the animals experienced loss of a previously learned conditioned reflex. Histologic examination of the spinal cord of rats that had received doses of 2,4-D for 5 consecutive days revealed regions of demyelinization in the pyramidal tract (Desi et al., 1962a). The authors concluded that 2,4-D acts directly on the cerebral cells via the blood stream, to alter the electrical activity of the brain. They also stated that 2,4-D may cause demyelinization in the spinal cord, which could lead to serious neurologic complications.

These studies are of limited value, as a complete description of the experimental procedures was not included in the reports. The use of control animals is not clearly stated and the purity and preparation of the 2,4-D

Table 6-7: ACUTE HUMAN EXPOSURES TO 2,4-D

Route	Dose	# of people	Asthenia	Hyporeflexia	Ataxia	Hypesthsia	Rigidity, Myotonia	Fatal	Reference
Cutaneous	--	1		X	X				Todd (1962)
Intravenous	3,600 mg	1		X	X		X	X	Seabury (1963)
Cutaneous	--	1	X	X		X			Foissac-Gegoux (1962)
Oral	30 ml	1	X			X			Berwick (1970)
Oral	--	1						X	Dudley et al. (1972)
Oral	30 ml	1	X	X	X	X		X	Brandt (1971)
Inhalation	--	1	X				X		Paggiaro et al. (1974)
Oral				X			X		Prescott et al. (1974)
Cutaneous	--	several			X		X		Tsapko (1966)
Cutaneous		11	X		X	X	X		Bezuglyi et al. (1979)
Cutaneous	--	1	X		X				Monarca and DeVito (1961)
Cutaneous	--	1	X	X	X		X		Wallis et al. (1970)
Cutaneous	--	3	X	X		X	X		Goldstein et al. (1959)
Cutaneous	--	1		X	X	X			Berkley et al. (1963)

compounds are not detailed. In addition, the authors did not consider other physical, biological, and environmental factors that may have influenced the EEG readings from the animals. These factors include the age, sex, and health of the animals, species differences, diet, ambient temperature, and possible traumatic effects caused by surgical emplacement of the EEG electrodes. In spite of these shortcomings, these experiments indicate that the EEG abnormalities observed in some humans exposed to 2,4-D (Kontak et al., 1973) may be similar to EEG patterns evoked in animals by administration of 2,4-D. It should be noted that 2,4-D may cause morphological damage in the central nervous system of humans and animals. Nielsen et al. (1965) found degenerative changes in ganglion cells of the cerebral cortex in a man who died from 2,4-D intoxication, and Desi et al. (1962a) found demyelinization in the spinal cord of animals. Both tentatively concluded that these morphological alterations may have been caused by 2,4-D.

6.5.2 2,4,5-T

One study is available that discusses the neurologic and behavioral effects of 2,4,5-T in rats (Sjoden and Soderburg, 1978). Adult rats that received oral doses of 2,4,5-T displayed reduced learning abilities and decreased food and water intake. Neurochemical analysis of brain tissue showed a decrease in the concentration of several enzymes. Neonatal rats exposed prenatally to 2,4,5-T exhibited hyperactivity and impaired ability to learn a maze and a conditioned response. The authors concluded that exposure to 2,4,5-T produced significant behavioral and neurochemical effects. These conclusions, however, are not definitive, since the purity of the 2,4,5-T was not specified and control animals apparently were not used. The authors did not discuss other factors that may have influenced the results, particularly for the behavioral effects. They did not relate their findings to human health effects or to effects produced in animals by similar compounds such as 2,4-D.

6.5.3 TCDD

One clinical study is available that discussed the neurological effects resulting from TCDD exposure in humans. Boeri et al. (1978) conducted neurologic examinations of people exposed to TCDD as a result of the Seveso accident. Individuals from zone A (high risk of acute exposure) were examined and results were compared to individuals from zone R (low risk). The study populations included both adults and children.

Individuals from both zones complained of numbness and weakness in their arms and legs, hyporeflexia, ataxia, and loss of coordination in the hands and fingers; nerve conduction velocities were also found to be reduced. Although people from both high- and low-risk zones exhibited these symptoms, a higher prevalence was observed in the high-risk population. The authors concluded that a higher prevalence of neuromuscular disorders was evident in the high-risk population because of a greater probable TCDD exposure. As described in chapter 5, other chemicals are likely to have been released in the Seveso accident, including chlorinated phenols and other components of the reaction mixture.

Neuromuscular disorders described in the above report are similar to those described in reports of acute human exposure to 2,4-D. Hyporeflexia, general muscular weakness, ataxia, and loss of manual dexterity were the main symptoms of 2,4-D poisoning in humans (Goldstein et al., 1959; Berkley et al., 1963; Wallis et al., 1970). TCDD and 2,4-D apparently produce similar neuromuscular effects in humans, indicating that the mechanism of action may be similar.

6.5.4 Diquat

Common symptoms of diquat poisoning include lethargy and coma (Thomas and Amor, 1968; Cobb and Grimshaw, 1979). Pupillary dilatation was also observed, in rats, along with a decrease in body temperature after subcutaneous administration of diquat (Clark and Hurst, 1970).

6.6 NUTRIENT ABSORPTION AND UTILIZATION

This section considers herbicides that cause deficiencies in nutrient utilization manifested as decreased body weight. Effects on several other organ systems, whose functions are related to maintenance of body weight, are considered in this section. These systems include the gastrointestinal tract and the endocrine system. Molecular manifestations of altered nutrient utilization, including metabolism of lipids and proteins, are also discussed in this section.

6.6.1 2,4-D and 2,4,5-T

Both of these compounds have been studied, sometimes in the same study, in terms of their effects on nutrient absorption and utilization. Since the amount of information on each compound is limited, the effects of both compounds will be described together in this section.

6.6.1.1 Body Weight and Food Consumption

Anorexia has been described as a symptom of toxicity of 2,4-D and 2,4,5-T. Weight loss has been measured in only one study. After a single oral dose of 100 mg 2,4-D/kg was administered to dogs, weight losses of 1.7-2.5 kg were observed in four dogs; two of four dogs survived for 14 days, while two died at 9 days (the time after the dose that the weight losses were noted was not stated). Weight losses of 0.1 to 2.0 kg occurred in dogs fed 250-400 mg 2,4-D/kg, which produced 100 percent mortality in 8 days. A single dose of 100 mg 2,4,5-T/kg produced weight losses of 0.4-1.0 kg and 75 percent survival, while higher doses of 250-400 mg/kg produced 1.1-1.4 kg weight losses and 100 percent mortality.

6.6.1.2 Effects on the Gastrointestinal Tract

In a brief report, a single oral dose of 100 mg 2,4,5-T/kg was described as having a stimulatory effect 24 hours later on mucosal uptake of iron, increasing this uptake by 8 percent in duodenal gut sacs in vitro. The authors pointed out the potential for this effect to produce adverse effects on iron absorption and metabolism. The implications of this effect on nutrient transport by the gut was not discussed and the dioxin content of the 2,4,5-T preparation was not reported (Manis and Kim, 1979). 2,4,5-T had no effect on glucose transport by the mouse intestine in vitro. However, the amount of glucose associated with the gut tissue was increased in the presence of 2,4,5-T in the culture system (Guthrie et al., 1974). These results do not support the concept that weight loss in 2,4,5-T-treated animals results from an effect on nutrient uptake by the gastrointestinal tract.

6.6.1.3 Effects on the Endocrine System

The uptake of ^{131}I by various tissues was evaluated in rats after a single oral dose of 100 mg 2,4,5-T/kg (with less than 1 ppm TCDD) was administered. A marked decrease in serum radioactivity and increases in thyroid and liver levels were observed within 3 days of 2,4,5-T treatment, although the amount of tissue uptake was inadequate to explain the loss in serum radioactivity. The authors presented these data as evidence that 2,4,5-T alters the permeability of cellular membranes, and suggested the thyroid, brain, and kidney as potential sites where deleterious effects from these changes could occur (Sjoden et al., 1977).

6.6.1.4 Alterations in Lipid Biosynthesis and Mitochondrial Respiration

Both 2,4-D and 2,4,5-T inhibited incorporation of radioactive precursors into cholesterol and fatty acids, in vitro. At levels of 4-9 mM, 2,4-D and 2,4,5-T (with less than 1 ppm dioxin) inhibited incorporation of ^{14}C-mevalonate into cholesterol and nonsaponifiable lipids, ^{14}C-acetate into fatty acids and cholesterol, and ^{14}C-isopentenyl pyrophosphate into cholesterol by rat liver homogenates. The authors indicated that these effects may not have any toxicological significance, however (Olson et al., 1974).

At $10^{-4}M$, both 2,4-D and 2,4,5-T produced uncoupling effects on rat hepatic mitochondrial respiration and reduced the respiratory control index (the ratio of succinate oxidation in the presence of ADP to the rate obtained after the acceptor is exhausted). The authors suggested that these effects are related to potential mechanism of actions of these compounds in plants and animals, but did not indicate what types of lesions would be produced from these biochemical changes (Abo-Khatwa and Hollingsworth, 1974).

6.6.2 TCDD

The effects of TCDD on nutrient utilization have been the focus of many studies. TCDD has been shown to produce severe weight loss in the animal

studies described in this section. The mechanisms by which TCDD produces this effect are unknown. Changes that have been investigated in other organ systems which could ultimately produce this effect are considered below. These changes involve nutrient absorption by the gastrointestinal tracts and changes in endocrine organs that participate in regulating nutrient utilization. Finally, biochemical events related to nutrient utilization are considered here which relate to changes in circulating metabolites of protein or lipid catabolism.

6.6.2.1 Effects on Body Weight and Food Consumption

An acute dose of TCDD produces weight loss in a variety of species, including the monkey, rat, mouse, guinea pig, rabbit, hamster, and chicken (Greig et al., 1973; Harris et al., 1973; McConnell et al., 1978a, 1978b; Luster et al., 1978; Vos et al., 1974, Olson et al, 1980). Weight loss is not a prominent feature in man and was seldom mentioned in workers after industrial exposure; in humans weight loss has sometimes been implied to be a consequence of anorexia, a neurologic disorder. In animals, weight loss, accompanied by depletion of fat deposits and the inability to utilize ingested nutrients, has been identified as the lesions responsible for death. In man, however, exposure to a lethal dose of TCDD has not been reported.

Several investigators have observed a biphasic pattern to the weight loss that results from a single exposure to TCDD. In the monkey, guinea pig, and mouse, an initial period of weight loss occurred for 7-10 days after TCDD was given, followed by a recovery in non-lethal poisonings or a second period of weight loss in lethal poisoning (McConnell, 381). Initial weight loss for 7-10 days after 100 ug TCDD/kg was administered to rats resulted in a decrease in body weight of 15-30 percent (Courtney et al., 1978). In the next 4-6 days this trend was reversed and 10-15 percent of the initial body weight was gained back. In lethal poisoning a second period of weight loss ensued and terminated in death. Food and water consumption were decreased in these rats, but forced administration of a balanced liquid diet, water, or an electrolyte solution failed to reverse the weight loss or death. In another experiment, TCDD-treated rats lost more of their body weight than was lost by pair-fed control rats (van Logten et al., unpublished, mentioned by Moore, 1978).

Lethal doses of TCDD have caused terminal weight losses of 15-50 percent in guinea pigs (McConnell et al., 1978b; Greig et al., 1973). At death, the gastrointestinal tract was empty and food consumption fell only in the terminal stages. Loss in body weight has been reported at doses that do not cause any other signs of toxicity in the guinea pig, indicating that it may be the most sensitive feature of toxicity in this species. Weight losses in the guinea pig, rat, and mouse are transient after nonlethal acute doses of TCDD (Harris et al., 1973; Vos et al., 1974).

Weight losses of 13-38 percent in monkeys and in mice have been reported following a lethal dose of TCDD (McConnell et al., 1978a; Luster et al., 1978). Food consumption has been variable and occasionally has shown a trend toward decreased consumption. These trends have not been significantly different between TCDD-treated and control groups, however, and the decreases

have not been large enough to account for the substantial weight losses observed (Greig et al., 1973; Harris et al., 1973; McConnell et al., 1978a; 1978b). Weight losses from TCDD treatment occur in rats after the adrenal or the pituitary gland is removed, indicating that these organs are not essential in mediating the toxic effects of TCDD (van Logten et al., 1980).

6.6.2.2 Effects on the Gastrointestinal Tract

Studies of gastrointestinal function have been carried out in rodents after an acute dose of TCDD was administered. Transport of iron was shown to increase in vivo and in vitro (Manis and Apap, 1979; Manis and Kim, 1979a; 1979b). In another study glucose transport was depressed, while transport of other nutrients was unaltered (Madge, 1977). An increased sensitivity of crypt cells compared to tip cells of the intestine to TCDD induction of enzyme activity has led to the suggestion that undifferentiated cells have high sensitivity to TCDD-mediated effects (Schiller, 1979). However, decreased ability of these cells to transport nutrients was not evaluated.

The importance of decreased glucose transport across the gut or nutrient utilization after TCDD treatment depends on whether this transport system is the limiting factor in supplying nutrients to organs. No evidence of this limiting role has been reported, and no strong evidence exists at present to suggest the contention that TCDD prevents nutrient utilization by blocking intestinal absorption of nutrients.

6.6.2.3 Lipid and Protein Metabolism

Several studies have considered the effect of TCDD treatment on protein and lipid biosynthesis. Radioactive precursors of lipids and protein were administered to TCDD-treated rats and the incorporation of these precursors into macromolecules in the liver was examined. Protein synthesis increased, while lipid biosynthesis appeared to decrease. However, concomitant changes in liver weights and specific activity of the radioactive precursors, as well as the use of inadequate techniques in quantitatively isolating pools of specific biochemicals, complicates the interpretation of these results. The authors concluded that TCDD caused an inhibition in the secretion of lipids from the liver (Cunningham and Williams, 1972).

In an abstract, Lovati et al. (presented at Workshop, Impact of Chlorinated Dioxins and Related Compounds on the Environment, Rome, October 22-24, 1980) reported that an acute dose of TCDD in rats resulted in elevated total plasma and high-density lipoprotein cholesterol levels. Hypercholesterolemia has been reported in humans after industrial and laboratory exposures, as well (see chapter 5 of this report). Lovati et al. also reported that TCDD treatment resulted in increased triglyceride levels in rabbits. However, a group of rabbits that became hypercholesterolemic by chronic cholesterol administration did not have altered triglyceride levels after TCDD was administered, but had severe atheromatous lesions.

Other investigators (Walker and Martin, 1979) have suggested that TCDD induces gamma-glutamyl transpeptidase activity which leads to abnormal lipid levels and predisposes exposed workers to ischemic vascular disease. They examined eight workers who were exposed to dioxins and found clinical signs of ischemic vascular disease in several workers, and raised triglyceride and transpeptidase levels in five workers. All eight workers had decreased levels of high-density lipoprotein cholesterol levels and elevated total cholesterol levels.

Acute TCDD treatment has produced changes in serum protein and lipid levels, although these changes have not been reported frequently and occur with high doses of TCDD. Changes in serum protein levels have been observed in rats after an acute dose of 200 ug TCDD/kg was administered (Greig et al., 1973). In monkeys a decrease in serum protein 30 days after an acute lethal dose of TCDD was shown to be caused specifically by a decrease in albumin. Serum cholesterol levels progressively fell to half their normal value at death, while serum triglycerides increased (McConnell et al., 1978a). Increased serum protein and cholesterol levels, and decreased serum triglyceride levels have been observed in hamsters administered doses of 1,000 ug/kg orally or intraperitoneally (Olson, et al., 1980). In the guinea pig, increased plasma levels of albumin, total protein, cholesterol, and triglycerides were observed after a 1 ug/kg intraperitoneal dose. These levels were increased above pair-fed controls, suggesting that the effect is not simply a manifestation of the effects of TCDD on fat depot utilization (Gasiewicz and Neal, 1979).

Both the presence of fatty acids and a deficiency in vitamin A have been suggested as mechanisms by which TCDD produces chloracne (see Section 6.2 of this report). The relationship between alterations in lipid metabolism by TCDD and the severe weight loss in experimental animals is speculative at this point. There is no compelling evidence to suggest that animals eat less or fail to absorb nutrients. TCDD is known to produce substantial chronic induction of enzymes and to alter parameters related to lipid biosynthesis. Investigations into the nature of this relationship may provide a basis for our understanding of weight loss, which has been implicated as the fatal lesion in some of the species most sensitive to TCDD.

6.6.3 Diquat

Loss in body weight has been reported following acute doses of diquat (Clark and Hurst, 1970). Food consumption has not been measured, although death does not occur in some animals for 14 days. Severe histopathologic changes of the gastrointestinal tracts, diarrhea, and alterations in corticosteroid levels all probably contribute to this weight loss. Neurological abnormalities, especially lethargy, that occur in these animals, could also contribute to the weight loss.

6.6.3.1 Effects on the Gastrointestinal Tract

The major histopathological changes seen after fatal diquat poisoning involve the gastrointestinal tract. Distension of the gastrointestinal tract,

with necrosis, exfoliation of the epithelium, and cellular infiltration were observed in monkeys (Cobb and Grimshaw, 1979). Distended abdomens were also observed in rats and other laboratory species after diquat poisoning (Clark and Hurst, 1970). In cattle, congestion, edema, and inflammation were observed in the gastrointestinal tract (Thomas and Amor, 1968).

Alterations in gastrointestinal function have been observed after diquat administration to rats. A single oral dose of diquat of 900 uM/kg (the LD_{50}) caused a rapid accumulation of water in the stomach and, associated with it, hemoconcentration. Rats that failed to gain weight in the 24-hour period after diquat administration usually died within 3 days of dosing, while survivors showed a 3 percent increase in body weight. Fluid accumulation in the stomach was delayed and less extensive after subcutaneous dosing. Prolonged inhibition of gastric emptying was observed after diquat administration by either route. Fluid accumulation was not considered to be a consequence of inhibition of gastric emptying because accumulation did not occur when diquat was administered subcutaneously to rats, following pyloric ligation (Crabtree, et al., 1977; Crabtree and Rose, 1978).

6.6.3.2 Effects on the Endocrine System

Diquat administration causes alterations in circulating corticosteroid levels in the rat. A large increase in plasma corticosteroid levels and increased AMP levels in the adrenals were observed 24 hours after diquat was administered. The increased plasma corticosteroid levels were assumed to result from increased synthesis, because metabolism and excretion were unaltered by diquat treatment. These increases were not observed after diquat treatment to hypophysectomized rats. Increases in liver glycogen synthesis and blood glucose levels after diquat treatment were blocked by adrenalectomy. The authors concluded that diquat stimulated ACTH release from the pituitary, which led to increased synthesis of corticosteroids by the adrenal. Free radical generation was not considered the likely mechanism by which diquat elicited this effect. The high circulating corticosteroid levels that diquat elicits could be responsible for the changes in the thymus, spleen, and adrenals observed by other investigators (Rose et al., 1974; Crabtree and Rose, 1976).

6.7 HEMATOLOGICAL EFFECTS

In this section, effects on blood components are described. Effects of herbicides and TCDD on the levels of circulating blood cells and bone marrow components as well as effects on thrombosis, incidents of hemorrhages, and changes in hemoglobin are considered here.

6.7.1 2,4-D

Kuz'minskaya and Bersan (1975) reported that a single dose of 2,4-D (1/2 the LD_{50}) produced enzyme changes in erythrocytes. The rate of glycolysis doubled and ATPase activity increased by 50 percent 5 days after dosing; the

levels were normal 15 days after dosing. Corresponding compromise in the physiologic function of erythrocytes was not studied, raising the question as to the toxicologic significance of these findings. Other hematologic effects of either 2,4-D or 2,4,5-T have not been described.

6.7.2 TCDD

Acute doses of TCDD have produced adverse effects on the hematopoietic system in the monkey, rat, guinea pig, and mouse. Hypocellularity of the bone marrow has been observed in the monkey (McConnell et al., 1978a) and guinea pig, but not in the mouse (McConnell et al., 1978b).

In the monkey, a relative increase in myeloid elements and a decrease in erythroid elements were observed in sternal bone marrow, while the lymphoid component was unchanged. Pancytopenic depletion was reported in the sternal bone marrow of guinea pigs, although cells were not counted.

Increases in red blood cell count and in hematocrit and hemoglobin content of the blood were reported in female rats 2-3 weeks after a single dose of 200 ug/kg was administered (Greig et al., 1973). Leukocytosis was also reported in these animals and in monkeys, due to an increase in neutrophils (McConnell et al., 1978a).

Hemorrhages have been observed in animals given an acute dose of TCDD. Death of a monkey 14 days after 70 ug TCDD/kg had been administered was attributed to bleeding (McConnell et al., 1978). Hemorrhages in the gastrointestinal tract have been observed in the mouse, rat, and guinea pig (McConnell et al., 1978b, Vos et al., 1974, Greig et al., 1973). Pulmonary bleeding also occurred in the rat and intraorbital bleeding and severe splenic atrophy were observed in the mouse.

6.7.3. Diquat

Acute administration of diquat to rats produced hemoconcentration. This effect was attributed to an accumulation of fluid in the stomach after oral treatment (Crabtree et al., 1977) and an alteration in renal hemodynamics (Lock, 1979). In monkeys an increase in the polymorphonuclear leukocyte count was observed. This effect was transient in monkeys that survived, but persisted in monkeys that died (Cobb and Grimshaw, 1979). Cardiac hemorrhages were observed in cattle that consumed a fatal dose of diquat (Thomas and Amor, 1968).

6.8 STRUCTURE AND FUNCTION OF LYMPHATIC TISSUES

Effects on the structure of the thymus and other lymphatic tissues and on the immunologic function of these tissues are described in this section. Most of the studies in this section investigated effects of TCDD.

6.8.1 TCDD

Thymic atrophy has been reported in many species from administration of a single dose of TCDD. No changes in thymic function or structure have been reported in man. After a lethal dose of TCDD was administered to monkeys, almost complete loss of the cortex of the thymus was observed at necropsy. Other lymphoid tissue also showed losses of lymphocytes, and lymphopenia was observed in the circulating blood one month after TCDD treatment (McConnell et al., 1978a). In guinea pigs, early changes in the thymus included scattered necrosis of lymphocytes of the cortex during the first two weeks after treatment, but was no longer observed by the third week. The cortex and medulla were not differentiable by the second week. The thymus of guinea pigs that survived for one month was normal histologically, but reduced in size. Lymphocytes were reduced in other lymphoid tissue. Changes in mice resembled those observed in guinea pigs (McConnell et al., 1978b). A dose of 3 ug TCDD/kg caused severe thymic atrophy and death in the guinea pig, while a single dose of 10 or 50 ug/kg to mice produced a transient decrease in thymic weight (evident only at 3 weeks after dosing) and is not lethal (Harris et al., 1973). Thymic atrophy, loss of differentiation between the thymic cortex and medulla, and lymphocyte depletion were also reported in mice by other investigators after an acute dose of TCDD was administered (Vos et al., 1974).

In the rat, a significant decrease in the weight of the thymus relative to body weight was observed 2 weeks after a single dose of 5 ug TCDD/kg was administered. This effect was transient and was not accompanied by a change in liver weight. A single dose of 25 ug/kg produced a more sustained decrease in thymic weight that was significant 3 days after dosing and was at its maximum 16 days after dosing (Harris et al., 1973). Thymic involution occurred in adrenalectomized rats after a single dose of 10 or 20 ug TCDD/kg was administered. Thymic involution from these doses of TCDD was more severe in hypophysectomized rats than in rats with intact pituitary glands. These results suggest that the adrenal and pituitary do not have essential roles in modulating the effect of TCDD on the thymus (van Logten et al., 1980).

Several studies have investigated the effects of brief exposure to TCDD on lymphocyte function. In one study, splenic lymphocytes from mice that received a single dose of 10 ug TCDD/kg were evaluated in vitro for their ability to incorporate ^{3}H-thymidine. Two weeks after treatment, ^{3}H-thymidine uptake was increased in the absence of mitogens. With phytohemaglutanin or poke weed mitogen present, ^{3}H-thymidine uptake was increased above non-mitogen-treated cultures. This response to mitogen was less for TCDD-treated lymphocytes than for control lymphocytes, partly because uptake with mitogens present was expressed relative to uptake without mitogens, and TCDD increased this last parameter. Other changes observed two weeks after treatment were a reduction in the size of the thymic cortex and an increase in liver weight. None of the in vivo or in vitro changes were observed four or eight weeks after treatment. Splenic lymphocytes from control mice incubated in the presence of TCDD failed to duplicate the effects of lymphocytes exposed in vivo; cells treated in vitro showed a cytotoxic response to TCDD and failed to respond to mitogens (Sharma and Gehring, 1979).

A second study of lymphocyte function examined the effects of brief submersion of mouse spleens in TCDD on lymphocyte responsiveness to mitogens. Submersion for 10 seconds in 2 x 10^{-7} M TCDD in DMSO resulted in uptake of 0.2 ng of TCDD into the spleen. Lymphocytes from the submerged spleen showed decreased incorporation of radioactive precursors into RNA, DNA, and protein. TCDD treatment also diminished DNA, RNA, and protein synthesis of mitogen-stimulated lymphocytes compared to DMSO-treated controls. The authors noted that these changes were more marked in the presence of T-lymphocyte specific mitogens (phytohemaglutanin and concanavalin A) than B-lymphocyte-specific mitogens (*E. coli* lipopolysaccharide), indicating that T-lymphocytes were more susceptible to TCDD than B-lymphocytes (Luster et al., 1979). The differences between responses, after different mitogens were added, actually were not large or consistent, and further experimentation would be needed to validate this difference in responsiveness between B- and T-lymphocytes. The authors found no effect of TCDD on ^{3}H-concanavalin A binding to lymphocytes or precursor incorporation in the absence of mitogens.

Mantovani et al. (1979) examined the effect of TCDD treatment on the cytotoxic activity of macrophages and of natural killer cells in vitro. The natural host-defense mechanisms of these cells were not altered by TCDD pretreatment in vivo. The pretreatment did lead to a reduction in the total numbers of spleen and peritoneal cells recovered, and to marked hypocellularity of the bone marrow. The cell-mediated immunity was thus compromised in that fewer cells were on reserve to fight infectious agents. The circumstances which would require these reserves are unknown.

In conclusion, TCDD produces severe thymic atrophy, and this effect can be produced from a single dose of TCDD in every laboratory species examined. In the rat decreased thymic weight was seen at a dose that produced no other effects. The effect is not mediated by hormones released from the adrenal or pituitary, and the mechanism of action remains unknown.

The implications of these structural changes on functional compromise of the thymus are not obvious. Immunocompetence has not been evaluated after acute doses of TCDD, although TCDD had the same lethal effects on aseptic rats as it did on rats housed in a normal environment (Greig et al., 1973), indicating that decreased resistance to infection is not the only cause of death from TCDD. Death has been attributed to pulmonary infections in rats on occasion, but many other causes of death have been noted in TCDD-treated animals. Functional compromise of the thymus or lymphocytes derived from the thymus does not appear to be as severe as the structural changes seen in the thymus, but the in vitro tests used to evaluate lymphocyte function may not have evaluated the parameters of immune function that are most sensitive to TCDD.

Other studies of immune function have been performed after subacute doses of TCDD were administered (see chapter 7). These studies have identified an increased susceptibility of immature mice to the effects of TCDD on the immune system and to bacterial endotoxin after TCDD treatment. These studies have not identified effects on the immune system that are comparable in severity to the effects on thymic structure, nor effects that would account for the lethal effects of TCDD.

No observations of thymic involution after human exposure to TCDD have been reported. Human sensitivity to this effect may be less than in other species and may be age-dependent. At the present time, however, one cannot rule out the possibilities that thymic effects were too subtle or too difficult to diagnose in patients or that the doses of TCDD required to produce this effect were not reached in cases where thymic atrophy could be evaluated.

6.8.2 Diquat

Acute subcutaneous doses of diquat to rats produced large reductions in organ weights and lymphocyte depletion in the cortex of the thymus and in the spleen (Clark and Hurst, 1970). Rose et al. (1974) suggested that these changes were a result of elevated plasma corticosteroid levels from diquat administration (see Section 6.6.3.2).

6.9 RENAL EFFECTS

The effects of herbicides and TCDD on the structure and function of the kidney are described in this section. Renal excretion of the herbicides are described in chapter 4.

6.9.1 2,4,5-T

The effects of 2,4,5-T on renal function have been reported. A single acute dose of 100 mg 2,4,5-T/kg has been shown in rat cortical slices to lead to a decrease in para-aminohippurate (PAH) accumulation (by 58 percent) and in tetraethylammonium (TEA) accumulation (by 34 percent) 24 hours later. A dose of 20 mg/kg had no effect after 24 hours, although after 4 hours it produced a 51 percent decrease in PAH accumulation into slices and a 24 percent decrease in PAH clearance in vivo (Stroo et al., 1979). 2,4,5-T also produced a decrease in renal transport of 2,4-D (Koschier and Berndt, 1976). 2,4,5-T did not alter N-methylnicotinamide efflux, however, and 2,4,5-T was not itself transported by the renal organic base secretory mechanism. The authors suggested that 2,4,5-T interference with organic transport is related to its high degree of binding to renal cortex tissue, and eliminated an effect of 2,4,5-T on tissue oxygen consumption (Koschier and Berndt, 1976; Koschier and Berndt, 1977).

6.9.2 TCDD

Effects of TCDD on the kidney have been reported for the rat and have been attributed to a general toxic effect of TCDD, rather than a specific lesion on the kidney. Renal cortical slices from rats treated intraperitoneally with 25 ug TCDD/kg 1 week prior to the in vitro experiment showed decreased uptake of para-aminohippurate and N-methylnicotinamide (Hook et al., 1978), but not of deoxyglucose (Pegg et al., 1976). Decreased ammoniogenesis and gluconeogenesis were also observed in renal slices from chronically acidic

rats after TCDD treatment (Hewitt et al., 1976). In vivo renal clearance of para-aminohippurate and inulin were also decreased one week after an intraperitoneal dose of 25 ug/kg of TCDD was given. No changes in the fractional reabsorption of sodium or of the renal response to volume expanders was observed and the authors concluded that the observed changes were not specific for renal function (McCormack et al., 1976).

Decreased renal DNA synthesis was observed after TCDD treatment of rats that were given folate or lead to simulate DNA synthesis by the kidney. TCDD treatment reduced the effects of lead and folate on renal DNA synthesis. The authors considered this effect of TCDD to be indirect, resulting from altered levels of inducible renal and hepatic enzymes (Greig et al., 1974).

6.9.3 Diquat

Severe renal changes have been observed in animals after diquat administration. In the monkey, exfoliation, vacuolation, and pycnotic nuclei were observed in the epithelial cells of the proximal and distal convoluted tubules, along with congestion of the glomeruli and hyperemic papillae. These changes were most severe in a monkey that died one day after diquat treatment. In animals that died after three to four days, these changes appeared to be regressing (Cobb and Grimshaw, 1979). No changes were observed in the kidneys of cows that died from diquat poisoning (Thomas and Amor, 1968) or in laboratory animals (Clark and Hurst, 1970; Burk et al., 1980), except in selenium deficient rats who also displayed hepatic lesions and high rates of peroxidation in response to diquat administration (see section 6.4.4).

Renal function was diminished after diquat treatment to rats. Glomerular filtration rates and clearances of acidic and basic compounds were reduced 24 hours after 540 uM/kg was administered orally (Lock, 1979). A 680 uM/kg dose produced proteinuria and glucosuria, and minor biochemical changes (Lock and Ishmael, 1979).

6.10 CARDIOVASCULAR EFFECTS

A low incidence of cardiovascular effects was observed after occupational exposure to TCDD in the early 1950s in Germany (Bauer et al., 1961). Acute exposure of the rat to a massive dose of TCDD also caused cardiovascular effects (Buu-Hoi et al., 1972). A clear cause-and-effect relationship between TCDD and cardiovascular disorders has not been convincingly demonstrated and these disorders may be elicited only after a substantial acute dose or after chronic doses are administered.

6.11 SUMMARY AND CONCLUSIONS

Single oral doses of about 350-800 mg of either 2,4-D or 2,4,5-T per kg are lethal to most species. Few accidental lethal ingestions of 2,4,5-T by animals or humans have been reported. In experiments in which fatal single doses were administered, a variety of nonspecific symptoms were produced. The cause of death is not always apparent. LD_{50} values given for 2,4,5-T in the

literature were published almost 30 years ago, when the possibility of TCDD contamination was not taken into account. The actual LD_{50} values may be higher than the published values.

About 15 accounts of human ingestion of 2,4-D have been reported. These ingestions involved accidental or suicidal use; cases of injection for therapeutic use have also been reported. The principal effect of these acute doses has been neurotoxicity. Acute toxicity in animals also involves neurotoxicity. Death is delayed, usually occurring within a week of administration. The cause of death is not usually apparent, as symptoms of neurotoxicity are usually accompanied by many non-specific effects.

The acute toxicity of TCDD is characterized by:

- Extremely low LD_{50} values (between 1-300 ug/kg)
- Large variation in LD_{50}s among species
- Long latency period to death, usually about 3 weeks
- Cellular toxicity, with no clear cause of death and different target organs for different species.

The LD_{50} for diquat is between 30 and 200 mg/kg for all mammalian species studied. Doses in this range produce severe gastrointestinal lesions and death occurs within 2 weeks. Doses four- to fivefold higher produce neurotoxicity, and death occurs in several hours.

Toxicity data for the remaining compounds are sparse and for tandex have not been published. Reported oral LD_{50} values for cacodylic acid range from 600-3,200 mg/kg in rats and 200 mg/kg in mice; reasons for the wide variation in reported values for the same compound and species have not been identified. Various organs are affected by cacodylic acid and death occurs within 2 weeks. The oral LD_{50}s for both monuron and diuron are about 1,000 mg/kg. Both compounds produce neurotoxicity, including central nervous system depression followed by stimulation. Death occurs 1 day after exposure from respiratory or cardiac failure.

Picloram and dalapon have low toxicities, with oral LD_{50}s ranging from 2,000-8,000 mg/kg. Fatal doses of dalapon cause death within several hours. The latency for picloram, and the cause of death and target organs for both compounds have not been described.

TCDD is the only compound of those covered in this report that produces dermal toxicity. Chloracne has been demonstrated in man and in several animal species including the rabbit, which provides a suitable animal model. Man appears to be less susceptible to the acnegenic effects of TCDD than the rabbit. Chloracne is not necessarily a local response to TCDD, as chloracne is produced following systemic administration. Pulmonary lesions are not produced by any of the herbicides under study. Differences in distribution to

the lung of diquat and its structural analogue, paraquat, explain the failure of diquat to produce pulmonary lesions characteristic of paraquat poisoning.

TCDD produces hepatic necrosis, lipid accumulation, hepatitis, and cholestasis. The nature and frequencies of these effects following lethal doses vary for different species, from being of minor importance to being lethal.

Weight loss is a common symptom for most of the compounds studied but its cause cannot be explained for any of the compounds. In cases of TCDD poisoning, biochemical lesions involving lipid metabolism may cause this effect, and in diquat poisoning severe gastrointestinal lesions may prevent nutrient utilization.

Various hematologic effects of TCDD have been reported. These effects vary with dose, species, and time after dosing, suggesting the likelihood that they are secondary effects.

Thymic atrophy is the only lesion of TCDD that is seen among all mammalian animal species studied. Despite the severity of this lesion, no correspondingly severe loss in immune function seems to occur. Diquat also produces atrophy of lymphatic tissue, which may be secondary to changes in ACTH and circulating corticosteroids.

Renal effects after treatment with 2,4-D, 2,4,5-T, and TCDD involve decreased renal function. These effects have not been shown to have toxicological significance, except at very high doses when elimination of the administered herbicide is impaired.

CHAPTER 6.

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CHAPTER 7

SUBACUTE AND CHRONIC TOXICITIES

Studies described in this chapter include reports of the effects of herbicides and TCDD exposure after more than one dose has been administered. In this chapter, subacute studies refer to studies that involved administration of more than one dose but less than chronic exposure, while chronic exposure refers to administration of compounds over a substantial proportion of the expected lifespan for that species. In animals, chronic studies usually involve exposure for at least 1 year, and in human studies industrial exposures for 20 years have been considered chronic exposures. In most of the subacute studies described below, the same parameters have been considered which were described for acute studies in chapter 6. For this reason, the present chapter is organized in the same format that was used for chapter 6. Oncogenicity has been addressed in most of the chronic studies, and these studies are considered in chapter 10 in detail and are mentioned here regarding other toxic effects. Descriptions of the potencies of herbicides and TCDD in producing mortality and the target organs affected are described in the first section of this chapter. In the remaining sections, effects of herbicide or TCDD exposure on specific organ systems are described. Only compounds that have been reported to affect a particular organ system are referred to in these sections.

7.1 MORTALITY

In this section, the subacute and chronic dosage regimens that have produced lethal effects are described. Nontoxic dosage regimens are also mentioned. The organ systems affected by each compound and the causes of death are discussed here, as well. Reviews of the subacute and chronic toxicities of 2,4-D and 2,4,5-T have been published (Esposito et al., 1980; NRC, 1974; IARC, 1977; Young et al., 1978; Dalgaard, Mikkelsen, and Poulsen, 1962; President's Scientific Committee, 1972).

7.1.1 2,4-D

Effects of human exposure to 2,4-D have been described and are considered in this chapter. These exposures resulted from occupational exposures in the manufacture or application of 2,4-D. Absorbed doses were not established in any of the studies. Subacute and chronic studies of 2,4-D have been carried out in several species. References on the subacute effects of 2,4-D are listed in table 7-1.

7.1.1.1 Ineffective and Lethal Dosages

2,4-D does not appear to be a cumulative toxicant in most species (Rowe and Hymas, 1954; Bjorn and Northen, 1948), although low doses may be cumulative in the dog. Oral doses of 100 mg/kg were lethal in the rat when administered daily for 10 consecutive days, but not when administered 5 days

TABLE 7-1. REFERENCES ON THE SUBACUTE AND CHRONIC TOXICITIES OF 2,4-D

Species	Route	Dose	Duration	Organs Affected or Studied:										Reference
				Skin	Liver	Kidney	Neural	Musc.	Blood	CV	Lungs	Gut	Body Wt.	
Rat	gavage	.1%	1 mo.	X	X	X	X	X						Hill and Carlisle, 1947
	NS[a]	125 mg/kg	NS		X				X					Szocs et al., 1970
	sc	80 mg/kg/d	several wks										X	Florsheim and Veloff, 1962
	gavage	2-5 g/kg	4-7 wk.		X									Chang et al., 1974
Mouse	NS	1/3-1/5 LD_{50}	2x/d.; 3 wk-3 mo.		X	X			X			X	X	Bucher, 1946
Guinea Pig	oral, inh.	1g total	12 d.	X	X	X	X	X						Hill and Carlisle, 1947
Rabbit	dermal		15x	X	X	X	X	X	X	X	X	X	X	Kay et al., 1947
Dog	iv	25 mg/kg	6x	X	X	X	X	X						Hill and Carlisle, 1947
	oral	2-20 mg/kg	13 wk.		X	X		X		X		X	X	Drill and Hiratzka, 1953
Sheep	oral	18 mg/kg/d	120 d.						X					Shavgulidze et al., 1976
	oral	100-500 mg/kg/d	481x		X	X	X	X	X			X	X	Palmer and Radeleff, 1964
Pig	oral	50 mg/kg	2-51x		X	X	X	X	X		X	X		Bjorklund and Erne, 1966
Cattle	oral	50-250 mg/kg/d	1-112	X			X	X	X		X	X		Palmer, 1963; and Palmer and Radeleff, 1964

[a]NS, not stated;

per week for 4 weeks. Daily doses of 75 mg/kg or lower produced no toxic effects. In one study (Hansen et al., 1971), rats fed diets containing 1,250 mg 2,4,-D per kg for 2 years exhibited no toxic effects, while in two other studies (Rowe and Hymas, 1954; Hill and Carlisle, 1947) slight toxicity was observed in rats fed 1,000 mg/kg feed for 30-113 days. In these studies, doses below 1,000 mg/kg feed were ineffective, and doses above 1,250 mg/kg feed or 1,000 mg/liter drinking water were toxic, but not lethal (Chang et al., 1974; Bjorklund and Erne, 1966).

In most species studied, repeated oral doses of 100 mg/kg/day of 2,4-D at levels of 1,000 ppm or higher produce toxicity, while lower doses are generally ineffective. Oral doses of 2,4-D were also administered to the dog and guinea pig. Oral doses of 20 mg/kg 2,4-D 5 days per week for 13 weeks were lethal to the dog, while 10 mg/kg doses were ineffective (Drill and Hiratzka, 1953). Dogs fed 500 mg 2,4-D per kg feed for 2 years also showed no adverse effects (Hansen et al., 1971). The effect of 50-100 mg of 2,4-D administered orally to guinea pigs for 10 days was difficult to evaluate because of unexpected mortality in the control groups (Hill and Carlisle, 1947).

In sheep, oral doses of 18 mg/kg/day for 120 days produced subtle changes on serum and liver enzymes (Shavgulidze et al., 1976). Doses of 100 mg/kg/day for 481 days produced no overt signs of toxicity, while doses of 250 mg/kg/day were lethal to sheep (Palmer and Radeleff, 1964). Cattle showed no effects from 112 doses of 50 mg/kg/day, slight toxicity from 88 doses of 100 mg/kg/day and lethality at 200 mg/kg/day after 44 doses (Palmer, 1963; Palmer and Radeleff, 1964). Only histopathological signs of toxicity were observed in pigs that were administered 300 mg/kg or diets containing 500 ppm 2,4-D for up to 12 months (Bjorklund and Erne, 1966).

Other routes of administration that have been employed in subacute 2,4-D studies include subcutaneous injections to mice, dermal applications to rabbits, intravenous injections to dogs, and inhalation exposure of guinea pigs (see table 7-1.) The effects of these experiments were not compared to effects of similar doses that were administered orally, precluding the determination of effects of route on 2,4-D toxicity.

7.1.1.2 Cause of Death and Target Organs

The health effects of occupational exposure to 2,4-D have been addressed in several studies. Festisov (1966) reported on effects of 2,4-D in workers. A group of 150 Russian workers was examined. These workers were between 18 and 47 years old, and were occupationally exposed to 2,4-D manufacture or application for 2-10 years. Symptoms reported by the workers were fatigue, headaches, abdominal pain, poor appetite, and impairment of sensory perception. The frequency of each complaint was not reported. Physical examinations were performed on another group of 292 Russian workers, comprised primarily of men between 21 and 40 years of age, exposed for up to 10 years (Bashirov, 1969). Complaints of fatigue, headache, dizziness, sweating, irritability, insomnia, and digestive disorders were reported by workers. Hypertension was detected in 20 percent of the workers and functional (asthenic) disorders of the autonomic nervous system in 61 percent. In a selected group of 50 workers examined, abnormalities in electrocardiograms,

and in gastric and liver function tests, were observed, compared to a control group of 20 workers. In another study, serum samples of workers exposed chronically to 2,4-D and 2,4,5-T during their manufacture were analyzed for cholinesterase, acetylcholinesterase, and tributyrinase activities. No differences in these levels were observed, compared to a group of paired controls (Bonderman et al, 1971). In another study, blood pressure of chlorophenoxy herbicide workers in Oregon was measured. A modest increase in the incidence of hypertension was observed in this group, compared to another group of pesticide workers not exposed to chlorophenoxy herbicides and to a group of control workers. This increase was attributed to a predisposition to hypertension, based on a high incidence of hypertension reported in family histories.

Subacute or chronic administration of 2,4-D to animals does not produce a clear syndrome of effects. At low doses, decreased weight gain and slight effects on the liver and gastrointestinal tract have been observed. Death has been attributed to infections and severe anorexia in some studies, (Palmer, 1963; Bucher, 1946; Rowe and Hymas, 1954), but in most studies no lethal lesions were mentioned. Renal changes, neurological changes, gastrointestinal problems, bleeding and hematologic disorders and, in the dog, hepatic changes have been reported (Hill and Carlisle, 1947). In dogs, bleeding of the gums was observed after subacute doses of 2,4-D were administered, but myotonia was not observed (Drill and Hiratzka, 1953).

7.1.2 2,4,5-T

Effects of occupational exposure to 2,4,5-T are considered in chapter 5 and, where reproductive effects were evaluated, in chapter 8. Animal studies of 2,4,5-T have been carried out, using several laboratory and domestic species. References on the subacute toxicity of 2,4,5-T are listed in table 7-2.

7.1.2.1 Ineffective and Lethal Dosages

Lethal effects have not been observed in rats or mice given subacute doses of 2,4,5-T. Oral doses of 334 mg/kg for 2 days or administration of 3,000 mg 2,4,5-T per kg feed for 90 days were not lethal to rats. Doses of 0.1 mg/kg per day to pregnant rats throughout gestation or 100 mg of 2,4,5-T per kg feed for 90 days produced slight toxicity in rats (Konstantinova, 1974; Coulston, 1970). Oral doses of 2,4,5-T administered to pregnant mice produced varied effects, depending on the strain (Highman et al., 1976a; 1976b). Doses of 120 mg/kg on days 6-14 of gestation had no effect on CRBL mice, while doses of 60 mg/kg to NCTR mice on the same days produced severe maternal toxicity.

Whether pregnant mice and rats show the same response to 2,4,5-T as their non-pregnant counterparts is not clear, although maternal toxicity was not considered to be the cause of teratologic effects observed in mice (Highman et al., 1976a). Comparing subacute doses to the LD_{50} for 2,4,5-T in these species (in tables 6-2 and 7-2) indicates that 2,4,5,-T is not a cumulative toxicant. Analogous to species differences in response to acute effects of 2,4,5-T, the dog seems to have a higher sensitivity to subacute doses of 2,4,5-T than other species. Oral doses of 20 mg/kg/day for 90 days were

TABLE 7-2. REFERENCES ON THE SUBACUTE AND CHRONIC TOXICITIES OF 2,4,5-T

Species	Route	Dose	Duration	Organs Affected or Studied										Reference
				Skin	Liver	Kidney	Neural	Musc.	Blood	CV	Lungs	Gut	Body Wt.	
Rat	gavage	10 mg/6g	6 d.		X								X	Rip and Cherry, 1976
	oral	20-100 mg/kg	2 wk.			X								Stroo et al., 1979
	gavage	2-5 g/kg	4-7 wk.		X									Chang et al., 1974
Mouse	oral	30-140 mg/kg	10 d.		X				X	X				Highman et al., 1976a
Dog	oral	2-20 mg/kg	13 wk.		X	X		X		X		X	X	Drill and Hiratzka, 1953
Sheep	oral	100-250 mg/kg/d	369		X	X						X	X	Palmer and Radeleff, 1964
Cattle	oral	25-250 mg/kg/d	to 7d, max		X	X						X	X	Palmer and Radeleff, 1964

lethal in the dog, while doses of 10 mg/kg/day for 90 days were ineffective (Drill and Hiratzka, 1953).

In cattle, two to three doses of a mixture of 2,4-D and 2,4,5-T at 500 mg/kg/day was not toxic, while three days of 1,000 mg/kg/day was lethal (Palmer and Radeleff, 1964). Seven daily doses of 250 mg 2,4,5-T/kg/day was fatal to sheep and cattle. Although 481 doses of 100 mg/kg of the salt were nontoxic to sheep, the ester form of 2,4,5-T was lethal at this dosage after 369 days. The differences in toxicity from different chemical forms of 2,4,5-T was not compared in other studies.

7.1.2.2 Cause of Death and Target Organs

As with 2,4-D, subacute doses of 2,4,5-T do not produce a characteristic syndrome of effects. The causes of death in animals subacutely exposed to 2,4,5-T overlap with those of 2,4-D and TCDD. Often the cause of death is not stated or could not be determined. Moribund animals as well as animals showing blood changes and weight loss have been observed after subacute 2,4,5-T treatment.

7.1.3 TCDD

The subacute and chronic toxicities of TCDD after human exposure are described in chapter 5 and reproductive effects from subacute and chronic exposures are described in chapter 8. Subacute and chronic animal studies of TCDD have been performed in a number of species and are described in this section. References on the subacute effects of TCDD are listed in table 7-3.

7.1.3.1 Ineffective and Lethal Dosages

Subacute and chronic administration of TCDD has been shown to be lethal in the monkey, rat, and mouse. Subacute doses of TCDD are cumulative in the monkey and the rat. Daily doses of 10 ug TCDD per kg for 30 days produced almost 100 percent mortality in the rat, and reduced weight gains were observed after 1 ug/kg was administered for 30 days (Gupta et al., 1973). The single oral LD_{50} for this strain of rat is between 50 and 100 ug/kg (Harris et al., 1973).

In monkeys, subacute doses of TCDD are far more effective than administering the cumulative dose as a single dose. TCDD levels of 500 ppt in feed--a cumulative dose of 3 ug/kg over 9 months--was lethal to five of eight monkeys (Allen et al., 1977); the estimated single-dose LD_{50} is about 70 ug/kg. Subacute doses of 6 ug/kg body weight, administered in feed over 61 days, or less than 10 ug/kg fed over 10 days, were also lethal to monkeys. The authors of this study predicted that only 1 ug/kg administered subacutely in food would probably be lethal to monkeys (McNultey, 1977). However, 0.9 ug/kg TCDD was not fatal to any of a group of eight monkeys when fed over 20 months at a level of 50 ppt in their feed (Schantz et al., 1979). This cumulative effect is limited and is not seen in rats when TCDD is administered over a period longer than a few weeks (Moore, 1978). The half-life of TCDD is

TABLE 7-3. REFERENCES ON THE SUBACUTE AND CHRONIC TOXICITIES OF TCDD

Species	Route	Dose	Duration	Organs Affected or Studied										Reference
				Skin	Liver	Kidney	Neural	Lymph	Blood	CV	Lungs	Gut	Body Wt.	
Monkey	gavage	2-20 ppb	58 d.					X				X	X	McNulty, 1977
	gavage	500 ppt	9 mo.	X	X			X			X	X		Allen et al., 1977
	gavage	(toxic fat)	445 d.	X	X				X	X		X		Allen and Carstens, 1967
Rat	oral	10 ug/kg/d	31 d		X	X		X					X	Harris et al., 1973
	oral	10 ug/kg/d	14						X					Weissberg and Zinkl, 1973
		1-10 ug/kg/d	31 d		X			X						Gupta et al., 1973
		5 ug/kg/wk	6 wk		X			X						Zinkel et al., 1973
	oral	1-10 ug/kg/d	30 d		X			X						
	oral	.2-5 ug/kg/wk	6 wk					X						Vos et al., 1973
	oral	.001-1 ug/kg/d	13 wk (5/wk)		X	X		X	X					Kociba et al., 1976
	oral	.1-1 ug/kg	2/wk x 12 mo.		X									King and Roesler, 1974
Mouse	oral	1.5-50 ug/kg/wk	4 wk					X	X				X	Vos et al., 1978
		25 ug/kg/wk	4 wk		X									Goldstein et al., 1973
	oral	.2-25 ug/kg/wk	2-6 wk				X	X	X					Vos et al., 1974
	oral	.5-20 ug/kg/wk	4 wk		X									Thigpen et al., 1975
	oral	.1-10 ug/kg/wk	8 wk		X			X	X					Sharma & Gehring, 1979
	ip	25 ug/kg/wk	11 wk		X				X				X	Sweeney et al., 1979
Guinea pig	oral	.2-10 ug/kg/wk	4-8 wk		X			X						Gupta et al., 1973
	oral	1 ug/kg/wk	8 wk		X			X					X	Harris et al., 1973
	oral	.008-1 ug/kg/wk	8 wk		X				X					Zinkel et al., 1973
Rabbit	dermal	.3-10 ug/kg/d	3d	X										Jones & Kizek, 1962
Horse	dermal	salvage oil	(1 yr)	X	X			X	X				X	Case and Coffmann, 1973

between 20-30 days; the cumulative effects seen within this period of time probably reflect the body burden of TCDD that is not cleared from the body in this time period. Daily oral doses of .001-0.1 ug/kg TCDD over 90 days were reported to produce no evidence of toxicity in the rat (Murray et al., 1979), and lifetime exposure of male and female beach mice to sand contaminated with a mean level of 164 ppt TCDD produced only a subtle increase in liver weights of pregnant females (VA, 1981).

The Environmental Protection Agency found that the monkeys used in the studies by Allen and Schantz had been exposed to polyhalogenated biphenyls in a previous experiment and the results of the TCDD experiments have not been generally accepted by the scientific community.

Crude industrial fats contaminated with dioxins (with TCDD comprising 64 percent of the dioxin content) were administered in feed to monkeys, rats, and chickens. The level of fat in feed that produced 50 percent mortality in chickens within 15 days was not lethal to monkeys within 100 days (the mortality rate of monkeys at 100 days was not stated). Rats were fed five times the dose of fat in feed that produced liver necrosis and ascites in the monkey and chicken. At 80 days, rats experienced 50 percent mortality from this level of feeding; the mortality rates were not reported for chickens and monkeys fed one-fifth the dose given to the rat (Norback and Allen, 1973).

Lack of information on mortality rates for all three species given the same dose, and the quantitatively imprecise content and contaminated nature of the samples administered, limits the usefulness of these data, but these results imply that the chicken is most sensitive to subacute exposure to dioxins, the rat least sensitive, and the monkey intermediate between the two. Presumably the same order would apply for TCDD, which in most species is the most toxic of the dioxins. A dose-related effect of toxic fat on mean survival time in the monkey was observed. Monkeys fed diets of 0.125 percent toxic fat survived an average of 445 days while those fed 10 percent fat lived 91 days (Allen and Carstens, 1967).

7.1.3.2 Cause of Death and Target Organs

In general, the symptoms observed after TCDD is administered acutely are also observed after subacute and chronic dosing (except for oncogenic effects of chronic dosing, which are discussed in chapter 10). Prophyria cutanea tarda appears to result in animals from long-term exposure, and not from acute exposure. The effects of TCDD on immune function have been studied after subacute doses were administered, although thymic atrophy and altered responses of splenic lymphocytes to mitogens have also been demonstrated after acute doses of TCDD were administered (see chapter 6).

7.1.4 Diquat

Two studies included experiments on the subacute toxicity of diquat. Twenty daily dermal applications of 20 mg/kg diquat to rabbits produced local erythema but no systemic effects, while four of six rabbits died after 8-20 daily applications of 40 mg/kg of diquat. Symptoms produced by this higher

dose included weight loss, unsteadiness and muscular weakness (Clark and Hurst, 1970). Rats, mice, rabbits, guinea pigs, and a dog were exposed to 1.1 ug of diquat aerosol per liter air for 15 6-hour periods, with no adverse effects noted. Rats exposed to 2 ug/liter of diquat for 15 6-hour periods showed a small decrease in weight gain and female rats of this exposure group experienced difficult breathing during the first few sessions only (Gage, 1968).

Chronic exposure to diquat results in cataracts. In rats, this effect was observed within 6 months in all rats fed diets containing 0.1 percent diquat, in 12 months in all rats fed 0.05 percent diquat, and in 18 months in all rats fed 0.025 percent diquat. One-quarter of the rats fed 0.01 percent and 0.005 percent diquat showed slight opacity in 12 months, while a diet of 0.001 percent diquat was ineffective in causing cataracts. Rats fed 0.05 percent diquat for only 8 weeks did not develop cataracts. In advanced conditions, opacity was accompanied by hemorrhages into the vitreous humor, synechiae, and retinal detachment. Rats fed ascorbic acid supplements or housed in darkness showed the same development of cataracts in response to diquat. The only other effect in these rats was reduced growth rates and food consumption in the highest dosage group (Clark and Hurst, 1970). Reduced glutathione levels remained high (Pirie et al., 1970) while ascorbic acid levels were low in eyes that developed diquat cataracts; distribution of diquat to the eye has been demonstrated (Pirie and Rees, 1970; Pirie et al., 1969).

Cataracts also developed in dogs from chronic exposure to diquat. Daily oral doses of 15 mg/kg of diquat produced cataracts in all treated dogs in 10-11 months, and 5 mg/kg doses produced cataracts in all dogs in 15-17 months. Daily doses of 1.7 mg/kg for 4 years or 0.4 or 0.8 mg/kg for 3 years were ineffective. No other changes were seen in these dogs (Clark and Hurst, 1970).

7.1.5 Picloram

The subacute doses of picloram administered to various species by several routes were usually too low to produce evidence of toxicity. Rats fed picloram (acid form) as 1,000 ppm in their diet for 90 days (equivalent to 75 mg/kg body weight per day) did not experience adverse effects. At levels of 3,000 and 10,000 ppm, moderate hepatic and renal changes were observed and females showed decreased weight gains. Picloram as the triisopropanolamine salt, at levels of 0.3 percent or less in feed, produced no alterations in rats over a 90-day feeding period. Doses of 100 mg of picloram per kg per day for 31 days produced no ill effects in sheep, while doses of 72 and 154 mg/kg for 31 days caused no adverse effects in cattle. Chronic exposure of dogs and rats to daily doses of up to 150 mg/kg of body weight for two years produced no clinical, gross, or microscopic alterations (McCollister and Leng, 1969). Dermal applications of undiluted picloram to rabbits followed by bandaging the site for 11 days, or ten repeated applications of a 25 percent solution over 14 days, produced only local effects, involving slight exfoliation and hyperemia (Lynn, 1965).

7.1.6 Dalapon

Local or nonspecific systemic effects have been observed in dalapon-treated animals. Ten consecutive dermal applications of dalapon to rabbits over 14 days produced local dermal effects, involving moderate hyperemia and slight necrosis, but no systemic toxicity. Doses of 1,000 mg/kg daily to dogs for 81 days caused gastrointestinal irritation (vomiting), but no other clinical or histopathological findings. Systemic effects of dalapon poisoning were produced in the rat and in cattle. Diets of .346 and 1.15 percent dalapon, fed to rats for 97 days, caused growth retardation, increased liver and kidney weights, and slight histopathologic changes in these organs. Ten oral administrations of 1,000 mg dalapon/kg to cattle caused anorexia, lassitude, diarrhea, weight loss, slowed pulse, and discharge from the eyes in one animal and no symptoms in the second animal. The only histopathological lesion that was observed involved the kidney of the second (unaffected) animal.

Few effects from chronic dalapon exposure were observed in animals. The only change produced after chronic exposure of rats and dogs to dalapon was increased kidney weight. This effect followed administration of 100 mg/kg/day of dalapon to dogs for 1 year and 50 mg/kg/day to rats for 2 years. Daily doses of 50 mg/kg to dogs for one year or 15 mg/kg to rats for two years produced no toxic effects (Paynter et al., 1960). The only effect observed in one sheep was a 6 percent loss in body weight after 10 daily doses of 100 mg/kg dalapon and 10 percent loss after 86 doses were administered; after a total of 481 doses were given, no other changes were observed. Ten daily oral doses of 500 mg/kg produced no effects in cattle, but weight losses of 6-17 percent in sheep (Palmer and Radeleff, cited in Kenaga, 1974).

7.1.7 Monuron

Monuron was administered to rats at levels of 0.25 percent, 0.025 percent and 0.0025 percent in their diets, for 2 years. Effects observed at the highest dosage were decreased growth rates, slight anemia, and increased liver and spleen weights; all tissues appeared normal, histologically, at autopsy. The mortality for all rats, including those in the control group, was 70-90 percent by the end of the experiment; this high rate was attributed to several epidemics of respiratory infections (Hodge et al., 1958).

Monuron was administered at doses of 2.5, 12.5, and 25 mg/kg body weight per day to dogs for 1 year. No treatment-related effects were observed on body weights, organ weights or histology or by analyses of blood and urine samples (Hodge et al., 1958).

7.1.8 Bromacil

Bromacil was administered in the diet at 1,250 ppm to rats for 2 years. Lower weight gains and decreased food consumption were observed in this group, compared to controls. Rats fed diets with 50 or 250 ppm bromacil for 2 years did not show these effects. None of the animals in the study showed any

clinical signs of toxicity and results of their hematologic, urine, and biochemical analyses were normal. Dogs fed the same dosages, as diets with 50-1,250 ppm bromacil, also showed no effects in any of these parameters over a 2-year period (Sherman and Kaplan, 1975). Midwest Research Institute (1975) reviewed bromacil toxicity studies.

7.1.9 Cacodylic Acid

No histopathological effects or clinical changes were observed from cacodylic acid, administered at dietary levels of 30 ppm for 90 days to dogs, or at dietary levels of up to 100 ppm, or at 280 mg/kg body weight for 20 days to rats. In rabbits a 77 percent cacodylic acid preparation was applied and exposure was continued for 12 hours per day, 5 days per week for 3 weeks. The effects from this exposure were local hyperemia, and other dermal lesions and rapid loss of conditioning, diarrhea, fluid accumulation in the gastrointestinal tract, congestion in the spleen, and distended bowels. Doses of 1 and 1.6 g/kg were not lethal to the single rabbit tested at each dose, while doses of 2.5 g/kg or higher were lethal (cited in Midwest Research Institute, 1975).

7.1.10 Herbicide White

Repeated dermal applications of a 5 percent solution of Herbicide White to man produced no skin irritation or sensitization. Sheep were administered daily oral doses of 0.55 ml of Herbicide White per kg for 5 days. Four of 11 sheep died and losses in body weight were noted in the remaining sheep. Doses of 0.11 ml/kg per day for 30 days produced neither mortality nor effects on appearance, behavior, or weight gain in sheep.

Rabbits were dermally administered 15 ml of a 5 percent solution of Herbicide White and the site of application was wrapped for 7 hours. After a total of 15 daily applications were made, no dermal or systemic effects were observed. Applications of undiluted herbicide in the same manner produced slight irritation (Lynn, 1965). Herbicide White was also applied to the skin and eyes of rabbits under conditions of high humidity and high temperature. Erythema from 0.02 ml of Herbicide White appeared more rapidly under adverse conditions than in temperate conditions, although the adverse conditions caused death to many of the control and treated rabbits (Weimer et al., 1970).

7.2 DERMAL LESIONS

7.2.1 2,4-D and 2,4,5-T

Both phenoxy acids, 2,4-D and 2,4,5-T, produce mild degenerative changes in the conditions of the fur of rodents and muzzle of cattle after subacute oral doses are administered. Doses that produce these changes also produce general debilitation. Dermal lesions were described in rabbits that were administered 2,4-D dermally. These changes involved local skin reactions and inflammation. However, these changes were also observed in rabbits that were treated with vehicles only (Kay et al., 1965).

7.2.2 TCDD

Chloracne has been shown to result from a single exposue to TCDD (see chapter 6). One study evaluated the effect of repeated small dermal doses of TCDD in the rabbit. In the rabbit ear bioassay 0.004 ug of TCDD in benzene, applied 5 days a week for 4 weeks, produced comedones. The effect of administering the cumulative dose (.080 ug of TCDD) as a single dose was not tested. Application of one-tenth of the effective dose of 0.004 ug for the same 4-week period of time did not produce chloracne (Schwetz et al., 1973).

Dermal lesions and chloracne were common effects of subacute exposure of monkeys to TCDD. Adult female rhesus monkeys fed diets containing 50 ppt TCDD for 20 months (0.9 ug/kg cumulative dose) experienced hair loss and hyperkeratosis, especially of the arms (Schantz et al., 1979). This exposure to TCDD was not lethal for any of the eight monkeys. Five of eight female rhesus monkeys died after 7 to 9 months of exposure to 500 ppt TCDD in feed (2-3 ug/kg cumulative dose). All eight monkeys developed acne, accentuated hair follicles, periorbital edema, swelling of the eyelids, loss of facial hair and eyelashes, and irregular nail growth. These changes appeared during the first 3 months of exposure. Hair loss and periorbital edema persisted in the survivors for 3 months after exposure ended. Keratinization of hair follicles, sebaceous glands, meibomian glands of the eyelids, and nails were observed at necropsy (Allen et al., 1977).

One male rhesus monkey who was fed 2 ppb TCDD in feed for 61 days (6 ug/kg cumulative dose) experienced no hair loss or acne prior to death on day 76 (death may have been caused by unidentified post-operative complications, although signs of severe TCDD intoxication were also present). Another monkey was fed 20 ppb TCDD and died after 12 days; in both of these monkeys, squamous metaplasia of sebaceous glands was observed at necropsy (McNulty, 1977). Alopecia and subcutaneous edema, which appeared first in the eyelids and then the face and other parts of the body, were observed one month prior to death in monkeys that were fed lethal doses of toxic fat in their diets. Dermal edema and keratinization of hair follicles and sebaceous glands were observed microscopically (Allen and Carstens, 1967).

Horses, dogs, cats, and mice at arenas that were sprayed with TCDD-contaminated salvage oils developed contact dermatitis and hair loss. Mild dermatitis of the face, limbs, and ventral surfaces became progressively severe, and eventually involved ulcerative lesions, as well as inflammation of the mucous membranes of the mouth and nasal passages. Hair loss in horses included extensive loss from the mane and tail (Case and Coffmann, 1973). The contribution of TCDD in the salvage oil to this condition, compared to other components which may have caused some of the deleterious effects, is unknown.

Acute TCDD treatment has been reported to cause a depletion in vitamin A storage levels in the liver to 30 percent of control levels, 8 weeks after a 10 ug/kg dose of TCDD was administered to rats (Thunberg et al. 1979). These authors noted that chloracne seen in man after TCDD exposure resembles dermal lesions observed in hypovitaminosis A.

7.3 HEPATOTOXICITY

7.3.1 2,4-D and 2,4,5-T

Subacute doses of 2,4-D or 2,4,5-T produce degenerative histological changes in the liver, enzyme changes, and liver enlargement. Focal necrosis of the liver was observed in dogs that received lethal subacute doses of 2,4-D or 2,4,5-T, but the authors of this study concluded that these lesions were of no toxicologic signficance, as their severity was not dose-related (Drill and Hiratzka, 1953). However, other investigators also observed centrilobular degeneration and atrophy in dogs that received lethal doses of 2,4-D (Hill and Carlisle, 1947). Multinucleated hepatocytes and degeneration of the liver parenchyma was observed in pigs that received 500 ppm 2,4-D in their feed for 12 months. This dose also produced locomotory disturbances and reduced growth rates, but was not fatal (Bjorklund and Erne, 1966). Degeneration of the liver was observed in sheep and cattle that received lethal subacute doses of 2,4-D or 2,4,5-T (Palmer and Radeleff, 1964).

The same types of serum and hepatic enzyme changes were observed in the rat after a single dose of 625 mg of 2,4-D per kg, or subacute doses of 125 mg/kg. These changes included decreased aldolase levels and increased glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, catalase, cholinesterase, and acid phosphatase levels, 21 days after treatment ended (the duration of treatments was not stated). Cellular infiltration of the liver was observed after subacute treatment, but not after an acute dose. However, livers were examined only within 2 days of the acute dose. The authors concluded that 2,4-D produces subacute hepatitis (Szocs et al., 1970).

Increased liver weights, increased cellular RNA and protein content, and decreased nuclear DNA content were observed in rats that were administered 2-5 g 2,4,5-T per kg (less than 0.05 ppm TCDD) over 4 to 7 weeks. The same dose of 2,4-D did not produce these changes, although both phenoxy acids caused a 50-100 percent elevation in liver glycogen content (Change et al., 1974). The effect on liver size was reversible, as liver size returned to normal after 2,4,5-T (less than 0.05 ppm TCDD) administration ended. Metabolism of 2,4,5-T was not ehanced in the enlarged livers and 2,4,5-T did not show strong hepatotoxic activity regarding enzyme induction (Rip and Cherry, 1976). In another study, sublethal doses of 2,4,5-T to rats produced liver enlargement accompanied by swelling parenchymal cells and slight centrilobular necrosis. Toxicity in these rats was mild, and included a small reduction in body weight and histological evidence of renal damage (Coulston, 1970).

7.3.2 TCDD

7.3.2.1 Structural Alterations

Subacute doses of TCDD were administered to monkeys in four studies (Schantz et al., 1979; McNulty, 1977; Allen et al., 1977; Allen and Carstens, 1967). Only one study, in which TCDD was administered as toxic fat, reported histological changes in the liver other than in the bile duct. Allen and Carstens (1977) reported that fatal doses of toxic fat produced enlarged livers, with focal necrosis, fat deposits, and multinucleated hepatocytes.

Proliferation of the smooth endoplasmic reticulum was observed in electron micrographs. Since these effects were not seen when lethal doses of purified TCDD were administered to monkeys, the possibility exists that other components of toxic fat caused the observed hepatotoxicity.

Hepatotoxicity was the principal effect observed in rats that were administered 0.1 or 1.0 ug of TCDD per kg per week of TCDD for 28 weeks; decreased weight gain was also observed in these rats. The major hepatic change was the accumulation of centrilobular fat deposits, which the authors suggested could be caused by impaired lipid transport or metabolism. In no case were the observed fatty changes considered severe. Fatty changes were first observed 16 weeks after treatment and regressed but did not completely disappear by the end of the 12-week recovery period. Other hepatic changes were described as subtle or insignificant, and included focal changes, multinucleation, and altered hepatic architecture. Female rats had less severe lesions than male rats (King and Roesler, 1974).

The severity of liver damage in rats is related to the dose of TCDD administered. Slight liver damage was observed in rats administered weekly doses of 5 ug/kg for 6 weeks, slight to moderate damage occurred from 31 daily doses of 1 ug/kg, while severe damage was observed after 16-31 daily doses of 10 ug/kg. A single oral dose of 2 ug/kg was ineffective in the same strain of rat, while 50 ug/kg produced severe damage. Marked necrosis of hepatocytes, multinucleation, and disturbance of the architecture of the liver were the hepatic lesions observed. In contrast to rats, liver damage was slight in guinea pigs that received four to five doses weekly of 1 ug TCDD per kg, which produced 100 percent mortality (Gupta et al., 1973).

TCDD causes an increase in liver size relative to body size in the rat. Ten or more daily doses of 0.1 or 1 ug TCDD per kg to rats resulted in increased absolute liver weights, while daily doses of 10 ug/kg for more than 10 days produced a progressive fall in absolute liver weights and a mortality rate above 90 percent. Weekly sublethal doses of 0.04 or 0.2 ug/kg to female guinea pigs did not produce significant changes in liver weight, although thymic weights were adversely affected by both doses, and body weights by the higher dose (Harris et al., 1973).

In another study, changes in liver weight was found to be the most sensitive response to TCDD. The only adverse effect observed in rats given 0.01 ug/kg/day for 13 weeks was a slight increase in liver weight relative to body weight, and the author associated no toxicological significance with this small change (Kociba et al., 1976).

At 0.1 ug/kg/day, liver degeneration occurred which involved mild changes in liver architecture and slight lipid accumulation. These hepatic changes were accompanied by lymphoid depletion and the decreased body weights. At 1.0 ug/kg/day, alteration in liver architecture and hepatocyte sizes, focal necrosis, mild fat accumulation, cellular infiltration, brown pigmentation, and multinucleation were observed. This dose produced 33 percent mortality and produced changes in hematologic and reproductive parameters as well as the effects seen at lower doses (Kociba et al., 1976).

As observed after subacute administration, chronic administration of TCDD to rats also produced hepatic changes. Feed containing 0.05 ppm TCDD or higher was lethal to all rats within four weeks and produced severe liver necrosis. Feed with 0.001-5.0 ppb TCDD was administered to rats for 65 weeks. Mortality rates were about 8 percent and all rats that died showed moderate liver alterations which were not described further (Van Miller and Allen, 1977). However, the laboratory practices used for this study were later found to be inadequate by the Environmental Protection Agency; thus, these results are questionable.

In another chronic study, proliferation of the smooth endoplasmic reticulum was observed in rats fed diets of 0.1 ug/kg/day (2,200 ppt) for 2 years (Kociba et al., 1978; 1979).

TCDD also produces histopathological changes and increased liver weights in the mouse. Liver weights relative to body weights were significantly increased 2 and 6 weeks after weekly doses of 1, 5, or 25 ug/kg were administered to mice. Lipid accumulation was detected after six weekly doses of 0.2 ug TCDD/kg. The effect was more severe at higher doses and involved centrilobular and midzonal hepatocytes. Necrosis and cellular infiltration were observed after doses of at least 5 ug/kg. Degenerated liver cells and nuclear changes were seen in the 25 ug/kg dosage group (Vos et al., 1974).

Young (VA, 1981) reported that the only toxic effect observed in field mice exposed chronically to sand containing a mean concentration of 164 ppt TCDD was increased liver-to-body weight ratios in pregnant females, compared to unexposed control field mice. This effect was seen consistently over a 5-year period of study.

Mice on an iron-deficient diet, after TCDD treatment (25 ug/kg/week for 11 weeks intraperitoneally) did not develop porphyria or hepatocellular lesions that were observed in mice with normal iron levels. These changes included altered liver architecture and hepatocyte sizes, focal necrosis, cellular infiltration, and mid-zone and peripheral vacuolation. Other degenerative changes in the conditions of the mice after TCDD were also absent in iron-deficient mice. The only difference in general appearance between untreated mice and TCDD-treated iron-deficient mice was the smaller size of the latter. Subtle changes observed after TCDD treatment included increases in liver weight and induction of microsomal enzymes in both the iron-deficient mice and mice with normal iron levels. The authors suggested that tissue iron (probably in another form) plays an essential role in the toxic changes induced by TCDD (Sweeney et al., 1979; Jones and Sweeney, 1979).

7.3.2.2 Serum Enzyme Changes

Changes in serum enzymes have not provided an accurate indication of TCDD hepatotoxicity, although increased serum glutamic pyruvic transaminase levels were detected in one study, in monkeys fed diets with a sublethal dose of 50 ppt TCDD for 20 months (0.9 ug/kg cumulative dose) (Schantz et al., 1979). Serum enzyme assays that are often used to indicate liver malfunction found no change in horses or in rats after TCDD exposure (Case and Coffmann, 1973; Kociba et al., 1976). Assays for biliary function, however, were altered by

TCDD treatment (see section 7.3.2.3). Rats fed diets with 2,200 ppt TCDD for 2 years showed increased serum enzyme levels as well as increased excretion of porphyrins and hepatic lesions (Kociba et al., 1978; 1979).

7.3.2.3 Biliary Excretion

TCDD produces adverse structural and functional effects on the biliary system. Dilation of the bile duct was observed at death in monkeys fed 500 ppt TCDD in feed for nine months (Allen et al., 1977). Proliferation and stratification of bile duct epithelium were observed in monkeys that died after subacute doses of toxic fat were administered in their feed (Allen and Carstens, 1967).

Prolonged bromsulphthalein clearance times were observed in horses after high and often lethal exposure to TCDD in salvage oil sprayed on arenas. Severe biliary cirrhosis was observed in all horses at autopsy (Case and Coffmann, 1973). Proliferation of bile ducts was observed in mice administered weekly doses of 25 ug TCDD/kg TCDD (Vos et al., 1974).

In rats that were administered 1.0 ug/kg/day of TCDD for 13 weeks, biliary hyperplasia was observed. Elevated serum alkaline phosphatase and direct bilirubin levels were also detected in these rats, indicating impairment of biliary functions. At a dose of 0.1 ug/kg/day for 13 weeks these changes were observed only in females, and at lower doses (0.01 and 0.001 ug/kg/day) these changes did not occur (Kociba et al., 1976). Chronic administration of feed with 0.001-5.0 ppb TCDD for 65 weeks produced mortality rates that were proportional to dose. Hyperplasia of the bile ducts was observed in all animals that died (Van Miller and Allen, 1977).

7.3.2.4 Porphyria

Porphyria is a disorder of hemoglobin metabolism. Porphyria cutanea tarda is a severe form of porphyria and has occurred in workers that were exposed to TCDD (see chapter 5). The suggested biochemical lesion in hepatic porphyria is an inhibition of the enzyme uroporphyrinogen decarboxylase. This enzyme converts uroporphyrinogen to coproporphyrinogen, intermediates in the hepatic conversion of delta-aminolevulinic acid to heme. Urinary porphyrin excretion is elevated in cases of hepatic porphyria.

In man, a relative increase in 7- and 8-carboxy-containing porphyrin metabolites over 4- to 6-carboxy-porphyrin metabolites is a more sensitive indicator of porphyria than the level of total porphyria excretion. In cases of porphyria cutanea tarda, total urinary porphyrins can be comprised of 45-70 percent uroporphyrin and 25-35 percent heptacarboxylic porphyrin, compared to much lower proportions in normal urine. Hepatic accumulation of these porphyrins occurs before clinical symptoms are manifest or urinary levels are elevated (VA, 1980).

Porphyria has been observed in male and female rats after 1 ug/kg/day of TCDD was administered for 13 weeks. Only females developed porphyria from 0.1 ug/kg/day doses for 13 weeks. Evidence of porphyria included elevated

excretion of total porphyrins, uroporphyrin, and delta-aminolevulinic acid. Mortality for the higher and lower doses were 33 percent and none, respectively (Kociba et al., 1976). Porphyria was also observed in the rat after chronic dosing (Greig et al., 1979; Kociba et al., 1978; 1979).

In another study, porphyria was not observed in any rats that died after 16-31 daily doses of 10 ug/kg of TCDD or guinea pigs that died from four to five weekly doses of 1 ug/kg TCDD. In this study, porphyria was evaluated by examining the liver under ultraviolet light for red fluorescence, an indication of excess amounts of porphyrins, and this method may have been too insensitive to detect the expected increase in porphyrins (Gupta et al., 1973). No changes in delta-aminolevulinic acid synthetase levels were detected in rat livers pretreated with up to 25 ug/kg TCDD 1 day prior to sacrifice, and induction of delta-aminolevulinic acid synthetase by allylisopropylacetamide (ALA) was not affected by TCDD over 28 days. Twenty-four-hour pretreatment of fetal or neonatal rats with TCDD also failed to alter ALA synthetase activity (Woods, 1973). In these experiments, TCDD was not administered chronically or at a high dose with a latency period greater than 1 day, which is probably necessary to allow enzyme changes to become manifest.

In mice, porphyria has been observed after four weekly doses of 25 ug/kg TCDD were administered. This dosage produced a 2,000-fold increase in the hepatic levels of 7- and 8-carboxyporphyrins, which were analyzed by thin layer chromatography. Total iron content in the liver was elevated significantly (to 150 percent of the control level), delta-aminolevulinic acid synthetase activity was increased, and histopathological changes in the liver were noted. A single lethal dose of 150 ug/kg of TCDD produced a 4,000-fold increase in hepatic uroporphyrins 21-25 days after treatment. Hepatic iron levels were elevated to about 250 percent of the level of the control group (Goldstein et al., 1973). In another study, the porphyrinogenic effects of intraperitoneal doses to mice of 25 ug/kg/week for 11 weeks included a 10-fold elevation in urine porphyrin levels and a reduction to 20 percent of uroporphyrinogen carboxylase activity, compared to control mice. As described above (section 7.3.2.1), iron-deficient mice with hemoglobin levels of 5.5 g/dl showed no signs of porphyria after the same dosage of TCDD was administered (Sweeney et al., 1979; Jones and Sweeney, 1979).

7.4 NUTRIENT ABSORPTION AND UTILIZATION

Changes in body weight and food consumption after herbicide and TCDD treatment are described here. Factors that can affect nutrient utilization are also considered in this section. These factors include absorption of nutrients from the gut, hormonal alterations that effect regulation of nutrient utilization, and biochemical factors that reflect levels of circulating protein and lipid precursors and metabolites.

7.4.1 2,4-D and 2,4,5-T

Decreased weight gain has been observed in most species studied, after low subacute doses of phenoxy acids were administered. Higher doses produced complete cessation of eating in some animals (see tables 7-1 and 7-2).

7.4.1.1 Body Weight and Food Consumption

Actual measurements of body weight and food consumption have been reported. Reduced food consumption and body weights were demonstrated in chicks that consumed 5,000 mg of 2,4-D or 2,4,5-T per kg of feed for one week. Two weeks after the chicks were returned to normal diets, their normal growth rates resumed (Whitehead and Pettigrew, 1972). In another study, three doses of 280 mg 2,4-D/kg of 2,4-D were administered per week for 4 weeks to chicks and a significant decrease in body weight of 20 percent was observed. Lower doses did not produce significant reductions in weight (Bjorn and Northern, 1948). In the dog, lethal subacute doses of 2,4-D and 2,4,5-T produced weight losses of up to 4 kg, while lower sublethal doses of 2,4,5-T produced smaller weight changes and sublethal doses of 2,4-D produced no weight changes (Drill and Hiratzka, 1953).

7.4.1.2 Effects on the Endocrine System

Florsheim and Velcoff (1962) described an effect of 2,4-D on the thyroid. Rats administered daily subcutaneous injections of 100 mg/kg 2,4-D showed decreased thyroid weights and body weights. At 80 mg/kg, these changes were not observed, but an increase in ^{131}I uptake by the thyroid was observed. This effect did not involve pituitary thyrotrophic hormone, and was observed in rats with normal thyroid function but not in hypophysectomized or iodine-depleted rats.

7.4.2 TCDD

7.4.2.1 Body Weight and Food Consumption

Reduction in body weight has been observed in many species following subacute TCDD exposure. TCDD caused weight loss in monkeys fed 50 ppt TCDD in feed for 20 months, 500 ppt for 9 months, 2 ppb for 61 days, or 20 ppb for 12 days. At death the monkey that was fed 2 ppb TCDD had lost 33 percent of its body weight in 76 days. For the monkey fed 20 ppb TCDD, food consumption progressively decreased, from the third day of feeding until day 12 when its body weight was decreased by 30 percent and death occurred (McNulty, 1977). Monkeys fed 500 ppt TCDD in their diet lost an average of 13 percent of their initial body weight at death (which occurred following at least 7 months of exposure) or at 1 year, for the survivors. Food consumption did not change in these animals during the treatment period, but was not compared to food consumption of untreated monkeys (Allen et al., 1977). Monkeys fed lethal amounts of toxic fat in their diets lost up to 1 kg in body weight at death (about 20 percent of their body weight) and food consumption was observed to be decreased. The authors did not clarify several points, including whether they actually measured consumption, whether the amounts consumed prior to death were lower than those of controls or than those of treated monkeys at the start of the experiment, or whether the observed decrease in food consumption could account for the decrease in body weight (Allen and Cartens, 1967).

Horses, cats, and dogs exposed to arenas that were sprayed with TCDD-contaminated salvage oil showed severe weight losses of up to 40 percent in

some horses. Absence of demonstrable body fat was a common finding at autopsy. Loss of appetite and preference for sweets were observed in these animals as well as in children who played in the sprayed arena (Case and Coffmann, 1973).

In the rat, body weight losses were observed in rats once the total dose administered exceeded 20 ug/kg; daily doses of 1 ug/kg for 1 month, weekly doses of 5 ug/kg, or a single dose of 25 ug/kg caused decreases in weight gains in the rat. This effect was reversible, as normal growth rates resumed several weeks after the dose regimens were terminated. Doses that failed to alter body weight included a single dose of TCDD of 5 ug/kg, weekly doses of 1 ug/kg over 6 weeks, or daily doses of 0.1 ug/kg over 30 days (Harris et al., 1973). In the guinea pig, a single dose of 1 ug TCDD/kg or weekly doses of 0.2 ug/kg over 8 weeks produced significant decreases in body weight, while doses of 0.04 ug/kg were ineffective. In the mouse, weekly doses of 25 ug/kg over 4 weeks were effective while weekly doses of 5 ug/kg or a single dose of 50 ug/kg were not (Harris et al., 1973). In another study, however, two weekly doses of 5 ug/kg caused significant weight reduction (Vos et al., 1974). These results indicate that effects on body weight, like the lethal effects, are cumulative, being produced when an effective amount of TCDD is administered as a single dose or in smaller doses over a period of about 1 month.

Chronic doses of TCDD that cause body weight reduction have been described for the rat. Male rats administered 1 or 5 ppb TCDD in feed for 65 weeks showed significantly decreased weight gain as well as 40-50 percent mortality. Rats fed 0.001-0.5 ppb TCDD in their diets had a mortality rate of 8 percent; no disturbances in body weight were mentioned for this group (Van Miller and Allen, 1977). Doses of 1.0 or 0.1 ug TCDD/kg, administered to rats for 13 weeks, caused reduction in weight gain and food consumption compared to controls, while doses of 0.01 ug/kg/day or less were ineffective (Kociba et al., 1976). Rats treated twice weekly for 28 weeks with 0.1 or 1.0 ug/kg/week had reduced body weights compared to controls. This effect was sustained in male rats, but not in females, during the subsequent recovery period of 12 weeks. Food consumption was not measured in this study (King and Roesler, 1974).

7.4.2.2 Effects on the Gastrointestinal Tract

Hyperplasia and ulceration of the gastric mucosa have been observed in monkeys after lethal subacute doses of TCDD were administered (McNulty, 1977; Allen and Carstens, 1967). The affected monkeys also showed large changes in body weight. Lethal subacute doses of TCDD also produced ulceration in the stomach of rats (Gupta et al., 1973). Gastrointestinal transport of nutrients was not evaluated in these studies. However, most organs in these animals showed effects from TCDD and no attempt has been made by the investigators to relate the observed intestinal lesions to weight loss in these animals.

7.4.2.3 Lipid and Protein Biosynthesis

No systematic study of protein or lipid levels following acute dosing of TCDD have been reported. Sporadic instances of altered protein or lipid

levels have been mentioned. Decreased serum protein levels and altered albumin-to-globulin ratios were observed in monkeys that were fed lethal amounts of toxic fat (Allen and Carstens, 1967). Decreased serum cholesterol levels were observed in monkeys fed sublethal amounts of TCDD (50 ppt in the diet for 20 months, Schantz et al., 1979). No connections between these altered serum levels and disturbed protein or lipid metabolism by TCDD were drawn by the authors. Lowered serum gamma globulin levels was one of the only abnormal results of clinical chemistry tests of horses exposed to arenas sprayed with TCDD-contaminated salvage oil. Serum lipid levels were not assayed, however (Case and Coffmann, 1973). Serum protein levels and globulin levels were reduced in mice that received weekly doses of 25 ug TCDD/kg for 6 weeks, while weekly doses of 5 ug/kg were not effective (Vos et al., 1974).

7.5 HEMATOLOGICAL EFFECTS

7.5.1 2,4-D and 2,4,5-T

Bleeding disorders, including increased bleeding of the gums in dogs and hemorrhages in the heart and gastrointestinal tract, have been observed in many species after phenoxy acids were administered subacutely.

2,4-D and 2,4,5-T have produced various hematological changes which are difficult to categorize. In sheep that were administered 18 mg/kg daily for 120 days, hematologic changes included increases in erythrocyte, hemoglobin, and leukocyte levels within the first months, followed by decreases in all of these parameters later in the exposure period. Biochemical changes in blood components and erythrocyte enzyme levels were also noted; these changes also showed bimodal patterns (Shavgulidze et al., 1976; Kuzminskaya and Bersan, 1975). None of these changes in blood cell counts were observed in dogs that received lethal doses of 2,4-D or 2,4,5-T, although a terminal fall in the relative proportion of lymphocytes to other blood cell types was noted (Drill and Hiratzka, 1953). In another study large decreases in white blood cell counts were observed in only some of the dogs that received lethal doses of 2,4-D. Mild decreases in red blood cell counts and hematocrits were also observed in these dogs (Hill and Carlisle, 1947). Hematocrit and hemoglobin levels were lowered in pigs that were fed 2,4-D at 500 ppm for 12 months (Bjorklund and Erne, 1966). Slight decreases in red cell counts and hemoglobin levels were observed in rats that were administered sublethal doses of 2,4,5-T orally (Coulston, 1970).

7.5.2 TCDD

Depletion of various types of circulating blood cells from high doses of TCDD in monkeys has been observed. TCDD at a sublethal level of 50 ppt in feed, administered to monkeys for 20 months, produced decreases in the hematocrit and white blood cell count (McNulty, 1977). At a level of 500 ppt in feed for 9 months, TCDD was lethal to five of eight monkeys. All five fatal cases developed pancytopenia prior to their deaths, which occurred 7-10 months after the experiment started. One of the survivors developed severe leukopenia and thrombocytopenia by the 12th month of the experiment. Hematocrits and hemoglobin levels began to fall by the sixth month in all monkeys and

subsequently showed a progressive fall. Hypocellularity of bone marrow was observed at necropsy (Allen et al., 1977). In monkeys that consumed toxic fat peripheral red and white blood cell counts were depressed; bone marrow hematopoiesis and activity of lymphoid tissue were also depressed in these animals as well (Allen and Carstens, 1967). However, neither hemoglobin levels nor hematocrit values were found to be useful parameters in monitoring the condition of horses exposed to TCDD in salvage oil (Case and Coffmann, 1973).

Rats that were administered 10 ug/kg TCDD daily were bled on days 10 and 14, and various hematologic parameters were evaluated. Hemoconcentration, as evidenced by elevated packed cell volumes and erythrocyte counts, observed, along with neutrophilia, lymphocytosis, and eosinopenia. These changes were considered to be nonspecific, reflecting the widespread toxicity of TCDD on various organs. Thrombocytopenia was found but no evidence of decreased platelet synthesis (based on normal marrow megakaryocyte levels), or losses by incorporation into microthrombi (since the level of serum fibrinogen degradation products was not elevated). Most platelet function tests were normal as well (Weissberg and Zinkl, 1973). In another study, hemoconcentration was observed terminally in rats given 10 ug/kg/day of TCDD for 30 days but not in rats that received 1 ug/kg/day or less, and was attributed to shock and dehydration. Thrombocytopenia occurred in all three groups while no significant changes occurred in leukocyte counts or in lymphocyte counts. Thrombocytopenia and decreased lymphocyte counts were observed in guinea pigs following eight weekly doses of 0.08-0.2 ug TCDD/kg (Zinkl et al., 1973).

Differences in hematologic effects of TCDD between males and females have been noted. Thrombocytopenia was observed in male and female rats fed 1.0 ug/kg/day of TCDD. Packed cell volume, red blood cell counts, and hemoglobin concentrations were elevated significantly for females only and decreased in males, compared to controls. White blood cell counts also showed these trends, although the decrease in males was not significant. At doses of 0.1 ug/kg/day or less, most changes in blood cell counts were not significant (Kociba et al., 1976).

Lethal hematologic changes have resulted from chronic exposure to TCDD. Death was attributed to aplastic anemia in 25 percent of the rats that succumbed to dosing at a level of .001-0.5 ppb TCDD in feed for 65 weeks (Van Miller and Allen, 1977). Hemoglobin and mean corpuscular hemoglobin levels were decreased in mice given 25 ug/kg/week of TCDD for 6 weeks, while leukocyte, lymphocyte, and erythrocyte counts were the same as controls (Vos et al., 1974).

Hemorrhages were observed in animals that were treated with high levels of TCDD. Monkeys fed 500 ppt TCDD in their diets for 9 months were found to have hemorrhages in many organs (Allen et al., 1977). Hemorrhages which proved lethal in 4 weeks were observed in the gastrointestinal tract of rats fed diets containing 0.05-1 ppm TCDD (Van Miller and Allen, 1977).

7.6 STRUCTURE AND FUNCTION OF LYMPHATIC TISSUES

7.6.1 2,4-D and 2,4,5-T

Highman et al. (1976a) have described changes in the structure of lymphatic tissues in mice that were administered 2,4,5-T (TCDD content less than .05 ppm). These changes involved atrophy of the thymus and spleen and hypocellularity of the bone marrow and lymph nodes. They resemble the changes that are usually seen in mice after TCDD treatment, but not after 2,4,5-T treatment.

7.6.2 TCDD

7.6.2.1 Structure of Lymphatic Tissues

Atrophy of lymphoid structures, especially the thymus and spleen, has been observed after subacute doses of TCDD were administered to various species. Lymph node atrophy, with loss of distinct germinal centers and sparse lymphocytes, was observed in monkeys fed lethal doses of TCDD (500 ppt in feed for 9 months) (Allen et al., 1977). At necropsy, the spleens of horses exposed to TCDD in salvage oil were found to be reduced to one-third the normal size, and most lymph nodes were small and inactive (Case and Coffmann, 1973). The thymuses of these horses and monkeys were not described in these reports. Lymphoid depletion of the thymus and other lymphoid tissues resulted from doses to rats of 0.1 ug/kg/day for 13 weeks. These changes were more severe in rats given 1.0 ug/kg/day for 13 weeks. The cortical region of the thymus was involuted from a decrease in the number of cortical thymocytes (Kociba et al., 1976). In mice that received 1 ug/kg/week of TCDD for 6 weeks, significant reduction of absolute thymus weights and thymus-to-body weight ratios were observed. At higher doses the reduction was more severe, and at 25 ug/kg/week, mean thymus weights were 20 percent of controls after 2 weeks. Microscopic examination indicated that the cortex of the thymus was depleted (Vos et al., 1974).

7.6.2.2 Immune Function

The effects of TCDD on immune function have been the subject of several reviews (Luster and Faith, 1979; Luster et al., 1979; Vos, 1977; 1978). In the guinea pig, TCDD suppressed cell-mediated immunity determined by measuring delayed hypersensitivity to tubercutin. Humoral immunity, determined by measuring the levels of antibodies produced against tetanus toxoid, was only slightly effected. TCDD did not produce an indirect immunosuppression by stimulating adrenocortical activity. In the mouse, cell-mediated immunity, determined by measuring graft versus host activity of donor spleen cells, was suppressed. In the rat however, no cell-mediated immunosuppression (based on delayed hypersensitivity response to tuberculin) occurred (Vos et al., 1973). TCDD at oral doses of 0.01 ug/kg/week for 2 weeks, stimulated splenic lymphocyte transformation; this effect reversed in a short period of time (Sharma and Gehring, 1979).

Doses of TCDD that produced no other clinical or pathological effects have been able to decrease the capacity for hose defense in mice, following salmonella infection; defense to pseudo-rabies virus was not altered, however (Thigpen et al., 1975; 1977). Hinsdall et al., (1979) also reported immunosuppression from low doses of TCDD in the ppb range. Vos et al., (1978) reported that the susceptibility to salmonella was the result of increased sensitivity to bacterial endotoxin in TCDD-treated animals. Thymic alrophy by TCDD in mice was not blocked by administering thymosin and serum zinc levels were not depressed in TCDD-treated mice. TCDD treatment also did not impair non-specific killing by macrophages or specific killing of Listeria (Vos et al., 1978).

7.7 RENAL EFFECTS

In some reports of subacute toxicity of 2,4-D and 2,4,5-T, renal lesions have been mentioned (see tables 7-1 and 7-2). Effects of 2,4,5-T on renal function were observed after acute administration to rats. However, the effects on inhibition of renal organic anion transport were not observed 24 hours after subacute doses of 2,4,5-T were administered and the authors concluded that chronic doses of 2,4,5-T were unlikely to produce a cumulative effect on renal function (Stroo et al., 1979). Histological changes of the kidney from subacute exposure to phenoxy acids have been described in the chicken (Bjorklund and Erne), but the relevance of these lesions to those observed in mammalian species has not yet been established.

7.8 CARDIOVASCULAR EFFECTS

Gangrenous necrosis of fingers and toes and extensive edema and ascites were observed in monkeys that were fed 500 ppt TCDD in their diets for 9 months. These lesions may have been manifestations of inadequate blood flow and changes in vascular permeability. Other cardiovascular problems that were observed in these animals included bilateral ventricular dilatation and cardiac enlargement (Allen et al., 1977). Cardiovascular disorders were also observed in monkeys that consumed lethal amounts of toxic fat. These disorders included blood vessel degeneration and cardiac edema (Allen and Carstens, 1967). The extensive number and severity of lesions in the animals from both studies indicates that some of the lesions, especially cardiovascular lesions that are not commonly observed after TCDD treatment, were produced by other compounds that these animals were administered, as mentioned previously in this chapter.

7.9 SUMMARY AND CONCLUSION

2,4-D and 2,4,5-T are not cumulative toxicants. Subacute doses of 2,4-D of at least 100 mg/kg/day or 1,000 ppm in feed are required to elicit toxicity. Symptoms produced by both compounds are nonspecific. Myotonia is not produced by subacute doses of 2,4-D, while bleeding of the gums has been noted in dogs. Other symptoms for 2,4-D and 2,4,5-T resemble those of acute toxicity. The phenoxy acids (in the absence of TCDD) also produced hepatotoxicity, including necrosis, hepatitis, and increased liver-to-body weight ratios.

Doses of TCDD administered within a month of each other show cumulative effects; doses administered more than 1 month apart do not. Monkeys show higher sensitivity to repeated doses of TCDD than to the cumulative dose when it is given in a single administration. Subchronic doses of TCDD have caused porphyria and lethal depletion of blood cells. Other symptoms of subacute toxicity resemble those observed after acute exposure. The cause of death from poisoning by the phenoxy acids or TCDD is usually not apparent. Dermal and thymic effects of TCDD after subacute exposure resembled those seen after acute exposure.

TCDD produced degenerative changes in the liver, which varied widely in intensity among the species studied. At doses producing no other effects, increased liver-to-body weight ratios were observed. Porphyria was observed in the mouse and the rat, although iron deficiency protects animals from this effect of TCDD.

All three compounds produced reduction in body weights, although the mechanisms involved are unknown for any of the compounds. All three compounds also produced hemorrhaging at high doses.

Data on the subacute and chronic toxicities of the remaining compounds are sparse and for some compounds nonexistent. Chronic exposure to diquat produces cataracts. Picloram in doses of 225 mg/kg/day (3,000 ppm in the diet) for 90 days produces only mild effects, including hepatic and liver changes. Daily doses of 1,000 mg/kg of dalapon also produced local or mild nonspecific effects.

CHAPTER 7.

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CHAPTER 8

REPRODUCTIVE TOXICITY

This chapter describes the effects of herbicide and TCDD exposures on reproductive parameters. Studies that used 2,4,5-T preparations known to contain less than 1 ppm TCDD are described together. Effects produced by these preparations have been attributed to 2,4,5-T. Studies that used 2,4,5-T preparations with unknown amounts of TCDD or that were known to have more than 1 ppm TCDD are described in a separate section, as the observed effects may have been caused by TCDD, 2,4,5-T, or both, in either an additive or synergistic manner.

The effects of each herbicide on animals that grazed on sprayed pastures, resulting in ingestion of an unknown quantity of herbicide, are mentioned at the end of the section. Data from avian species are also mentioned only briefly, since their route of exposure and fetal isolation from maternal sources of metabolism render them to be inadequate models for mammalian teratogenicity. Aspects of reproductive effects of phenoxy acids and TCDD have been reviewed recently (Anonymous, 1980; Allen et al., 1979; Greig, 1979; Tschirley and Melvin, 1979; Gehring and Betso, 1978; Joint IARC/NIEHS Working Group, 1978; Nelson and Holson, 1978; Young et al., 1978; IARC, 1977; Wilson, 1977; Epstein, 1973; Johnson, 1971; Robson, 1970).

8.1 2,4-D

One study evaluated the relationship between human male exposure to 2,4-D and the incidence of spontaneous abortions in their wives. Questionnaires pertaining to these two factors were mailed to almost 15,000 people who were employed in occupations involving the manufacture or use of herbicides. From the respondents, a group of 134 cases of miscarriages were selected and compared to a control group of 311 cases of live births. The cases were analyzed to determine the incidence of paternal exposure to 2,4-D prior to conception, and the incidence of other relevant confounding factors, such as smoking, illnesses, and drug use. The data were obtained or verified by telephone interviews. No positive association was established between 2,4-D exposure and abortions, for the entire study population. For a group of 21 cases of wives of young forest and commercial workers, an association was suggested, but was not found for any other subgroup. Further investigations were suggested to clarify whether this relationship resulted from biases in study design or reflected a real increase among this small subset (SRI International, 1981).

The effects of 2,4-D administration during gestation have been studied in the rat, mouse, and hamster (see table 8-1). In the rat, 2,4-D at high doses produced a decrease in fetal weight in four separate studies. Malformations of the skeletal system, which were not severe or incompatible with life, were also reported in one of these studies (Khera and McKinley, 1972). In the second study, abnormal cranial development was noted after treatment with the butyl ester, but not the sodium salt, of 2,4-D (Buslovich et al., 1976). No

TABLE 8-1: REFERENCES ON THE REPRODUCTIVE EFFECTS OF 2,4-D IN MAMMALS

Species	Route	Daily Dose	Exposure Period[a]	Maternal Toxicity	Fetal Death	Decreased Growth	Malformations			Reference
							Gross	Visceral	Skeletal	
Rat (SD)	oral	8.7mg/kg	6-15	-	-	+	-	-	-	Schwetz et al., 1971
(Wistar)	oral	100mg/kg	6-15		-	+	-	-	+	Khera and McKinley, 1972
(Osborne-Mendel)	gavage	1500ppm[b]	all	-	-	+				
		($\frac{1}{2}LD_{50}$)	4-13[c]	-	+	+		cranial		Buslovich et al., 1976
Mouse	oral oral, sc	1mM/kg	12-15	+	-	+	cp[d]			Courtney, 1977 Bionetics, 1968[e]
Hamster	oral	100mg/kg	6-10		+	-	-			Collins and Williams, 1971

[a] days of gestation

[b] level of 2,4-D added to the diet

[c] on only one of these days

[d] CP, cleft palate

[e] various strains of mice, routes and doses were used; effects were reported for some treatment groups

Code: (+) a deleterious effect in this parameter was observed;
(-) this indicated parameter was analyzed, but no effect was observed;
( ) this indicated parameter was not analyzed;

malformations were observed in the third and fourth studies (Schwetz et al., 1971; Hansen et al., 1971). In the study by Hansen, three consecutive generations of rats were continuously fed diets of 100, 500, or 1,500 ppm 2,4-D; in the highest group, decreased survival of neonates to 21 days of age was observed, although litter sizes were not decreased. A fifth study also reported embryotropic effects after 2,4-D administration, but neither the conditions of administration nor the effects were described (Konstantinova et al., 1978).

In the mouse a small increase in the incidence of cleft palate and a decrease in fetal growth were caused by 2,4-D, at a dose that also produced increased maternal liver-to-body weight ratios (Courtney, 1977). A study by Bionetics (1968), found variable effects of 2,4-D in the mouse, depending on the chemical form administered, the route, the strain of mouse, and the dose and parameters studied. In the hamster, a small increase in fetal death was observed after 2,4-D administration, although this effect was not dose-related, and no malformations or effect on fetal growth occurred at doses of up to 100 mg 2,4-D/kg (Williams and Collins, 1971). These mammalian studies have shown that 2,4-D is capable of affecting fetal growth, but does not affect fetal survival and does not reproducibly cause malformations.

Cattle and sheep have had deleterious reproductive effects after grazing pastures that were recently sprayed with 2,4-D (Sadykov et al., 1972; Bodai et al., 1974). Adverse teratological effects were observed in eggs that were treated with 2,4-D, although other lots of 2,4-D failed to reproduce these effects (Dunache and Fletcher, 1967; Whitehead and Pettigrew, 1972).

In sum, the primary observed effect of 2,4-D was a decreased rate of fetal growth for exposed gestating mice. No link has been drawn conclusively between exposure of men to 2,4-D and either their fertility or the rate of spontaneous abortion in their wives.

8.2 2,4,5-T

In this section only studies of 2,4,5-T preparations with less than 1 ppm TCDD contamination are discussed. Studies of 2,4,5-T with higher levels of TCDD and studies that used 2,4,5-T which were not analyzed for TCDD are described in section 8.4. None of the cases of suspected reproductive effects in humans from alleged exposure to 2,4,5-T involved situations in which concomitant TCDD exposure could be eliminated, so they are all discussed in section 8.4 as well. All reproductive studies of only male exposure to 2,4,5-T similarly involved 2,4,5-T preparations of unknown levels of TCDD contamination and are described in section 8.4. One mouse study involved exposure of males to 2,4-D, 2,4,5-T, and TCDD simultaneously. One group of mice was exposed to the phenoxy acids in the presence of 0.16 ppm TCDD, and no reproductive or teratogenic effects were observed (Lamb et al., 1980). The results from this entire study are discussed in section 8.4.

Evidence that 2,4,5-T reaches the fetus after it is administered maternally has been produced in the mouse and guinea pig. Lindquist and Ullberg (1971) demonstrated by whole-body autoradiography that 2,4,5-T administered

late in gestation is distributed evenly among fetal tissues and to the same levels as in maternal blood. 2,4,5-T was concentrated in the yolk-sac epithelium after administration either early or late in gestation. Dencker (1976) confirmed that 2,4,5-T uptake by the fetal mouse occurred only late in gestation (after day 12). No uptake of 2,4,5-T administered prior to day 12 was observed in the fetal hamster, and uptake in the hamster at later times was not studied. In the mouse, fetal concentrations of 2,4,5-T were highest eight hours after an oral dose was administered maternally on day 13 of gestation, although distribution to the palate could not be demonstrated (Courtney et al., 1977). In contrast, in the guinea pig, the maternal rate of 2,4,5-T excretion was so high that 2,4,5-T did not reach the fetus (Ebror and Courtney, 1976).

All of the studies of the reproductive effects of 2,4,5-T (without significant TCDD contamination) involved administration only during gestation. Five mammalian species have been used for these studies: the monkey, rat, mouse, rabbit, and hamster. References on these experiments are listed in table 8-2.

2,4,5-T did not produce any malformations in rhesus monkeys that were administered up to 10 mg/kg doses during gestation (Dougherty et al., 1973; Dougherty et al., 1975; Dougherty et al., 1976). No maternal toxicity was observed in this experiment. The rate of fetal death, including abortions and stillborns, was 20 percent for groups of 10 monkeys given 10 mg/kg and 1 mg/kg doses, and 10 percent for the control group. This difference is probably not significant, because the number of animals per group was small (10 per group) and a spontaneous abortion rate of 15 percent is usually considered normal for untreated monkeys.

In the rat, 2,4,5-T at doses of 50 mg/kg or less produced no adverse effects on maternal toxicity, or on fetal survival, growth, and development (Emerson et al., 1971; the same experiment was reported in abstracts by Emerson et al., 1970; Thompson et al., 1971; Khera et al., 1971; Sparschu, 1971; Khera and McKinley, 1972). Dilated renal pelvises were observed in 7-45 percent of Wistar rats that received 2,4,5-T, but the incidence was not treatment-related, as the control group also had a high incidence of this defect, with 20-30 percent of fetuses affected (Khera and McKinley, 1972). This defect was observed in mice and rats after TCDD treatment, as well (see section 8.3).

Related doses of 100 mg/kg to Wistar rats during gestation produced increases in fetal deaths, growth retardation, and skeletal abnormalities, but no maternal toxicity (Khera and McKinley, 1972). This dosage produced severe maternal toxicity and fetal death in Sprague-Dawley rats. The incidence of dilated renal pelvis in the few fetuses that survived was two out of the six fetuses examined, compared to 21 percent of the 110 control fetuses that were examined (Sparschu, 1971; also reported in an abstract by Thompson et al., 1971).

Behavioral effects, which were measured as alterations in open-field behavior, were observed in Wistar rats that were exposed to a single dose of 100 mg/kg 2,4,5-T in utero. This dose did not produce any other effects

TABLE 8-2: REFERENCES ON THE REPRODUCTIVE EFFECTS OF 2,4,5-T (less than 1ppm TCDD)

Species	Route	Daily Dose mg/kg	TCDD Level	Exposure Period	Maternal Toxicity	Fetal Death	Decreased Growth	Malformations				Reference
								Gross	Visceral	Skeletal	Behavioral	
Monkey	oral	10	.05	22-38	-	+	-	-	-	-	-	Dougherty et al., 1975
Rat (SD)	oral	24	.5	6-15	-	-	-	-	-	-		Emerson et al., 1971
(SD)	oral	50	.5	6-15	-	-	-		-	-		Sparschu, 1971
(SD)	oral	100	.5	6-10	+	-	-	-	renal	-		Thompson et al., 1971
(Wistar)	oral	50	.5	6-15	-	-	-		-	-		Khera & McKinley, 1972
(Wistar)	oral	100	.5	6-15	-	+	+		-	+		Khera & McKinley, 1972
(Wistar)	oral	100	1	7,8,9		+		-	-		+	Sjoden & Soderberg, 1972
(Wistar)	oral	100	1	8,9,10							+	Sjoden & Soderberg, 1975
Mouse (NMRI)	oral	35	.1	6-15	-	-	+	cp		+		Roll, 1971
(NMRI)	sc	50	1	6-14	-	+	-	cp	-	-		Sage et al., 1973
(NMRI)		60	.05	6-15	-	+	+	cp				Neubert & Dillman, 1972
(NMRI)	oral	80	.1	6-15	+	+	-	+				Frohberg et al., 1975
(NMRI)	inhal	216 mg/m	.1	6-15	+	+		+				Frohberg et al., 1975
(CD-1)	oral	60	.05	6-14				cp	renal			Higham et al., 1977
(CD-1)	oral	100	.5	6-15	+	+	+			+		Beck, 1981
(CD-1)	oral	1mM/kg	.1	12-15	+	+	+	cp				Courtney, 1977
(CD-1)	oral	250-300	.01	7-15[a]		+	+	exencephaly short muzzle	-	-		Hood et al., 1979
Hamster	oral	100	.5	6-10		+	+	exencephaly abnormal eyes	GI hem.	+		Collins & Williams, 1971
Rabbit	oral	40	.5	6-18	-	-	-	-	-	-		Emerson et al., 1971
Sheep	oral	100	1	14-36		-		-				Binns and Balls

[a] dose given for 3 consecutive days between d 7-15; other mice were administered 800-900 mg/kg on a single day between d. 8 and 15.

(although visceral and skeletal examinations were not mentioned). The significance of these transient behavioral defects in relation to effects in man has not been demonstrated. The authors suggested that these effects resulted from an effect of 2,4,5-T on the thyroid but did not attempt to demonstrate any thyroid deficiency in these animals (Sjoden and Soderberg, 1972, and 1975).

2,4,5-T produces cleft palate in NMRl and CD-1 mice. This effect was produced by doses of 35 mg/kg, administered during days 6-15 of gestation, which did not produce obvious maternal toxicity (Roll, 1971). Decreased fetal growth occurred in groups given lower doses (20 mg/kg), while higher doses (60-90 mg/kg) produced maternal toxicity and fetal deaths. Skeletal malformations (table 8-2) involved decreased rates of ossification of the skeleton and were observed along with decreased fetal body weights. In another experiment in NMRI mice, higher doses of 2,4,5-T than the 10 mg/kg dose that caused retarded fetal growth (20 mg/kg) were also needed to cause an increase in cleft palate; neither dose produced maternal toxicity (Neubert and Dillman, 1972). Subcutaneous and inhalation exposures to 2,4,5-T during gestation also produced cleft palate in NMRI mice (Bage et al., 1973; Frohberg et al., 1975).

In CD-1 mice, exposure to 2,4,5-T on day 11 of gestation was the most likely day to result in cleft palate, while exposure on days 14-15 were the most likely days to cause decreased fetal growth (Hood et al., 1979). Since days 11 and 14-15 are closest to the days when the palate closes and rapid growth occurs, respectively, these results are in accord with the rapid distribution and clearance of 2,4,5-T. In the CD-1 mouse, the isobutyl and isooctyl esters were found to be equivalent in producing cleft palate, fetal growth retardation, and fetal deaths. Maternal weights were not altered at oral doses (100 mM/kg) that caused these adverse fetal effects, but the ratio of maternal liver-to-body weights was elevated (Courtney, 1977). Since most teratology studies evaluated maternal body weights but not maternal liver weights, this subtle maternal effect was usually missed.

Few malformations other than cleft palate were observed in mice after 2,4,5-T treatment. Beck (1981) reported that mice exposed to 2,4,5-T in utero showed a high incidence of skeletal anomalies in adulthood. Seventeen of 88 traits examined were more frequently encountered in rats treated with 100 mg 2,4,5-T/kg before birth than in controls. However, this dose produced high maternal toxicity, and a lower dose of 20 mg/kg did not result in skeletal anomalies in adulthood. Highman et al. (1977) observed a delay in renal development in CD-1 mice following administration of 60 mg 2,4,5-T/kg. This dose also produced cleft palate, which was not observed in the control group.

Doses of 100 mg 2,4,5-T/kg administered to hamsters on days 6-10 of gestation were embryotoxic, and malformations and decreased fetal weights were observed in the surviving offspring. The conditions of the mothers were not described. In the hamster, malformations involved abnormal development of the head, but not the cleft palate which was observed in the mouse (Collins and Williams, 1971). However, 2,4,5-T administration after day 10 in gestation may be required to provide an effective dose at the palate just prior to closing (which is complete on day 12 in the hamster). In the rabbit, the highest dose tested (40 mg/kg) was ineffective in altering fetal growth or

development (Emerson et al., 1971). These mammalian studies indicate that 2,4,5-T is teratogenic, producing cleft palate in several strains of mice at doses that elicit no maternal toxicity (or only a subtle decrease in liver-to-body weight ratios). 2,4,5-T causes fetal growth retardation at even lower doses. The effects were not observed in any other species.

Studies of 2,4,5-T effects on chicken development have shown an effect on behavioral development (Sanderson and Rogers, 1981), but not on other parameters of structural development of eggs sprayed with 2,4,5-T (Somers et al., 1978a; 1978b; 1978c,), although decreased viability was observed in eggs that were immersed in 2,4,5-T (Gyrd-Hansen and Dalgaard-Mikelsson, 1974). Cardiovascular abnormalities were observed in fish, following exposure of eggs to 2,4,5-T (Schreiveirs and Murray, 1976).

Significant effects of mammalian exposure to 2,4,5-T included increased incidence of cleft palate in mice at doses that caused no maternal toxicity, and decreases in fetal growth at lower doses. Teratogenicity and other embryotoxic or lethal effects were not observed in other species studied.

8.3 TCDD

In this section, studies of the reproductive effects of TCDD are described. Human exposure to TCDD resulted from industrial accidents and occupational exposures, which have been described in chapter 5. Only reproductive data from these incidents are described in this section. The reproductive toxicity of TCDD has been studied in several laboratory species, as described below.

8.3.1 Human Exposure

Evaluation of reproductive effects of TCDD have been made after TCDD exposure of male workers in Czechoslovakia and after TCDD exposures of residents near Seveso. The reproductive performance and frequency of abortions among wives of the Czechoslovakian workers were reported to be normal (Pazderova-Vejlupkova et al., 1980), although no systematic study of reproductive parameters among these workers or expected values of parameters for a matched control group were reported.

The reproductive parameters of the residents near Seveso were never systematically studied, either. The total number of abortions were reported, but they were from zones A, B, and R, and the expected or actual incidence of abortions for each exposure group was not presented. Paternal exposure and the estimated length of exposure prior to mating were not described. An estimated 150 women in the area near Seveso were in the first trimester of pregnancy when the explosion occurred. Most of the 125 abortions were elected, after the mothers became aware of the potential for having a child with birth defects. This knowledge alone, and not necessarily exposure to TCDD, would be likely to cause a large decrease in the birth rate and increase in the rate of abortions. No study design has taken these factors into account.

Information on the teratology of 34 fetuses that were spontaneously or therapeutically aborted by women in the Seveso area was published by Rehder et al. (1978). (The remaining abortions were not officially approved and had to be performed elsewhere.) Only three of the 34 mothers were reported to be residents of zone A where they would be likely to have received a relatively high exposure to TCDD. Five were from zone B, 13 from zone R, and nine from outside the contaminated areas. The report by Rehder et al. states that the ages of the fetuses were 4-20 weeks and that they were removed within 2 1/2 months of the accident. There is no way to know how many cases involved exposure which occurred only after conception or before and after conception, and whether one or both parents were exposed prior to conception.

Although no structural abnormalities attributable to TCDD exposure were reported, the authors (Rehder et al., 1978) indicated that many of the fetuses were mutilated from the procedures used to perform the abortions. The authors did not state how many fetuses were suitable, based on age and condition, for evaluation of cleft palate, renal abnormalities, or other suspected malformations. Therefore, the teratologic data from Seveso do not provide enough information to know whether the fetuses from mothers that were exposed to TCDD were old enough and adequately preserved to detect any specific birth defects. Unless all of the known aborted fetuses (numbering 125) were examined, it seems unlikely that enough well-preserved fetuses from parents exposed to TCDD (during organogenesis, 3-8 weeks after conception) could be examined to identify any nonspontaneous malformations. Three of the four mothers who experienced spontaneous abortions were from zone R. The report did not indicate the time period between the Seveso accident and the spontaneous abortions and found no evidence for or against any causal relationship.

8.3.2 Animal Exposure Prior to Mating

An unpublished report by Townsend et al., (1981) described a study conducted in workers at Dow Chemical Company who were involved in manufacturing processes that could potentially have resulted in their exposure to TCDD. These employees were identified by company records and their wives were interviewed to determine incidences of various reproductive parameters. A control group of wives of workers who were not likely to have been exposed to TCDD or other dioxins was also interviewed. No increases in the incidences of spontaneous abortions, stillbirths, infant deaths, or congenital malformations were identified in wives of the exposed group. The exposed group included pregnancies that occurred at any time after the father was exposed. Only male exposures that occurred near the time of conception are relevant to the outcome of a particular pregnancy (based on the male reproductive cycle). Thus, data limited to cases of male exposure close to conception would have provided more relevant information, although the study group used is more analogous to American veterans, who may have been exposed to dioxins many years before they were ready to become fathers.

One animal study of reproductive effects from male exposure to TCDD prior to mating has been reported (Murray et al., 1979). Male rats that were fed 0.01 ug/kg body weight per day for 1 year were mated with younger, untreated

females. Sixty percent of the 20 females became pregnant, with an average of 10 implants per female and a 9 percent rate of resorption of implants. Male rats that were fed a control diet for 1 year were mated with untreated, younger females; 58 percent of the females became pregnant with nine implants per female and a 10 percent resorption rate. Matings between untreated young male and female rats resulted in a 90 percent pregnancy rate, and matings of male and females fed control diets for one year produced a 30 percent pregnancy rate. These results indicate that the older rats showed diminished fertility, precluding any evaluation of the effects of TCDD on reproduction in these rats.

In another study, male mice were exposed to a combination of 2,4-D, 2,4,5-T, and TCDD (0.16-2.4 ug/kg) prior to mating, and no adverse reproductive effects were observed (see section 8.3).

Other studies have considered the effects of exposure of both males and females to TCDD prior to mating. In a three-generation reproductive study in rats, TCDD exposure started 90 days prior to mating and was maintained for three generations. Decreased fertility, increased numbers of stillbirths and neonatal deaths, and smaller litter sizes resulted from exposure to 0.1 or 0.01 ug TCDD/kg/day while exposure to .001 ug/kg/day produced no effects. Malformations were not studied. The litter sizes of beach mice that were progeny of an estimated 50 generations of male and female mice that lived in TCDD-contaminated sand were the same as those of control beach mice (VA, 1981).

Alterations in the male reproductive system by TCDD have been described. Induction of aryl hydrocarbon hydroxylase activity and of cytochrome P-448 has been shown in rat testicular and prostate tissue (Lee and Dixon, 1978) and decreased catabolism of testosterone after TCDD treatment has been demonstrated in the rat (Nienstedt et al., 1979). The effects of these alterations on male reproductive potential or on the DNA of germ cells have not yet been demonstrated.

Structural changes in male reproductive organs have been observed in the guinea pig and mouse, but were observed only in cases of lethal poisoning for both species. In the guinea pig, the changes involved reduction in the size of the testicles and loss of seminiferous components, while in the mice necrotic spermatocytes and reduced spermatogenesis occurred (McConnell et al., 1978).

The effects of female exposure to TCDD prior to mating have been studied in the monkey and the mouse. Monkeys were fed diets with 500 ppt TCDD for 6 months prior to mating and during gestation. This dosage resulted in substantial maternal toxicity, including altered steroid hormone levels consistent with reduced fertility. A decreased number of conceptions and an increased rate of abortions were observed after mating. No gross malformations were mentioned in the aborted fetuses or in the few offspring (Barsotti et al., 1979). Female mice fed diets containing 5 ppb TCDD for 4 weeks prior to mating, during gestation, and after birth did not produce maternal toxicity, reduced fertility, fetal deaths, or decreased fetal growth. After 3 to 4 weeks of nursing, facial alopecia, periorbital edema, decreased spleen

size, and immune suppression were observed in the offspring (Thomas and Hinsdill, 1979). These changes are consistent with toxicity observed in adult mice and suggest a higher suseptibility of newborns to TCDD toxicity, compared to adults.

8.3.3 Animal Exposure During Gestation Only

The remaining studies of the reproductive toxicity of TCDD all involve administration of TCDD to females after conception has taken place. These studies are listed in table 8-3. Maternal toxicity was observed in monkeys that received a total of 1 ug TCDD/kg between days 20-40 of gestation. This dosage also produced cleft palate and an increased incidence of abortions. Only brief reports of these studies have been published (Zingeser, 1979; Colby, 1978).

TCDD has been shown to reach the fetus after it was orally administered to pregnant rats on day 14, 18 or 21 of gestation (Moore et al., 1976). Teratologic effects of rats were reported in one study. A high frequency of kidney abnormalities was observed in fetuses of CD rats given 0.5 ug/kg/day of TCDD subcutaneously on days 6-15 of gestation. No maternal toxicity, or fetal death or decreased growth occurred at this dosage (Courtney and Moore, 1971). In another study, a lower dose (0.125 ug/kg/day) administered to rats orally during day 6-15 caused decreased fetal growth and signs of toxicity in the fetuses, but no malformations or increased fetal death (Sparschu et al., 1971; Sparschu et al., 1970). The remaining rat studies describe toxic effects in fetuses that are not considered teratogenic. The studies of immune function included TCDD exposure on day 18 of gestation and after birth. The effects observed in these rats were the same as in other rats that were exposed only postnatally, suggesting that prenatal exposure was not necessary for the observed decrease in cellular immunity (Faith and Moore, 1977; Faith et al., 1978; Faith and Luster, 1979; Vos and Moore, 1974). Few teratogenic effects would be expected from exposure on day 18, when most developmental processes and all organogenesis are complete.

Teratology experiments in the mouse indicate that TCDD produces cleft palate and renal abnormalities after oral or subcutaneous administration on days 6-15 of gestation. Cleft palate was produced by daily doses of only 1 ug TCDD/kg a dose which produced no evidence of maternal toxicity, or fetal death or growth retardation (Smith et al., 1976). However, the incidence of cleft palate was low at this dose and a higher dose of 3 ug/kg/day not only increased the frequency of cleft palate, but also caused an increase in fetal death and evidence of maternal toxicity (Courtney and Moore, 1971; Neubert and Dillman, 1972; Smith et al., 1976). Cleft palate was also produced in strains of mice that have been shown to respond to the enzyme inductive effects of TCDD and to have a high capacity for TCDD-receptor binding, but was not produced in other strains that did not show induction and binding (Poland and Glover, 1980).

Renal abnormalities have also been noted in mouse fetuses following TCDD exposure. These changes have been described as hydronephrosis, and resemble a slowed rate of renal development. Some evidence indicates that these changes

TABLE 8-3: REFERENCES ON THE REPRODUCTIVE EFFECTS OF TCDD EXPOSURE TO FEMALES

Species	Route	Dose ug/kg/d	Days of Exposure	Maternal Toxicity	Fetal Death	Decreased Growth	Malformations			Reference
							Gross	Visceral	Skeletal	
Man			1-140		-		-	-		Rehder et al., 1978
Monkey	oral	1 ug/kg total	20-40	+	+					Colby, 1978
	oral	1 ug/kg	20-40				cp			Zingeser, 1979
	gavage	500ppt[a]	pre, all	+	-		-			Barsotti et al., 1979
Rat	oral	.125	6-15	-	-	+	-	(GI;edema)[b]	-	Sparschu et al., 1971
(SD)	s.c.	.5	6-15	-	-	-	-	renal		Courtney & Moore, 1971
			13-15				-	(hepatic)[c]	-	Becker, 1973
		5	11, 18		+	+		(spleen)[d]		Vos & Moore, 1974
		5	18, post			+		(spleen)[d]		Faith & Luster, 1979
		5	18, post			+		(spleen)[d]		Faith & Moore, 1977
Mouse (C57Bl/6)	oral	1	10-13				-	renal		Moore et al., 1973
(C57Bl/6)	oral	3	10-13				cp	renal		Moore et al., 1973
(CF-1)	oral	1	6-15	-	+	-	cp	renal		Smith et al., 1976
(CF-1)	oral	3	6-15	-	-	-	cp	-	-	Smith et al.,1976
(C57Bl/6)	s.c.	3	6-15	+	-	-	cp	renal (GI)[e]		Courtney & Moore, 1971
(DBA/2J)	s.c.	3	6-15	+	-	-	cp	renal (GI)[e]		Courtney & Moore, 1971
(CD-1)	s.c.	3	6-15	-	-	-	cp	renal (GI)[e]		Courtney & Moore, 1971
(NMRI)	oral	3	6-15		-	-	cp			Neubert & Dillman, 1972
(NMRI)	oral	9	9-13		-	-	cp			Neubert & Dillman, 1972
(NMRI)	oral	9	6-15		+	-	cp			Neubert & Dillman, 1972
(CD-1)	oral	25	7-16	+	-	-	-	renal		Courtney, 1976
(CD-1)	s.c.	25	7-16	+	+	-	cp, club-foot	renal		Courtney, 1976
	oral		14, 17, post			+		(spleen)[d]		Vos & Moore, 1974
	gavage	5ppb[a]	pre, all, post				(facial)[f]	(spleen)[d]		Thomas & Hinsdall, 1979

a Level in feed
b GI hemarrhages; subcutaneous edema
c fatty degeneration
d decreased size of spleen and decreased cellular immunity
e GI hemarrhages
f facial alopecia; periorbital edema

are transient, as renal abnormalities do not persist in young mice who have had no exposure to TCDD after birth (Moore et al., 1972). Both cleft palate and renal abnormalities were produced in every strain of mouse that has been studied for these changes (see table 8-4).

TCDD administered as toxic fat or as the isolated chemical in acetone, caused fetal deaths in chicken eggs. Structural malformations were not examined (Higginbotham et al., 1968; Allred and Strange, 1977).

TCDD

Reproductive or teratogenic effects of TCDD exposure in humans have not been substantiated, in part due to inadequate study designs. Chronic exposure of three generations of male and female rats has shown adverse reproductive effects that include low fertility; this may be the result of using animals with low fertility prior to exposure, or to doses that produced severe maternal toxicity. Reproducible teratologic effects in maternally exposed mice were primarily cleft palate, with some incidence of renal abnormalities.

8.4 COMBINATIONS OF 2,4-D, 2,4,5-T, AND TCDD

In this section, the effects of 2,4,5-T with unknown levels of TCDD contamination and 2,4,5-T samples known to have at least 1 ppm TCDD are discussed. Studies of other combinations of 2,4-D, 2,4,5-T and TCDD are also included in this section.

8.4.1 Human Exposure

Several instances of birth defects, abortions, and impotence in the human population have been purported to be associated with herbicide use. No quantitative assessments of herbicide exposure of the individuals involved has been made in any of the cases. Except for the cases of male impotence described below, either or both parents may have been exposed to herbicides, although the exposure periods, in relation to the time of conception, are unknown, or in some cases chronic exposure is assumed. Although 2,4,5-T, and in some cases its TCDD contaminant as well, have been the suspected teratogenic agents, concomitant exposures to many other substances were likely in each instance. In fact, unusually high exposure to 2,4,5-T of parents of affected children has never been demonstrated. In summary, all of the human cases described below involve reproduction abnormalities which retrospectively have been suggested to be caused by exposure to herbicides.

Cutting et al. (1970) conducted a study of the incidences of abnormal births among the Vietnamese population relative to the extent of herbicide spraying during the same period of time. During the period of low military use of herbicides, between 1961 and 1965, the incidences of stillbirths, hydatidiform moles (spontaneous abortion of resorbed placental tissue), and malformations were 36.1, 6.6, and 5.5 per 1,000 live births, respectively.

TABLE 8-4: REFERENCES ON THE REPRODUCTIVE EFFECTS OF 2,4,5-T (with unknown levels of TCDD)

Species	Route	Daily Dose (mg/kg)	Exposure Period	Maternal Toxicity	Fetal Death	Decreased Growth	Malformations			Reference
							Gross	Visceral	Skeletal	
Monkey	oral	10(3/wk)	20-48		-	+	-		±	Wilson, 1972
Rat	oral	0.1	all	+	+	-			-	Konstantinova, 1974
(SD)	oral	4.6	10-15	-	+	-		renal (gi)[c]		Bionetics, 1968
	oral	10[a]	10-15		+			renal (gi)[c]		Courtney et al., 1970
(SD)	iu	12-24	12-16		-		cp			King et al., 1973
	gavage	1000ppm[b]	all	+	-	+				Hall, 1972
Mouse (C57B1/b)	oral	46.4		+	-	-	-	renal		Bionetics, 1968
(C57B1/b)	oral	113[a]	6-14		+		cp	renal		Courtney et al., 1970
	oral	113[a]	9-17	+	-	+	cp	renal		Courtney et al., 1970
(AKR)	oral	113[a]	6-15	+	+		cp	-	-	Courtney et al., 1970
(NMRI)	oral	60[d]	6-15		-	+	cp			Neubert & Dillman, 1972
(CDI)	sc	10	6-15				-	-	-	Hart & Valerio, 1972
CD-1,AJAX,C3H C57B1/b, BALB	gavage	30	6-14				cp			Gaines et al., 1974
(CD-1)	gavage	60	6-14					renal		Highman et al., 1976
(CD-1)	oral	100		+	-	+	cp	renal		Courtney and Moore, 1971
(CD-1)	oral	100	all					(enzymes)		Courtney, 1979
Hamster	IV	2	8				-			Gale and Ferm, 1973

[a] 30 ppm TCDD

[b] level in feed

[c] hemorrhages in GI tract

[d] 5 ppm TCDD = 0.3 ug/kg body weight

During the period of high military use of herbicides, between 1965 and 1969, all of these rates fell, to 32.0, 5.6, and 4.5 per 1,000 live births, respectively. Despite difficulties in data collection and biases based on the sampling procedures, these results do not support the premise that herbicide use is correlated with elevated rates of fetal death or malformations in the exposed population.

Tung et al. (1980) reported that birth defects were frequent among Vietnamese children whose fathers had been soldiers in South Vietnam. However, exposure specifically to herbicides was never established; neither experimental nor control groups were defined clearly (even in terms of military service) and other relevant factors, such as maternal health (or exposure), family predisposition, or drug use, were not considered in the study.

A consultative council was appointed in Australia to determine whether a series of premature births and malformations in the Yarram district of Australia was higher than expected and whether these abnormal events could be attributed to exposure to 2,4-D or 2,4,5-T (Allred et al., 1978). Statistical analysis of these reported incidences of birth defects, which included three defects in neural tube development (two cases of spina bifida and one of anencephalus) and one case of renal agenesis, revealed that their rates per total number of births were not significantly different from the incidences in other regions of Australia which have lower usage of 2,4-D and 2,4,5-T. Other abnormal births were eliminated from the study because the mothers had not resided in the Yarram district prior to the births. Specific exposure to herbicides could not be established in any of the eight cases that were brought to the attention of the committee. The committee also presented estimates of the amount of contaminated food or water consumption required in a pregnant woman to reach the lowest dose of 2,4-D, 2,4,5-T, or TCDD that was teratogenic in animals (assuming contamination at the highest reported levels). These values ranged from 15,000-900,000 liters of water per day or 6,000-90,000 kg of meat per day.

Another study of the incidence of neural tube defects among births in Australia, compared with 2,4,5-T usage during the previous year, showed a positive correlation between the two parameters. In the brief report of these data, the authors avoided drawing a causal relationship between these two factors (Field and Kerr, 1979).

Cases of neural tube defects in New Zealand were also suggested to be caused by exposure to 2,4,5-T (Sare and Forbes, 1972). The New Zealand Department of Health reviewed a total of 20 cases of neural tube defects that were referred to them (McQueen et al., 1977). The Department was unable to demonstrate exposure to 2,4,5-T prior to neural tube closure in most of the cases or even a higher than expected incidence of these birth defects for the three regions where these cases occurred. In some cases, familial predisposition to these types of birth defects was established, although in most cases the etiology of the malformations could not be identified. Another study that compared the incidence of various birth defects in New Zealand with the rate of 2,4,5-T spraying found no association between 2,4,5-T usage and malformations in general, or neural tube defects, cleft palate, or other

specific defects in particular. The only exception was a correlation between 2,4,5-T usage and talipes (malformations of the foot), a malformation that has not been associated with 2,4,5-T or TCDD administration in other studies (Hanify et al., 1981).

Two studies were conducted in the U.S. to evaluate the relationship between herbicides spraying and reproductive events. The incidence of spontaneous abortions over a 6-year period in Alsea, Oregon, was found to be higher than the rates in two other regions of Oregon that had lower rates of 2,4,5-T usage (EPA, 1979). A cyclic pattern in the incidence of abortions in the Alsea group correlated positively with the pattern of annual 2,4,5-T spray usage. In another study, however, the incidence of cleft palate over a period of 32 years in Arkansas was not related to the usage of 2,4,5-T. The incidence of cleft palate for regions with a low proportion of rice acreage (indicating low exposure to 2,4,5-T) and for regions with high 2,4,5-T exposure increased over the years studied. This trend was attributed to better detection of cleft palate and not to herbicide usage (Nelson et al., 1979).

Several cases of male impotence were identified among farm workers in England (Espir et al., 1970). Four out of a crew of five male workers experienced symptoms of impotence, which in each case began in April or August of 1967. No other symptoms were present in any of the men. The authors of this report were unable to identify the cause of these cases of impotence. All men were treated with methyltestosterone and terminated exposure to the chemicals they had been using in their work, which included 2,4-D as well as other phenoxy acids and various other pesticides. Symptoms cleared within 2 to 3 months in three cases and in 1 year in the fourth case. The authors suggested that exposure to pesticides caused the symptoms in these men, but they were unable to substantiate this claim with evidence from any other study of impotence as a sole symptom of exposure to any pesticide.

8.4.2 Animal Exposure Prior to Mating

The exposure situation relevant to a consideration of reproductive effects of herbicides in Vietnam on American veterans is the situation in which exposure is limited to male exposure. Animal studies of male exposure to herbicides are considered in this section.

8.4.2.1 2,4,5-T With Known or Possible TCDD Contamination

Buselmaier et al. (1972) studied the effect of male exposure to 2,4,5-T on reproductive parameters in the mouse (NMRI strain). Mice were administered 100 mg 2,4,5-T/kg of intraperitoneally at 10 weeks of age. They were then mated with unexposed females over the subsequent 6 weeks. Females were examined for evidence of pregnancy and, on day 14 of gestation, for numbers of fetuses, implantation sites, and corpora lutea. No increase in losses prior to or after implantation were observed in mice that were mated with 2,4,5-T-exposed males, compared to mice mated with unexposed males.

Changes in testosterone metabolism have been observed in 2,4,5-T-treated mice. Corresponding alterations in reproductive function have not been tested, however. Treated mice showed decreased accumulation of testosterone by the prostate gland, although hepatic metabolism of testosterone and androgenic responses of the testes and accessory sex organs were not altered (Lloyd et al., 1973; Thomas, 1974).

8.4.2.2 Combinations of 2,4-D and 2,4,5-T

Lamb et al., (1980) exposed male C57BL/6 mice to feed with various combinations of 2,4-D, 2,4,5-T, and TCDD for 8 weeks. The levels of these components resulted in daily doses of 40 mg 2,4-D/kg, 40 mg 2,4,5-T/kg, and 2.4 ug TCDD/kg to one group; 40 mg/kg of each phenoxy acid and 0.16 ug TCDD/kg to the second group; and 20 mg/kg of each phenoxy acid and 1.2 ug TCDD/kg to the third group. The levels of 2,4-D, 2,4,5-T, and TCDD were not periodically analyzed to confirm the concentrations being administered. When these mice were mated with unexposed females, mating frequency, fertility, fetal deaths, and malformations were found to be the same as for matings of male mice administered diets with vehicle only. Toxicity in the treated males, which involved decreased thymus and body weights and increased liver weights, was temporary, with recovery after termination of exposure. Sperm concentration, motility, and abnormalities were not adversely affected by treatment. This study protocol is relevant to the question of potential for reproductive risk from military use of these compounds. The negative result leaves open the possibility that the doses were too low to be effective through male exposure or that other species might be more sensitive to these compounds after male exposure than this strain of mouse (which is sensitive to the teratogenic effects).

8.4.3 Animal Exposure During Gestation Only

The majority of reproductive studies of herbicides and TCDD in animals involved exposure only of the female after conception. This protocol is not relevant to an assessment of reproductive effects in American servicemen in Vietnam. The findings in the studies in this section have been used to design a number of epidemiological studies in which the incidence of cleft palate and of spontaneous abortions were examined in human populations.

8.4.3.1 2,4,5-T with Known or Possible TCDD Contamination

Fetuses of monkeys that were treated with 10 mg/kg of 2,4,5-T three times per week between days 20 and 48 of gestation were smaller than control fetuses. Delayed ossification and small bones were noted upon examining their skeletons (Wilson, 1972). At doses of 40 mg/kg one of five monkeys aborted, compared to none of the five control monkeys. However, the 20 percent rate of abortion is probably not significantly higher than the normal abortion rate of about 10-15 percent given the small sample size. No malformations were observed in the 2,4,5-T-treated groups.

In the rat, 2,4,5-T at low doses (4.6-10 mg/kg) produced cystic kidney and intestinal hemorrhage (Courtney et al., 1970; Bionetics, 1968). Only one study reported that 2,4,5-T produced cleft palate in the rat, but the incidence was very low (9 of 2,231 fetuses) and was produced by intrauterine administration of 2,4,5-T (King et al., 1973). In other studies, 2,4,5-T administered throughout gestation produced maternal toxicity and either fetal deaths or decreased fetal growth. In both experiments, lower doses produced no maternal or fetal effects (Konstantinova, 1974; Hall, 1972).

In the mouse, 2,4,5-T produced cleft palate and cystic kidney. Two studies reported that both malformations occurred in C57BL/6 mice from doses of 113 mg/kg, while a dose of 46 mg/kg produced cystic kidney, but not cleft palate. In AKR mice, doses of 113 mg/kg produced cleft palate without cystic kidney (Bionetics, 1968; Courtney et al., 1970). In another study that used large quantities of animals, cleft palate incidence was elevated by 2,4,5-T doses of only 30 mg/kg to C57BL/6, BALB, C3H, and CD-1 mice on days 6-14, and of 15 mg/kg to A/JAX mice (Gaines et al., 1974). A lower dose of 10 mg/kg administered subcutaneously to CD-1 mice, however, was ineffective (Hart and Valerio, 1972).

Combinations of 100 mg 2,4,5-T/kg and 1 ug TCDD/kg produced higher incidences of cleft palate and cystic kidney than either compound alone, but the effect was less than additive (Courtney and Moore, 1971). Neubert and Dillman (1972) found that TCDD levels of 1.5 ppm or less (0.1 ug/kg) were inadequate to potentiate the effect of cleft palate caused by doses of 60 mg 2,4,5-T/kg while higher TCDD doses of 0.3 ug/kg (5 ppm) did cause potentiation. In the hamster, the incidence of malformations, which involved abnormal cranial development, increased with increasing levels of TCDD.

Doses of 80 mg 2,4,5-T/kg, with TCDD levels of 0, 0.5, and 45 ppm, caused 0, 40, and 100 percent, respectively, of the exposed litters to be abnormal. The protocol did not permit an evaluation of potentiation between the two compounds (Collins and Williams, 1971). Cleft palate was rarely encountered in 2,4,5-T-treated hamsters in this or in another study by Gale and Ferm (1973) in which only a single, low dose of 2,4,5-T was administered. In avian species, 2,4,5-T has been shown to be embryolethal, but not teratogenic (Lutz and Lutz-Ostertag, 1973; Strange et al., 1976; Strange and Kerr, 1976).

8.4.3.2 Combinations of 2,4-D and 2,4,5-T

Mixtures of 2,4-D and 2,4,5-T have been administered to pregnant mice. Herbicide Orange, comprised of the butyl esters of 2,4-D and 2,4,5-T, was administered to CD-1 mice at a dose of 1 mM/kg on days 12-15 of gestation. This combination (which had 0.4 ppm TCDD) produced a lower incidence of cleft palate than 1.0 mM/kg doses of either compound alone. All three preparations produced maternal toxicity and decreased fetal weights to the same extent while only 2,4,5-T produced fetal mortality (Courtney, 1977). Another study found that a 2:1 mixture of 2,4-D:2,4,5-T produced almost as high an incidence of cleft palate and dilated renal pelvis, and the same effect of fetal weight and fetal mortality, as the same dose of 2,4,5-T alone (Bage et al., 1973). Another study, which was described briefly in an abstract, indicated that

2,4,5-T, whether administered alone or combined with 2,4-D, produced a very low incidence of cleft palate (9 of 2,231 fetuses) in the rat (King et al., 1973).

8.5 DIQUAT

The reproductive effects of male exposure to diquat have been studied. Anderson et al. (1976) administered 10 mg diquat/kg orally for 5 days to male CD-1 mice. This dosage produced no alterations in fertility, or in pre- or post-implantation deaths. A higher dose corresponding to the LD_{50} was given as a single intraperitoneal dose to male Swiss-Webster mice (Pasi et al., 1974). These mice were then mated with new females weekly for 8 weeks. This treatment did not affect pre- or post-implantation deaths, but caused a reduction in fertility. The temporal pattern of this antifertility effect indicated that spermatogenesis was disrupted in premeiotic early and late spermatocytes and in all postmeiotic maturation.

An increased incidence of fetal deaths has been observed in rats, following diquat administration during gestation. Single intravenous doses of 15 mg diquat/kg were administered on a single day, between day 7-21 of gestation (Bus et al., 1975). This dose produced 20 percent maternal mortality and 57 percent fetal mortality. Distribution of radioactivity to the fetus was demonstrated after ^{14}C-diquat was administered intravenously on days 13, 16, or 21 of gestation. In a brief abstract, Khera and Whitta (1968) reported skeletal and total fetal growth retardation after a dose of 7 mg diquat/kg was administered on a single dose between day 6 and 14 of gestation. These effects were more severe, and were accompanied by increased fetal mortality at a dose of 14 mg/kg.

8.6 PICLORAM

Administration of doses of 500, 750, or 1,000 mg picloram/kg/day on days 8-17 of gestation did not cause adverse effects on litter size, fetal deaths, or fetal weights. The two highest doses produced maternal deaths and retardation of skeletal development in fetuses (Thompson et al., 1972). Picloram, at levels up to 3,000 ppm in the diet, was also reported to cause no adverse effects on fertility, fetal survival, fetal growth, structural development, or lactation in a three-generation study. Exposure to a diet with 0.01 percent picloram for 4 days prior to and 14 days after mating also had no effect on fertility. These experiments were not described further and whether one or both parents were treated prior to mating was not indicated (McCollister and Leng, 1969).

8.7 DALAPON

Administration of 30-300 mg/kg/day doses of dalapon by gavage for 110 days to each of three consecutive generations of rats produced no effect on fertility, or on fetal or offspring survival or growth (Paynter et al., 1960). Doses of 250-2,000 mg/kg/day were administered to pregnant rats on

days 6 to 15 of gestation. Maternal toxicity was produced at 1,500-2,000 mg/kg and reduced fetal viability was also observed. No effects, including gross malformations, were detected at doses of 1,000 mg/kg or less (Thompson et al., 1971, cited in Kenaga, 1974). In another study, doses of 500-1,500 mg/kg/day were administered on days 6-15 to rats (Emerson et al., 1971, cited in Kenaga, 1974). At doses of 1,000 mg/kg or above, maternal and fetal weights were depressed, but no visceral or significant skeletal malformations were observed in any group.

8.8 BROMACIL

A three-generation study in rats showed no adverse reproductive effects in any generation from chronic feeding of diets containing 250 ppm bromacil (Sherman and Kaplan, 1975). Fertility, survival of fetuses and offspring, and growth were unaffected in the exposed group. No gross, visceral, or skeletal malformations were observed in fetuses. In a brief abstract, bromacil, included in the diets (level not indicated) of male mice for 7 weeks, produced no adverse effects on the fertility of these mice during the subsequent 8 weeks (Jorgensen et al., 1976).

8.9 DIURON

Doses of 135-500 mg diuron/kg/day administered to rats on days 6-15 of gestation did not alter fetal viability, but caused a signficant decrease in fetal weight. The incidence of pregnancy was decreased from 19 to 20 in controls to between 14 and 15 of 20 rats given either 250 or 500 mg/kg doses. Both doses caused reductions in maternal weights, although this change was significant only at the higher dose. Skeletal abnormalities of questionable significance were observed at these doses, but no gross or visceral malformations were observed (Khera et al., 1979).

8.10 CACODYLIC ACID

The effects of 1 and 10 ppt cacodylate on development of isolated fetal mice were investigated. Eighty-six percent of embryos exposed to the higher dose failed to undergo normal cell divisions, while neither dose produced a statistically significant reduction in DNA division, based on radioactive precursor incorporation (Williams et al., 1979). The validity of this novel system for testing teratogens awaits the results of in vivo tests to determine the reproducibility of these effects.

8.11 SUMMARY AND CONCLUSIONS

2,4-D has been studied for reproductive effects in man and in the rat, mouse, hamster, and in grazing animals and avian species. Industrial exposure in man was not associated with an increased rate of spontaneous abortions in the wives of the entire study group but in one subset of workers an increased risk was implicated. In other mammalian species, exposure was limited to the

female during gestation, and the only effect of 2,4-D was a decreased rate of fetal growth. In mammalian studies administration of 2,4,5-T with less than 1 ppm TCDD was also limited to pregnant females. This exposure produced cleft palate in the mouse at doses that produced no significant maternal toxicity; a decrease in fetal growth was produced in mice at lower doses of 2,4,5-T than those causing cleft palate. Teratogenicity or other embryotoxic or lethal effects were not observed in the other mammalian species studied (monkey, rat, hamster, and rabbit).

No evidence of reproductive or teratogenic effects has been observed after human exposure to TCDD. However, systematic testing of these parameters, in a manner that could evaluate these effects in a significant number of people with documented exposures, has not been carried out.

In a three-generation reproductive study, chronic exposure of male and female rats to 0.1 or 0.01 ug TCDD/kg/day resulted in decreased fertility. Female mice fed 5 ppb TCDD in feed before and during gestation showed normal fertility and gave birth to normal offspring. Other experiments of TCDD exposure prior to mating either evaluated reproductive structures but not fertility, used animals with low fertility prior to exposure, or used doses that produced severe maternal toxicity.

The only reproducible effects of TCDD administration during gestation were teratologic effects in the mouse. Cleft palate was observed in several strains of mice, and renal abnormalities that may be transient were also consistently produced.

Cases of reproductive abnormalities in the human population have never been shown convincingly to correlate with usage of herbicide mixtures or of 2,4,5-T with possible TCDD contamination. One possible exception involved an increased incidence of abortions in Oregon at times and places where 2,4,5-T usage was high. However, this study did not establish 2,4,5-T exposure of the individual who had abortions and did not investigate other factors that may have predisposed this group to an increased incidence of abortions. These factors were evaluated in the Australian and New Zealand cases of birth defects; exposure during the relevant time in gestation could not be established in most of these cases, while familial predisposition and other potential causes of birth defects were identified.

Exposure of males only to 2,4,5-T with possible TCDD contamination alone or in combination with 2,4-D and known amounts of TCDD, has not been shown to produce antifertility effects.

In the mouse, combinations of 2,4-D and 2,4,5-T, or 2,4,5-T and TCDD were not additive or synergistic in producing cleft palate, except possibly for 2,4,5-T doses above 60 mg/kg with TCDD levels above 5 ppm.

Diquat exposure of males or pregnant females produced some adverse reproductive effects, but the doses causing these effects were also highly toxic to adult animals. Dalapon exposure to pregnant rats produced fetal deaths and reduced fetal growth, and diuron caused reduced growth, but only at doses that elicited maternal toxicity as well. Bromacil and picloram produced no significant reproductive effects at any dose.

CHAPTER 8

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CHAPTER 9

MUTAGENICITY

This chapter discusses the cytogenetic and mutagenic effects of Vietnam herbicides in humans, mammals, and in vitro test systems. Major information gaps exist in the published literature on this subject. Only 2,4-D, 2,4,5-T, and TCDD have been studied for induction of aberrations in human or mammalian chromosomes. Only short-term in vitro testing has been performed on other Vietnam herbicides. The situation is further complicated by inconsistencies within and between test systems. Table 9-1 summarizes the results of short-term tests of Vietnam herbicides.

Table 9-1. SUMMARY OF SHORT-TERM TEST RESULTS

COMPOUND	Human Chromosome Effects	Mammalian Chromosome Effects	Host Mediated Assay	Recessive Lethal-Drosophila	Bacterial Systems	Yeast Systems
2,4-D	1/1 [a]	1/3	0/1	1/2	0/4 [b]	4/4
2,4,5-T	1/1	2/3	0/2	2/3	0/2	1/1
TCDD	0/1	1/2	N	N	3/3	2/2
Picloram	N	N	N	N	2/5	0/6
Cacodylic Acid	N	N	N	N	0/2	0/1
Diquat	N	N	N	N	3/5	2/2
Monuron	N	N	N	N	0/4	0/1
Diuron	N	N	N	N	N	0/3
Dalapon	N	N	N	N	0/3	0/6
Bromacil	N	N	N	N	0/3	0/1

a = number of studies positive/number of studies performed
b = assayed at pH 4.5
N = no information

9.1 HUMAN CYTOGENETIC STUDIES

The information available on the effects of herbicides on human chromosomes is very limited. Table 9-2 lists the available studies concerning the clastogenic effects associated with 2,4-D, 2,4,5-T, TCDD, and herbicide mixtures. Five studies examined the effects of compounds under controlled

Table 9-2: CYTOGENETIC EFFECTS OF HERBICIDES ON HUMAN CELLS

COMPOUND	TEST SYSTEM	END POINT	RESULTS	REFERENCE	COMMENTS
2,4-D	Human peripheral lymphocytes	Chromosome aberrations	Increase in chromosome aberrations	Pilinskaya et al. (1974)	2,4-D added to cells in culture at 0.002-50,000 ug/ml
2,4-D	Human lymphocytes	Chromosome aberrations	Negative	Wild (1975)	Cited by Seiler (1978[a])
2,4-D dimethylamine salt	Human embryonic fibroblasts	Chromosome aberrations	Increase in chromosome aberrations	Berin et al. (1973)	Cited by Seiler (1978[a])
2,4,5-T	Human spleen fibroblasts	Chromosome aberrations	Not reported	Babitt et al. (1973)	Occupational exposure
	Human lymphocytes	Chromosome aberrations	Not reported		
2,4,5-T (0.09 ppm TCDD)	Human peripheral leukocytes	Chromosome aberrations	Increase in chromosome aberrations	Fujita et al. (1975)	2,4,5-T (0.09 ppm TCDD) added to cells in culture at 10^{-7}M to 10^{-4}
TCDD	Human peripheral lymphocytes	Chromosome aberrations	Negative	Tenchini et al. (1979)	Industrial accident - Seveso, Italy
	Cultured human fetal tissue	Chromosome aberrations	Negative		Industrial accident - Seveso, Italy
Herbicide mixtures[a]	Human peripheral lymphocytes	Sister chromatid exchanges	Negative[b]	Crossen et al. (1978)	Occupational exposure study; 3 individuals using no protection had elevated SCE's
Herbicide mixtures[c]	Human peripheral lymphocytes	Chromosome aberrations	Increase in chromosome aberrations during *spraying season*	Yoder et al. (1973)	Occupational exposure

[a] Includes 2,4-D, 2,4,5-T, bromacil, diquat

[b] Overall exposed versus unexposed

[c] Includes 2,4-D, 2,4,5-T, diquat, picloram

conditions. Four other studies describe the clastogenic effects associated with accidental or occupational exposure to herbicides. Since these latter studies could not document the amount and type of herbicide exposure, they are of limited value in determining the clastogenic effects of the herbicides.

The limited data available suggests that both 2,4-D and 2,4,5-T have the potential to induce clastogenetic effects in human cells in vitro. Whether these compounds will react similarly in vivo cannot be determined from the available data. TCDD has not been conclusively associated with any clastogenic effects in humans. Although studies which evaluated the effects of occupational exposure to a variety of herbicide sprays were generally unconsistent, there appears to be a disturbing trend in the data suggestive of a potential human genetic hazard.

9.1.1 2,4-D and 2,4,5-T

In a review by Seiler (1978), it was noted that 2,4-D has been associated with both negative (Wild, 1975) and positive (Pilinskaya et al., 1974) results in human lymphocyte culture. Pilinskaya et al. (1974) demonstrated that 2,4-D concentrations of 0.02 and 0.2 ug/ml induced statistically significant increases in chromosome damage. Cytotoxicity was observed at 2,4-D doses of 2.0 ug/ml and above while no chromosome damage was detected at 0.002 ug/ml. Chromatid-type aberrations prevailed over chromosome-type aberrations in a ratio of 4:1. The authors concluded that 2,4-D was a potential genetic hazard to humans. According to Seiler (1978), 2,4-D dimethylamine salt (0.4 mg/ml) has also been demonstrated (Berin et al., 1973) to induce an increased frequency of chromosome aberrations in human embryonic fibroblasts.

It appears that 2,4-D has the potential to induce chromosome damage in human cells in vitro (Pilinskaya, 1974; Seiler, 1978). However, these results do not indicate that 2,4-D is a potential genetic hazard in vivo.

Fujita et al. (1975) described the effects of 2,4,5-T on human chromosomes. The herbicide appeared to induce increasing single chromatid breaks with concentrations of 2,4,5-T ranging from 10^{-7} to 10^{-4} M. Since the preparation used in the study contained 0.09 ppm TCDD as a contaminant, the authors noted that it was not clear whether the 2,4,5-T, the contaminating TCDD, or the combination of both was responsible for the results. However, if the 2,4,5-T presently in existence is always accompanied by TCDD, it may be irrelevant which of these possibilities is responsible for the results.

Babitt et al., (1973) reported a study on the effects of 2,4,5-T on chromosomes of two human cell lines, spleen fibroblasts and lymphocytes. No experimental results were described in this abstract and no subsequent reports were published by these authors.

9.1.2 TCDD

Reggiani (1979) and others (Homberger et al., not date; Reggiani, 1980; and Pocchiari et al., 1979) described chromosome analyses performed on individuals exposed to TCDD in the Seveso accident and in ICMESA plant

workers. Peripheral lymphocytes were examined from persons receiving acute and chronic exposure to TCDD at the ICMESA plant, and from Seveso children with and without chloracne. No increases in chromosome abnormalities were observed in the sample population. The frequency of gaps and breaks was reported to be within the normal range.

Effects on human chromosomes from accidental exposure to TCDD was also described by Tenchini et al. (1979), and results are in agreement with Reggiani (1979) and others. The authors analyzed peripheral blood samples and aborted fetal tissues from women who were exposed to TCDD during the Seveso accident. No increase in chromosome aberrations were observed in the peripheral blood samples. In fetal tissues, large numbers of chromosome aberrations were observed. However, no unexposed tissues were included in the experiment to control for the effects of culturing these tissues in a laboratory. It is possible that culturing conditions were responsible for the large numbers of chromosome aberrations observed. The effect of TCDD on fetal chromosomes is therefore unclear.

Based on these studies, there is no indication that accidental exposure to TCDD is associated with detectable clastogenic effects in the peripheral lymphocytes. Although fetal tissues appear to be more susceptible to chromosome aberrations following the accidental exposure to TCDD, the evidence is inconclusive.

9.1.3 Herbicide Spray Mixtures

Yoder et al. (1973) performed chromosomal analyses on lymphocytes from persons occupationally exposed to herbicides and insecticides, including 2,4-D, 2,4,5-T, diquat, and picloram. No documentation of type and quantity of pesticide exposure was available. Lymphocyte chromosomes of 26 herbicide-exposed individuals and 16 unexposed controls were analyzed during the peak spraying season and during the midwinter off-season. The herbicide-exposed group had approximately twice the number of chromatid gaps and four times the number of chromatid breaks compared to controls. Surprisingly, during the midwinter sampling the herbicide-exposed group had about one-half the number of chromatid gaps and one-fourth the number of chromatid breaks compared to the unexposed group. The exposed group had nearly four times as many chromatid gaps per person and 25 times as many chromatid breaks per person during the peak spraying season as compared to the off-season sampling. The authors speculated that enhanced chromosome repair may be taking place at this time as compensatory protection. No statistical analysis of the results was presented and the ranges of chromatid gaps as well as chromatid breaks overlapped in all groups tested. The data presented do not permit any conclusions about effects specifically associated with the herbicides of interest.

Another study on occupational exposure to herbicides and pesticides was described by Crossen et al. (1978). Documentation of type and quantity of chemical exposure was not possible. However, the chemical exposures most commonly encountered included 2,4-D, 2,4,5-T, bromacil, and diquat. Peripheral lymphocytes from 57 pesticide and herbicide sprayers were analyzed for sister chromatid exchanges (SCE). When the means of SCE in control and exposed subjects were compared, no statistically significant differences

between the two groups were observed. However, five sprayers had a SCE rate three standard deviations outside the mean of the controls. Upon further analysis, it was noted that a high SCE rate seemed to be associated with lack of protection during spraying in those individuals who had been spraying for longer than one year. When the exposed group was divided into categories of no protective clothing, some protective clothing, and full protection, the group with no protection was statistically different than the unexposed group. Similarly, when the exposed group was divided into exposure categories of one year or less of spraying exposure or greater than one year of exposure, the group with greater than one year's exposure had a tendency toward elevated SCE rate ($p=0.5$) compared to controls. This observation supports the concept that the increased frequency of SCE was associated with herbicide exposures rather than the pesticide exposures. The authors concluded that there is a genetic hazard arising from the careless use of herbicides and pesticides. No difference in SCE rates were observed between the groups that sprayed only herbicide and the group that sprayed both herbicide and insecticide. Although there appears to be a genetic hazard associated with the careless use of herbicides, the hazard associated with any individual herbicide or herbicide combination cannot be evaluated with this study.

Tung et al. (1971) described a survey of chromosome aberrations in Vietnamese people exposed to herbicide spraying. Peripheral leukocytes were cultured from three experimental groups:

- Group 1: six individuals affected with asthenia who lived two to three years in sprayed areas
- Group 2: five normal North Vietnamese
- Group 3: five normal South Vietnamese.

The type and extent of herbicide exposure that was received by Group 1 was not determined or approximated by the authors. In Group 1 the rate of chromosome aberrations was observed to be 5.88 percent, while in control Groups 2 and 3 only 1.14 percent chromosome aberrations were observed. Because each subject was examined for "hematologic, metabolic, medicinal, and radiological" factors in order to exclude other causes of chromosome aberrations, the authors concluded that the increased incidence of chromosome damage was due to herbicide spraying. However, sample size in this experiment was very limited and herbicide exposure was not documented; therefore, no firm conclusions can be made regarding the clastogenic effects of herbicide spraying on the Vietnamese people. The authors also examined chromosomes of children with congenital malformations whose mothers had been exposed to herbicide spraying. All the individuals examined had high rates of chromosome aberrations. This, however, is not sufficient evidence to link herbicide spraying with induction of chromosome aberrations.

None of these studies provide information on the effects associated with exposure to a specific herbicide or herbicide combination. Although each study associates general herbicide exposure with human chromosome aberration, the evidence presented is inconclusive. However, the trend in the data

indicates that occupational herbicide spray exposure may present a potential genetic hazard to humans.

9.1.4 Other Vietnam Herbicides

No information was found in the available literature concerning the effects of other Vietnam herbicides on human chromosomes.

9.2 CYTOGENETIC AND HOST-MEDIATED STUDIES IN MAMMALS

Investigations of the cytogenetic effects of herbicides on mammals are sparse and the results are inconsistent. Table 9-3 lists the cytogenetic and host-mediated studies in mammals. No information was available in the literature describing the mutagenic effects of the other Vietnam herbicides other than 2,4-D, 2,4,5-T and TCDD in mammals. Without additional substantiating data, no firm conclusions regarding the clastogenetic potential of 2,4-D, 2,4,5-T, or TCDD are appropriate.

9.2.1 2,4-D and 2,4,5-T

Pilinskaya (1974) described the cytogenetic effects of 2,4-D in mouse bone marrow cells. Groups of six mice received a single oral dose of 10, 50, 100, or 300 mg of 2,4-D per kg body weight. Twenty hours later the animals were killed and bone marrow cells were examined for chromosome aberrations. Statistically significant increased frequencies of chromosome aberrations were observed at 100 and 300 mg/kg. These doses also caused pronounced symptoms of intoxication in the animals. The authors concluded that 2,4-D was weakly mutagenic.

Jenssen and Renberg (1976) tested both 2,4-D and 2,4,5-T (<1ppm TCDD) in a micronucleus test in mice. The micronucleus test measures the chromosome damaging ability of a substance. Male CBA mice received a single 100 mg/kg intraperitoneal injection of either 2,4-D or 2,4,5-T. Animals were killed 24 hours or 7 days later and bone marrow cells were analyzed for numbers of polychromatic erythrocytes with micronuclei. No increase in polychromatic erythrocytes with micronuclei was observed in animals treated with 2,4-D or 2,4,5-T after either 24 hours or seven days. However, a weak toxic effect on mitotic activity was observed in treated animals. Both compounds appeared in peripheral blood and bone marrow within four hours after injection. 2,4-D and 2,4,5-T levels in blood plasma declined by 24 hours after injection. No more than 5 percent of the plasma levels of phenoxy acid were found in cell fractions. Because the 2,4-D and 2,4,5-T do not enter the target cells to an appreciable extent, the authors concluded that results of the test did not reliably indicate a lack of mutagenicity. However, since the data were in agreement with the known rapid excretion of these compounds, the authors also concluded that no cytogenetic hazard is connected with 2,4-D and 2,4,5-T.

In an abstract, Bongso and Basrur (1973) also reported that 2,4-D did not increase chromosome aberrations in bovine peripheral blood cells. However, embryonic bovine kidney cells exposed to 2,4-D for longer than 48 hours showed

Table 9-3: SUMMARY OF CYTOGENETIC AND MUTAGENIC EFFECTS OF HERBICIDES ON MAMMALS

COMPOUND	TEST SYSTEM	END POINT	RESULTS	REFERENCE	COMMENTS
2,4-D	Mouse bone marrow cells	Chromosome aberrations	Increase in chromosome aberrations	Pilinskaya (1974)	Oral doses of 100 & 300 mg/kg caused intoxication and chromosome aberration; doses of 10 & 50 mg/kg had no effect.
2,4-D	Micronucleus test--mice	Induction of micronuclei	Negative	Jenssen and Renberg (1976)	Intraperitoneal injection of 100 mg/kg
2,4-D	Bovine peripheral blood cells	Chromosome aberrations	Negative	Bongso and Basrur (1973)	Abstract; no data
2,4-D	Host mediated assay--rats	Induction of S. typhimurium revertants	Negative	Styles (1973)	Mouse serum + S. typhimurium; abstract; no data
2,4,5-T(<1ppm TCDD)	Micronucleus test--mice	Induction of micronuclei	Negative		
2,4,5-T	Mongolian gerbil bone marrow cells	Chromosome aberrations	Increase in chromosome aberrations	Majumdar and Hall (1973)	Clastogenic effects at $\geq$ 250 mg/kg; results disputed (Ramel, 1978)
2,4,5-T butoxy ethyl ester (commercial preparation, Hormoslyr 500-r) (TCDD<0.1 ppm)	Mouse bone marrow cells	Chromosome aberrations	Increase in chromosome aberrrations	Davring and Hultgren (1977)	Emulsifier & solvent in the commercial preparation also caused chromosome aberrations
2,4,5-T and 2,4,5-T n-butyl ester	Host mediated assay--mice	Induction of mutations in S. typhimurium and S. marcescens	Negative	Buselmaier et al. (1972)	
2,4,5-T	Host mediated rats	Induction of S. typhimurium revertants	Negative	Styles (1973)	Mouse serum + S. typhimurium; abstract; no data
TCDD	Rat (male) bone marrow cells	Chromosome aberrations	Negative	Green and Moreland (1975)	
TCDD	Rat (male & female bone marrow cells	Chromosome aberrations	Increase in chromosome aberrations	Green, Moreland, and Sheu (1977)	

an increase in nucleolar size and number, and an increase in multipolar spindles and polyploid mitotic stages. Although the authors speculated that 2,4-D has an effect on spindle protein synthesis, no data were presented.

Davring and Hultgren (1977) tested a commercial 2,4,5-T ester preparation (Hormoslyr 500-T, TCDD <0.1ppm), its components, and 2,4,5-T acid for their ability to induce chromosome aberrations in mice. Animals received either a single intraperitoneal injection or five daily intraperitoneal injections of 2,4,5-T ester preparation, 2,4,5-T acid, the commercial solvent, or the commercial emulsifier. All of the substances tested, including the solvent and the emulsifier, caused increased frequencies of chromosome aberrations. The positive and negative controls were associated with appropriate results. (chromatid gaps, breaks, deficiencies, and fragments).

Majumdar and Hall (1973) investigated the clastogenic effects of 2,4,5-T in Mongolian gerbil bone marrow cells. Male and female animals received daily intraperitoneal injections of 10, 30, 50, 70, or 100 mg of 2,4,5-T (no detectable TCDD) per kg, a total of 50, 150, 250, 350, or 500 mg/kg over the five-day period. According to the authors, chromosome damage (chromatid gaps and breaks) increased significantly at 250 mg/kg total dose. However, Ramel (1978) reported that these results were questioned by Natarajan in a statement in the Swedish Products Control Board:

> "While I cannot dispute the quantitative aspects of the authors' data, their classification of aberrations as well as the types illustrated are very confusing. According to my judgment of the figures, what they call breaks (which incidentally are termed as breaks and fragments in the table, and I cannot say how they differentiate between these two types) are really gaps. The so called deletions in the figures are certainly artifacts due to the spreading of the chromosomes. It is difficult to say as to how many such misjudgments have gone into the making of the table, which shows a very high frequency of abnormal cells in animals treated with high doses."

Styles (1973) tested both 2,4-D and 2,4,5-T in a host mediated assay in rats. Animals received a single oral dose (unspecified) of 2,4-D or 2,4,5-T. Serum samples from these animals were incubated with histidine requiring mutants of S. typhimurium; the frequency of revertants to prototrophy was measured as an indicator of mutagenicity. Neither compound caused an increase in revertant numbers. This result is in agreement with Jenssen and Renberg (1976), who observed a negative result in a micronucleus test in mice. However, Pilinskaya (1974) concluded that 2,4-D was a weak mutagen.

Buselmaier et al. (1972) tested 2,4,5-T and 2,4,5-T n-butyl ester in a host mediated assay in mice using S. typhimurium G46 and S. marcescens a21 Leu as the indicator organisms. NMRI mice were injected subcutaneously with 0.2 ml of the test substance immediately after being injected intraperitoneally with a suspension of the bacterial strains. Three hours later the animals were killed and intraperitoneal fluid collected and plated on selective media for detection of mutant colonies. Neither compound had a mutagenic effect.

The data concerning the clastogenic potential of 2,4-D appear to be conflicting. Chromosome aberrations were observed in murine bone marrow cells following oral dosage as low as 100 mg/kg (Pilinskaya, 1974). However, two studies of clastogenic effects in peripheral blood cells (Jenssen and Renberg, 1976; Bongso and Basrur, 1977) were negative. Whether these negative results represent a difference in cell type sensitivity, differential absorption rates due to route of exposure, the study of an ineffective dose level, or simply unsubstantiated claims (abstract by Bongso and Basrur, 1977) cannot be determined at present. Therefore, the report by Pilinskaya (1974) provides the only data that associate 2,4-D exposure with clastogenic effects. Any firm conclusions regarding the clastogenetic nature of 2,4-D will require additional substantiating data.

While 2,4,5-T butoxyethyl ester appears to induce a clastogenic effect in mouse bone marrow cells (Davring and Hultgren, 1977), there are no undisputed results which indicate that 2,4,5-T has similiar biological activity in vivo. Any firm conclusions regarding the clastogenic potential of 2,4,5-T also require additional substantiating data.

The last mediated assays were included in their section due to the in vivo aspects of these bacterial mutagenicity tests. These assays were universally negative for both 2,4-D and 2,4,5-T. These data support the conclusion that these phenoxy acids lack mutagenic activity in vivo.

9.2.2 TCDD

Green and Moreland (1975) investigated the effects of TCDD on chromosomes of bone marrow cells in male rats. Daily for 5 days, animals received 10 ug TCDD per kg by intubation; 5, 10, or 15 ug/kg intraperitoneally; or 20 ug/kg orally. No increases in chromosome aberrations were observed in any of the treatments. In a followup study, however, Green, Moreland, and Sheu (1977) administered 0.25, 0.5, 1, 2, or 4 ug TCDD/kg body weight by gavage twice a week for 13 weeks. No controls were included on the study. TCDD produced statistically significant increases in the number of cells with chromosome abnormalities at 2 and 4 ug/kg in male rats and at 4 ug/kg in female rats. However, results of both the 2 ug/kg dose level in males and the 4 ug/kg dose in females fell within the normal frequency range of abnormalities (2-3 percent). Only the 4 ug/kg dose in males appeared to be mutagenic. This study indicates that TCDD appears to be a weak clastogen, but substantiating evidence is lacking.

9.2.3 Other Vietnam Herbicides

No information concerning the mutagenic effects of other Vietnam herbicides in mammals was found in the available literature.

9.3 MUTAGENICITY STUDIES IN DROSOPHILA

Several studies have been conducted on 2,4-D and 2,4,5-T in Drosophila. Table 9-4 lists the mutagenicity studies on Drosophila found in the available

Table 9-4: SUMMARY OF MUTAGENIC EFFECTS OF HERBICIDES ON DROSOPHILA MELANOGASTER

COMPOUND	END POINT	RESULTS	REFERENCE
2,4-D	Nondisjunction Chromosome loss Recessive lethal	Negative Negative Positive	Magnusson et al. (1977)
2,4-D	Recessive lethals	Negative	Vogel and Chandler (1974)
2,4-D	Appearance of mutant male flies in genetically stable and genetically unstable strains of flies	Positive, unstable strain; negative, stable strain	Rasmuson and Svahlin (1978)
2,4,5-T	Recessive lethals	Positive	Majumdar and Golia (1974)
2,4,5-T		Negative	
2,4,5-T	Appearance of mutant male flies in genetically stable and genetically unstable strains of flies	Negative, both strains	Rasmuson and Svahlin (1978)
2,4,5-T (TCDD <0.1)	Recessive lethal	Positive	Magnusson et al. (1977)
2,4,5-T butoxyethyl ester, commercial preparation (TCDD < 1 ppm)	Nondisjunction Chromosome loss	Negative Negative	Magnusson, et al. (1977)

literature. Of the two reports of sex-linked recessive lethal studies with 2,4-D in Drosophila, one reported an increase in sex-linked recessive lethals. Of the three sex-linked recessive lethal studies with 2,4,5-T, two reported an increase in sex-linked recessive lethals. Although results among these studies were inconsistent, 2,4-D and 2,4,5-T appear to have the potential inducing mutational events in Drosophila. Substantiating data is necessary before any firm conclusion can be drawn. None of the other herbicides of interest have been reported to be tested in Drosophila.

9.3.1 2,4-D and 2,4,5-T

Magnusson et al. (1977) tested 2,4-D, 2,4,5-T (TCDD<0.1ppm), and a commercial preparation of 2,4,5-T butoxyethyl ester (TCDD<1ppm) for induction of nondisjunction, chromosome loss, and sex-linked recessive lethals in Drosophila melanogaster. In the chromosome loss and nondisjunction tests, males and females were treated during their entire larval period with 250 ppm 2,4,5-T butoxyethyl ester or 100 ppm 2,4-D. No increases in chromosome loss or nondisjunction were induced by 2,4,5-T or 2,4-D. In the recessive lethal test, adult wild type Karsnas 60 strain males were treated with 1,000 ppm 2,4-D or 2,4,5-T containing less than 0.1 ppm TCDD. After two weeks of treatment males were mated to Muller 5 strain females. When F_1 and F_2 results were combined, both 2,4-D and 2,4,5-T induced significant increases ($p<0.01$) in recessive lethals. However, when data from each generation were tabulated separately, only 2,4-D induced a statistically significant increase in recessive lethals in F_2.

Majumdar and Golia (1974) also studied the effect of 2,4,5-T in the sex-linked recessive lethal test in Drosophila. Male Oregon K flies were fed 250 or 1,000 ppm 2,4,5-T for 15 days and then mated to untreated females. The authors reported that 2,4,5-T induced a statistically significant increase in recessive lethals at 1,000 ppm but not at 250 ppm.

Vogel and Chandler (1973), however, obtained negative results with both 2,4-D and 2,4,5-T in a sex-linked recessive lethal test in Drosophila. Adult two-day-old Berlin K males were fed 2,4-D (4.5 or 9.0 mM) or 2,4,5-T (3.6 or 7.2 mM) for three days. Following treatment, the males were mated to two females for three days (Brood 1). Males were mated with new females for an additional three-day-brood (Brood 2) and a four-day-brood (Brood 3). Results of the study were analyzed by the X^2 test with criteria for a positive result being $p<0.01$. Neither 2,4-D nor 2,4,5-T induced increased numbers of recessive lethals compared to controls. However, a decline in fertility in Broods 2 and 3 was observed in flies treated with 7.2 mM 2,4,5-T.

Rasmuson and Svahlin (1978) used a sex-linked genetically unstable strain of Drosphila to test 2,4-D and 2,4,5-T for their ability to induce somatic mutations. The results in the genetically unstable strain were compared with results in a genetically stable wild-type strain. 2,4-D induced mutational events in the genetically unstable strain but not in the stable strain, while 2,4,5-T was negative in both strains.

Based on these studies, both 2,4-D and 2,4,5-T appear to be capable of inducing mutational events in Drosophila. However, additional substantiating data is needed.

9.3.2 TCDD

No information on the mutagenic effects of TCDD in Drosophila was found in the available literature.

9.3.3 Other Vietnam Herbicides

No information on the mutagenic effects of other Vietnam herbicides in Drosophila was found in the available literature.

9.4 MUTAGENICITY IN IN VITRO SYSTEMS

Most of the mutagenicity testing of Vietnam herbicides occurred in short-term in vitro assays. Results of these assays are not clear-cut. The phenoxy acids 2,4-D and 2,4,5-T were demonstrated to be weakly mutagenic in a variety of short-term in vitro test systems. TCDD was associated with mutagenic activity in all the in vitro assays in which it has been tested. However, these data on TCDD are suspect and any firm conclusions concerning TCDD would be inappropriate. Of the other herbicides discussed in this report most gave negative results in a variety of test systems. The exceptions to this are diquat, which was mutagenic in most systems, and monuron, which affects DNA synthesis in mammalian test systems. Tables 9-5 and 9-6 summarize the results of in vitro testing of herbicides.

9.4.1 2,4-D and 2,4,5-T

Zetterberg (1977) reported that 2,4-D induced mitotic gene conversion and mitotic crossing over in Saccharomyces cerevisiae. The herbicide was mutagenic in this organism at pH $\leq$ 4.5 only. At pH 4.6, 2,4-D was not mutagenic. From these results and the pKa of 2,4-D, the authors speculated that yeast cells take up only the undissociated form of 2,4-D, which occurs at pH 4.5 or lower.

Zetterberg et al. (1978) also reported that in a test for reverse mutation in yeast, 2,4-D induced increased numbers of histidine revertants in the RAD18 strain of Saccharomyces cerevisiae. As in the previous study, mutagenicity was observed at pH 4.5 or less. However, 2,4-D was not mutagenic in four strains of Salmonella typhimurium in reverse gene mutation assays (Ames test) at either pH 4.3 or 6.8 (Zetterberg, 1977). Similarly, Anderson et al. (1972) reported that 2,4-D gave negative results in the Ames test, and Nagy et al. (1975) also reported negative results with 2,4-D in a reverse mutation assay in E. coli.

Ahmed et al. (1977) investigated the potential of 2,4-D, diquat, and 11 other pesticides for inducing unscheduled DNA synthesis in human cells in culture. The cell line VA-4, a human fibroblast cell line transformed by the virus SV-40 grown on cover slipps, was used as the cell culture system. Unscheduled DNA synthesis measured by [^{3}H]-TdR incorporation and autoradiographic analysis was determined in cell cultures with and without rat (S9) metabolic activation. Results of the assay were analyzed by a t-test

Table 9-5: ASSAYS OF HERBICIDES ON IN VITRO MUTAGENICITY ASSAYS

REFERENCE	COMPOUND TESTED	TEST SYSTEM	END POINT	RESULT
(1) Anderson et al. (1972)	Bromacil (a) Cacodylic acid (b) 2,4-D (c) Dalapon (d) Diquat (e) Diuron (f) Monuron (g) Picloram (h) 2,4,5-T (i)	S. typhimurium E. coli B + T4 phage E. coli B + phage mutants	$his^- \rightarrow his^+$ reversion to wild type	- a through i - a through i - a through i
(2) Aulicino et al. (1976)	Dalapon (a) Picloram (b)	A. nidulans	$8\text{-aza}^S \rightarrow 8\text{-aza}^r$ non-disjunction Mitotic crossing-over	-a,b -a,b
(3) Bignami et al. (1977)	Dalapon (a) Picloram (b)	A. nidulans	$8aza^S \rightarrow 8\text{-aza}^r$ Non-disjunction Mitotic crossing-over	-a,b -a,b
(4) Bignami & Crebelli (1979)	Diquat	S. typhimurium	$8\text{-aza}^S \rightarrow 8\text{-aza}^r$	+
(5) Carere et al. (1976)	Dalapon (a) Picloram (b)	S. typhimurium S. coelicolor	$his^- \rightarrow his^+$ $strep^S \rightarrow strep^r$	-a,b +b;-a
(6) Carere et al. (1978)	Dalapon-NA (a) Picloram (b)	S. typhimurium S. coelicolor	$his^- \rightarrow his^+$ $strep^S \rightarrow strep^r$	-a,b +b;-a
(7) Hussain et al. (1972)	TCDD	E. coli sd-4 S. typhimurium E. coli K-39	$strep^- \rightarrow strep^+$ $his^- \rightarrow his^+$ Prophage activation	+ + +
(8) Nagy et al. (1975)	2,4-D	E. coli WP2 trp^- (hcr^+ and hcr^-)	$trp^- \rightarrow trp^+$	-
(9) Shirasu et al. (1976)	Bromacil 2,4-D Diuron Monuron 2,4,5-T	B. subtilus	rec assay	- - - - -
(10) Seiler (1973)	TCDD	S. typhimurium	$his^- \rightarrow his^+$	+
(11) Seiler (1979)	Monuron	S. typhimurium	$his^- \rightarrow his^+$	+

Table 9-5: CONTINUED

REFERENCE	COMPOUND TESTED	TEST SYSTEM	END POINT	RESULT
(12) Siebert & Lemperle (1974)	Diquat	S. cerevisiae	Mitotic gene conversion	+
	2,4-D			+
(13) Zetterberg et al. (1977)	2,4-D Na salt	S. cerevisiae	Mitotic gene conversion	+ pH 4.5
		S. typhimurium	Mitotic recombination	+ pH 4.5
			$his^- \rightarrow his^+$	-
(14) Zetterberg et al. (1978)	2,4-D	S. cerevisiae RAD 18	$his^- \rightarrow his^+$	+ pH 4.5
	2,4,5-T			+ pH 4.5
(15) Simmon et al. (1977)	Bromacil (a) Cacodylic acid (b) Monuron (c)	Human fibroblast cells	Induction of unscheduled DNA synthesis	-a,b;+c
		S. typhimurium	his- → his+	-a,b,c
		E. coli WP2	trp- → trp+	-a,b,c
		S. cerevisiae	Mitotic recombination	-a,b,c
(16) Bronzetti et al.	TCDD	S. cerevisiae	Reverse mutation	+
			Mitotic gene conversion	+
(17) Seiler (1978b)	Diuron (a) Monuron (b)	Testicular DNA synthesis-mice	Inhibition of DNA synthesis (DSI test)	+a,b
		S. typhimurium	$his^- \rightarrow his^+$	+a,b
		mouse bone marrow cells	micronucleus test	-a,b
(18) Benigni et al. (1979)	Diquat	S. typhimurium	$his^- \rightarrow his^+$	-
		S. typhimurium	Repair assay	+
		S. typhimurium	$8\text{-aza}^s \rightarrow 8\text{-aza}^r$	+
		A. nidulans	$8\text{-aza}^s \rightarrow 8\text{-aza}^r$	+
		A. nidulans	$meth^+ \rightarrow meth^-$	
		A. nidulans	Induction of recessive lethals	+
(19) Seiler (1979b)	2,4-D (a) 2,4,5-T (b) 2,4,5-T isooctyl ester (c)	Testicular DNA synthesis-mice	Inhibition of DNA synthesis	+a,b,c
(20) Ahmed et al. (1977)	2,4-D	Chinese hamster cells	Induction of gene mutations	+
(21) Ahmed et al. (1977)	2,4-D (a) Diquat (b)	Human fibroblast cells	Induction of unscheduled DNA synthesis	+a,b

Table 9-6: RESULTS OF IN VITRO ASSAYS FOR DETECTING MUTAGENICITY OF HERBICIDES

TEST SYSTEM / COMPOUND	Bacteria Reverse Mutation	Bacteria Forward Mutation	Rec. Assay	Bacteria Repair Assay	Yeast-Gene Mutation	Yeast-Mitotic Gene Conversion	Yeast-Mitotic Crossing Over	Prophage Activation	Mammalian Cells - Gene Mutation	Mammalian Cells Unscheduled DNA Synthesis	Inhibition of Mouse Testicular DNA Synthesis
2,4-D	-1,8,13[a]		-9		+14	+12,13	+13		+20	+20	+19
2,4,5-T	-1		-9		+14						+19
TCDD	+7,10	+7			+16	+16		+7			
Bromacil	-1,15		-9			-15				-15	
Cacodylic Acid	-1,15					-15				-15	
Dalapon	-1,5	-5			-2,3	-2,3	-2,3				
Diquat	-1,18	+4,18		+18	+18	+12				+18,20	
Diuron	-1,17		-9								+17
Monuron	-1,11,15,17		-9			-15				+15	+17
Picloram	-1,5,6	+5,6			-2,3	-2,3	-2,3				

[a] Number designate reference found in table 9-5

comparing the mean number of silver grains in controls versus treated cells. Both diquat- and 2,4-D-treated cultures had statistically significant increases in mean numbers of silver grains at all concentrations tested. No metabolic activation was necessary for this effect. In addition, the authors also measured 2,4-D-induced 313 nm photolysis of BudR containing repaired regions of DNA to determine the form of DNA repair induced. The shape of the curve plotting induced breaks for 2,4-D resembled the shape of curves obtained in similar experiments using ionizing radiation and alkylating agents such as ethyl methane sulfonate. This result suggests a similarity between alkylating agents and 2,4-D in the mechanism of induction of unscheduled DNA synthesis.

In another experiment, Ahmed et al. (1977b) studied the mutagenicity of 2,4-D in an assay for detection of forward mutations in Chinese hamster V79 cells. 2,4-D was tested in triplicate at a concentration of 19 uM, which yielded about 40% survival. The forward mutation frequency of 2,4-D-exposed cells was calculated to be 25.5 per 10^6 survivors, while the mutation frequency of control was 1.8 per 10^6 survivors. The authors concluded that 2,4-D was a weak mutagen in this test system.

Anderson et al. (1972) evaluated the mutagenicity of bromacil, cacodylic acid, 2,4-D, dalapon, diquat, diuron, monuron, picloram, and 2,4,5-T in four in vitro mutagenicity assays in bacteria and viruses. The test systems used were:

- Test 1 - eight strains (unspecified) of Salmonella typhimurium that revert from histidine dependence to histidine independence when exposed to a mutagenic chemical
- Test 2 - T_4 bacteriophage in Escherichia coli B cells that form morphologically distinguishable mutant plaques when exposed to a mutagen
- Tests 3 and 4 - mutants of T_4 bacteriophage, AP 72 and NI7, that revert to the wild type with mutagen treatment.

The S. typhimurium assay was carried out as a "spot test" in which the herbicide to be tested was added as a liquid or as crystals to the surface of the agar plate. No metabolic activation system was used; therefore, only directly acting mutagens could be detected. In bacteriophage assays the test compound was incubated in liquid suspension followed by plating. All of the herbicides tested in the four assays gave negative results.

Nagy et al. (1975) evaluated the mutagenicity of 2,4-D in Escherichia coli WP 2 try. Two bacterial strains which detect reversion from tryptophan-requiring to non-requiring were used. One strain (hcr^-) was repair-deficient in addition to requiring tryptophan; the other strain (hcr^+) was repair-proficient. 2,4-D was tested in a spot test without metabolic activation as either 1-3 mg crystals or 20-25 ul liquid. Both positive and negative controls were included in the assay. According to the authors, 2,4-D gave negative results in this assay. However, since numerical values of results were not reported the authors conclusions could not be evaluated.

Seiler (1979) tested 2,4-D and 2,4,5-T in a mouse testicular DNA synthesis inhibition test. One hour before treatment, male mice (number unspecified) received 1 uCi of ^{14}C-thymidine intraperitoneally. Herbicides were administered in a single dose: 200 mg of 2,4-D per kg; 200 mg of 2,4,5-T acid per kg; and 50, 100, 200, and 400 mg of 2,4,5-T isooctyl ester per kg. The animals were given intraperitoneal injections of 10 uCi of ^{3}H-thymidine, from three to 96 hours later. The ratios of ^{3}H counts per ug DNA and per ^{14}C counts were then compared to controls. Criteria for a positive result was that thymidine uptake was depressed significantly (statistical test not specified). All three compounds showed statistically significant inhibition of testicular DNA synthesis. The author concluded that, in light of their indicator of mutagenic activity, the potential for carcinogenicity of these compounds should be investigated.

Seibert and Lemperle (1974) tested commercial preparations of dalapon, bromacil, diuron, diquat, 2,4,5-T amyl ester, 2,4-D, and 24 other herbicides in a mitotic gene conversion assay in Saccharomyces cerevisiae using strain D-4. Neither positive nor negative controls were reported by the authors. However, according to the authors, diquat and 2,4-D induced a significant increase in mitotic gene conversion compared to controls. Both compounds were tested at pH 4.5. 2,4-D increased convertants fivefold at the ade 2 locus and 6 fold at the trp 5 locus, while diquat induced a 7-fold increase at the ade 2 locus and 4-5 fold at the trp 5 locus. Bromacil (ph 4.5) and 2,4,5-T (pH 7.0) amyl ester increased convertants slightly, but not significantly, over controls. Convertant frequencies induced by dalapon (pH 4.5) or diuron (pH 4.5) did not differ from controls.

Shiraus et al. (1978) studied the DNA damaging and mutagenic capabilities of bromacil, 2,4-D, diuron, monuron, and 2,4,5-T, using three bacterial assays: a rec assay in Bacillus subtilus, and reverse mutation assays in Escherichia coli and Salmonella typhihmurium. All of the herbicides were negative for mutagenicity in all three assays.

Although bacterial reverse mutation assays were universally negative with 2,4-D and 2,4,5-T, a variety of other in vitro assays were positive. There are sufficient data to associate 2,4-D and 2,4,5-T with mutagenic potential.

9.4.2 TCDD

Hussain et al. (1972) evaluated the mutagenicity of TCDD in three bacterial systems: 1) reversion to streptomycin independence in Escherichia coli Sd-4; 2) reversion to histidine independence in Salmonella typhimurium; and 3) prophage induction in E. coli K-39. TCDD (99 percent purity) in DMSO was added to suspension cultures of lag phase E. coli Sd-4 at 0.5, 1, 2, and 4 ug/ ml for one hour. Mutation frequencies appeared to increase at 2 ug/ml. However, the two replicate cultures reported had wide variation in frequencies of mutation (34×10^{-8} and 256×10^{-8}), but not in survival (18 percent and 11 percent, respectively). Since further testing was done, this wide discrepancy in replicate makes it difficult to interpret the test. In addition, lag phase cells were exposed in this experiment. Other investigators have found that this is not the most sensitive stage for mutagenic effects to occur.

In Salmonella typhimurium TA1530, no increased numbers of revertants were observed at 1 and 10 ug/ml TCDD for one hour. Survival values were 90 percent and less than one percent respectively. In strain TA1532, increased mutation frequency was observed when TCDD caused the survival rate to decrease to about one percent . No numerical data were presented to analyze the author's results. However, in this test a survival rate of less than 10 percent is not generally recognized as being a reliable indicator of a positive result. In the prophage induction experiment, TCDD in DMSO was incubated at 0, 0.5, 1, 1.5, and 2.5 ug/ml for 30 minutes with E. coli K-39 cells. E. coli K-49 cells were used as indicator cells and incubated with the treated, washed K-39 cells for two hours. Numbers of replicate cultures were not reported. The solvent DMSO appeared to have had an effect on prophage induction. In the controls without DMSO, 7.3×10^{-5} plaques/ml were observed while only 1.9×10^{-5} plaques/ml were observed with DMSO. The significance of this effect could not be calculated because of lack of data. At 0.5 ug/ml, TCDD induced 5.4×10^{-5} plaques/ml, about a twofold increase compared to DMSO controls. No other concentration of TCDD increased the numbers of plaques observed. The authors conclude that TCDD is mutagenic in Salmonella typhimurium TA1532 and is a weak inducer of prophage. However, this conclusion is suspect, due to insufficient data from replicate cultures, no statistical analysis of results, and poor presentation of experimental design.

Seiler (1973) also studied 2,3,7,8-TCDD in Salmonella typhimurium strains his G-456, TA1530, TAA1531, TA1532, and TA1534. In this system, the tester strain reverts from histidine dependence to histidine independence when treated with a mutagen. According to criteria set by the author, a strong mutagenic response was defined as a relative mutagenicity of greater than 10 (number of revertants from treated plates per 10^8 bacteria/spontaneous reversions per 10^8 bacteria). A medium mutagenic response was defined as a relative mutagenicity of 5-10, a weak response was a relative mutagenicity value of 3-5, a doubtful response was a relative mutagenicity of 1-2, and a negative response was a relative mutagenicity of 1. 2,3,7,8-TCDD was a strong mutagen in strain TA1532, and a doubtful mutagen in strains TA1531 and TA1534, strains which are reverted by frameshift mutagens. The compound was a directly acting mutagen; that is, it did not require exogenous metabolic activation for a mutagenic effect. 2,3,7,8-TCDD was negative in G46 and TA1530, which are reverted through base pair substitutions. No numerical data for chemically-induced revertants or spontaneous revertants were presented by the authors. This prevents a proper evaluation of the test system in that laboratory. The author also did not include the numbers of replicate plates used in the experiment, the dose of TCDD used, or the number of times the experiment was repeated. Failure to include this information severely limits the value of the data since no quality of experimental design can be evaluated. The positive mutagenicity data concerning TCDD is weakened by deficiencies in the reported studies. Although it appears that TCDD is mutagenically active, a firm conclusion to that effect is inappropriate at present.

9.4.3 Other Herbicides

Benigni et al. (1979) studied the mutagenicity of diquat in several in vitro mutagenicity assays, ranging from prokaryotic bacterial cells to eukaryotic mammalian cells. This battery included:

- Ames test, Salmonella typhimurium strains TA98, TA98, TA100, TA1535, TA1537, and TA1538, with and without metabolic activation, measuring reversion from histidine dependence to histidine independence
- Forward mutation test in S. typhimurium measuring induction of 8-azaguanine resistance in a sensitive strain
- DNA repair test in S. typhimurium measuring preferential killing of DNA repair deficient cells compared to DNA repair proficient cells
- Forward mutation test in Aspergillus nidulans measuring induction of 8-azaguanine resistance and methionine suppression
- Induction of recessive lethals in A. nidulans
- Induction of unscheduled DNA synthesis in a human cell line.

This study represents a thorough in vitro test battery to study the mutagenicity of diquat and reveals much valuable information. Diquat was mutagenic in forward mutation tests in bacteria and yeast, DNA repair tests in bacteria and mammalian cells, and a recessive lethal test in yeast. Reverse mutation tests in bacteria were negative. The authors suggested the reason for these results may be that diquat is unable to induce frameshift or base-pair substitution-type mutations, but may be able to cause other damage, such as deletions, strand breaks, or cross links at the gene level. Diquat was shown to cause damage at the chromosomal level (recessive lethal test) and to damage DNA (unscheduled DNA synthesis).

Rocchi et al. (1980) studied the action of diquat on scheduled and unscheduled DNA synthesis in rat thymocytes and human lymphocytes. Diquat (95 percent purity) in DMSO was added to rat thymocyte cell cultures to determine the concentration of diquat that gave 50-70 percent DNA synthesis inhibition. This dose was then used to determine the effect of diquat on DNA synthesis in human lymphocytes. For four hours, diquat (500 ug/ml) was added to cultures of human lymphocytes with and without hydroxyurea. During this culture period, the cells were labeled with tritiated thymidine to measure DNA synthesis. Cells treated with ultraviolet radiation were used to compare scheduled and unscheduled DNA synthesis. At 500 ug/ml, diquat produced approximately the same amount of inhibition of scheduled DNA synthesis in rat thymocytes as in human lymphocytes. In this system, inhibition of scheduled DNA synthesis was comparable to inhibition of unscheduled DNA synthesis. The authors did not discuss the relevance of these data. However, it appears from the data presented that diquat at the dose tested in human lymphocytes did not induce unscheduled DNA synthesis compared to controls.

Carere et al. tested dalapon and picloram for mutagenicity in Salmonella typhimurium and Streptomyces coelicolor. S. typhimurium strains TA1535, TA1536, TA1537, and TA1538 were used in the assay with and without rat liver S-9 metabolic activation. A streptomycin-sensitive strain of S. coelicolor A3(2), hisA1, was used in a forward mutation assay. (Mutagenic effects of a chemical are measured by induction of streptomycin resistance.) Both assays were performed as a spot test, i.e., absorbent paper saturated with solutions of the chemical to be tested were placed on agar seeded with bacteria. Neither compound induced increased numbers of revertants, with or without metabolic activation, in any of the four tester strains of S. typhimurium. In the S. coelicolor forward mutation assay, picloram-treated plates had approximately 40 times the number of resistant colonies compared to controls, while dalapon treatment did not induce an increase in numbers of resistant colonies. The authors studied the mutagenicity dalapon, picloram, and tordon in Aspergillus nidulans. Three types of mutagenic events were studied: forward point mutation from 8-azaguanine sensitivity to 8-azaguanine resistance in haploid strain 35; mitotic crossing over in the diploid strain p; and mitotic nondisjuction in the diploid strain P. In all three, a spot test was employed. Dalapon and picloram were negative in all three mutagenicity assays.

Seiler (1978) evaluated the mutagenicity of diuron and monuron in the mouse testicular DNA synthesis inhibition (DSI) test and the Ames test. In the DSI test, each herbicide was administered in a single oral aequeous dose given by stomach tube to four male mice (age and strain unspecified) per dose level. Both monuron and diuron produced a statistically significant inhibition in testicular DNA synthesis. Each compound was also screened for mutagenicity in the Ames test. Data for only one tester strain (S. typhimurium TA1535) were reported. Rat liver S9 was used as the metabolic activation system. Both compounds induced dose-dependent increases in numbers of revertants in the plate assay, and also in a number of tubes with microbial growth in the fluctuation assay. Testing presented in this paper cannot be viewed as reliable evidence of mutagenic effects, primarily because the author fails to present methodology, description of animals used and controls, and criteria used in evaluating the results of this study.

Simmon et al. (1977) reported a study of twenty pesticides including bromacil, cacodylic acid, and monuron for mutagenic activity in several in vitro test systems. The test systems included a mammalian cell unscheduled DNA synthesis, Salmonella typhimurium reverse mutation Escherichia coli WP2, Escherichia coli W3100/p 3478, and Bacillus sublilis H17/M45, and Saccharomyces cerevisiae DE. All of the tests had appropriate controls and sufficient detail was presented to substantiate the author's conclusions. Bromacil and cacodylic acid were negative in all of the assays. In the presence of metabolic activating enzymes, monuron (10^{-5}-10^{-3} M) was positive in the mammalian cell unscheduled DNA synthesis assay. It was negative in this test without metabolic activation. With respect to this positive data on monuron, the authors concluded that this compound should be tested more extensively in vivo to evaluate its carcinogenic potential.

Mutagenicity tests on other Vietnam herbicides were presented by Anderson et al. (1972), Seibert and Lemperle (1974), and Shirasu et al. (1978). These studies are described in detail in Section 9.4.1.

Diquat and monuron are the only herbicides which have been studied, other than 2,4-D, 2,4,5-T, and TCDD, that have demonstrated positive results in at least two different in vitro test systems. Diquat was positive in the majority of the assays in which it was tested. These results are sufficient evidence to suggest in vivo evaluation of the biological activity of these compounds is appropriate.

9.5 CONCLUSIONS

2,4-D and 2,4,5-T appear to be weak mutagens based on positive results in Drosophila and yeast assays. Some test results in human and mammalian cells also showed these compounds to be weak mutagens.

TCDD was reported to be mutagenic in all bacterial and yeast assays in which it was tested, however, there are serious deficiencies in this data. TCDD appears to be a weak clastogen in rats dosed by gavage, however substantiating evidence is lacking. A survey of subjects exposed to TCDD in the Seveso accident associate no increase in chromosome aberrations with that exposure.

Of the other herbicides studied for mutagenic potential, only diquat and monuron were positive in at least two different in vitro assay systems. This suggests that in vivo testing of these two compounds is warranted. There is, however, a disturbing trend in the data on human exposure to a variety of herbicides which is suggestive that such exposure may present a potential genetic hazard to humans.

CHAPTER 9.

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CHAPTER 10

CARCINOGENICITY

This chapter reviews human and animal studies concerned with the carcinogenic potential of herbicides used in Vietnam by the armed forces. The primary focus of the chapter is the assessment of the carcinogenicity of 2,4-D and 2,4,5-T (the primary constituents of Herbicide Orange), the contaminant TCDD, and other herbicide formulations. Some limited data are also presented for picloram, monuron, and cacodylic acid.

The chapter is divided into three main sections. The first deals with available epidemiologic data. This is followed by a discussion of animal bioassays that have been conducted on the individual components of the herbicide formulations. The final section summarizes the human and animal data and assesses the potential carcinogenic risk to humans posed by exposure to the various herbicides.

10.1 EPIDEMIOLOGIC STUDIES

10.1.1 Study of Cancer Prevalence in Vietnam

Tung et al. (1973) reported an increase in the number of persons with liver cancer in proportion to the number of cases of all cancers in North Vietnam during the period 1962 to 1968, during which time herbicides were used in South Vietnam. A total of 159 cases of liver cancer out of 5,492 cases were reported for the period 1955 to 1961, prior to the start of herbicide spraying in South Vietnam. Between 1961 to 1968, 791 cases of liver cancer were found among 7,911 total cancer cases. The authors attributed the increased prevalence of liver cancer to the herbicide application, explaining that the cases appeared in the North either by population mixing or by transport of the herbicide by contaminated vectors. No evidence was presented to support this suggestion, nor were other possible etiologies adequately ruled out. Serious limitations in the report make a definitive conclusion regarding any relationship between the prevalence of liver cancer and herbicide spraying impossible.

10.1.2 Studies of Industrial Exposures

The incidence of cancer in workers accidentally exposed to high levels of trichlorophenol (TCP) and TCDD has been investigated in two studies.

Thiess and Frentzel-Beyne (1977) reported six cancer deaths that had occurred during a 24-year period among a group of 75 men who were exposed following an accident at a BASF factory in Germany in 1953. Three of these deaths were attributed to carcinoma of the stomach. The incidence of stomach cancer in the exposed group was found to be significantly higher than that in three different age-matched control groups; however, this finding was significant only for those persons between the ages of 65 and 69 years. The

incidence of mortality from all malignant neoplasms in the study group was not found to be significantly different from any of the control groups. It was also reported that among a group of BASF workers who were not involved in the accident, four had died of cancer. None of these deaths were due to stomach cancer. Comparisons between this group and the study population were not subjected to statistical analysis.

In 1980, Zack and Suskind investigated cancer deaths among 121 male workers who had developed chloracne following TCP and TCDD exposure in an accident in 1949 at the Monsanto plant in Nitro, West Virginia. Nine deaths due to cancer occurred during the 29-year observation period. Five of these deaths were from lung cancer, three from neoplasms of lymphatic or hematopoietic tissue, and one death was caused by a malignant fibrous histiocytoma presumed to be of dermal origin. No deaths from stomach or liver cancer were found. The incidence of death from cancer was not found to be significantly higher than among age-matched U.S. males. The authors concluded that there was no apparent excess of cancer deaths resulting from the accidental high exposures to TCP and TCDD. The results of this study do not support the findings of Thiess and Frentzel-Beyne (1977) in the 65 to 69-year-old cohort, despite the larger cohort and longer observation time used by Zack and Suskind (1980).

10.1.3 Studies of Swedish Railway Workers

In a preliminary study, Sundell et al. (1973) found no excess number of cancer deaths among a group of 194 Swedish railway workers exposed to a variety of herbicides including phenoxyacetic acids. In a followup report by Axelson and Sundell (1974) on a cohort of 207 workers exposed to phenoxyacetic acids this finding was confirmed. Only workers who had been known to be exposed primarily to phenoxyacetic acids for a period greater than 45 days were included in the cohort. The authors also recorded eight cases of cancer among members of the cohort who were still alive at the time of the study. This was not found to be significantly higher than the five cases that were expected for the general Swedish population. The authors attributed the slight increase in tumor morbidity in phenoxyacetic acid-exposed individuals to concurrent exposure of these workers to other herbicides, including amitrol. The authors found significant increases in cancer mortality and morbidity among workers exposed primarily to amitrol.

The preliminary report (Sundell et al., 1973) and the completed report (Axelson and Sundell, 1974) each listed the herbicide formulations known to be in use during the study period, 1957 to 1971. According to these listings, the phenoxyacetic acids 2,4-D and 2,4,5-T were always used in combination with other herbicides, including atrazin, mecoprop, and dichloropropionic acids. It is not known to what extent the 2,4,5-T was contaminated with TCDD, nor in what proportions these herbicides were used in the formulations.

In 1977, Axelson and Sundell reevaluated the data presented in the 1974 study. The authors reported that two of the cancer cases were difficult to evaluate with respect to exposure; both were initially included in the amitrol-exposed cohort but it was possible that exposure to phenoxyacetic acids alone took place. No details supporting this suggestion were provided.

After including these two cases in the phenoxyacetic acid-exposed cohort, Axelson and Sundell (1977) found a significant increase in tumor morbidity as compared to the general population. It was concluded that this reevaluation supported the suspicion that exposure to phenoxyacetic acids leads to an increase in the incidence of tumor morbidity. However, the authors recognized the difficulty in interpreting their findings presented by mixed exposures.

Another shortcoming of the studies by Axelson and Sundell (1974, 1977) is the short latency period used by the authors. Calculations of significance were based on 3- and 5-year latency periods. In an unpublished report, Axelson et al. (undated) performed a follow-up investigation which allowed for the use of a 10-year latency period. Six deaths from cancer were found among the study cohort, compared with 3.14 expected cases. This difference was not significant. Two of these deaths were reported to be from stomach cancer, the incidence of which was found to be significantly higher than the 0.33 cases expected in the general population. Data on the incidence of tumor morbidity were not presented in the report. As with the previous reports by Axelson and Sundell (1974, 1977) it is impossible to draw any conclusions regarding a causal relationship between exposure to particular phenoxyacetic acids and cancer incidence, because of the confounding factor of mixed and multiple exposures to several herbicides among members of the study cohort.

10.1.4 Studies of Swedish Forestry and Agricultural Workers

The first indication that forestry and agricultural workers in Sweden may be at increased risk from soft-tissue tumors arose following a report by Hardell (1977), in which he described the employment histories of seven patients hospitalized in Umea and diagnosed with malignant mesenchymal tumors. Five of the seven patients were forestry workers with confirmed exposure to phenoxy acids. The remaining two were believed to be indirectly exposed through their work, which involved the clearing of phenoxy acid-treated areas. Although Hardell (1977) stated that this report was not conclusive, he believed that there were several reasons for suspecting that exposure to phenoxy acids might have been a factor in the development of the tumors. The reasons cited by the author included the observations that the tumor type is relatively rare and the latent period of 10 to 20 years agreed with the suspicion of chemical involvement. Additionally, without providing details, the author reported that the sex ratio of patients diagnosed as having mesenchymal tumors in Umea, a heavily wooded area, deviated from national statistics.

The report by Hardell (1977) prompted a formal investigation into the occurrence of malignant mesenchymal tumors among forestry workers exposed to phenoxy acids. Results of a case-control study of patients in northern areas of Sweden who were diagnosed as having malignant mesenchymal soft-tissue sarcomas during the period 1970-1977 appeared in two publications (Hardell and Sandstrom, 1978; Hardell and Sandstrom, 1979). The patients selected for study were all males between the ages of 26-80. For each patient alive at the time of the study, eight controls matched for age, sex, and place of residence were selected. Ten matched controls were selected for each deceased patient. Exposure of the patients to phenoxy acids was assessed by means of a questionnaire sent to the patients or their next of kin and persons selected

as controls. Employers of the patients were also contacted for information regarding the patients' exposure to phenoxy acids.

The authors reported that phenoxy acids in wide use during the study period included 2,4-D, 2,4,5-T, and 4-chloro-2-methylphenoxyacetic acid (MCPA). However, exposure to phenoxy acids was difficult to evaluate; replies from employers were obtained for less than half of the patients involved in the study. It was also reported that employers did not keep records of individual employees, and the answers received were based on memory. While conducting a parallel study on chlorophenol exposure within the study group, the authors received a 97 percent response from the patients' employers and good agreement between the employers' responses and patients' responses. Based on this finding, Hardell and Sandstrom (1978, 1979) concluded that patients' responses to questions regarding exposure to phenoxy acids were probably adequate.

It was found that 13 of a total of 45 patients had been exposed to phenoxy acids. Exposure times varied from 3 days to 49 months and latency periods varied from 3 to 27 years. Of 201 controls, 14 had indicated past exposure to phenoxy acids of a duration of at least 1 day. The relative risk of exposure to phenoxy acids was calculated to be 5.3. A similar finding was reported for patients exposed to chlorophenols either alone or in combination with phenoxy acids. According to the authors, confounding factors, such as smoking habits or exposure to DDT or chain saw exhaust fumes, did not contribute to the increase in relative risk, but the data supporting this conclusion were not reported. The influence of exposure to diesel oil or other herbicides could not be assessed due to a lack of data. The authors concluded that the use of phenoxy acids and chlorophenols contributed to an increased risk of soft-tissue sarcomas, although no evaluation of the effect of specific substances could be made.

In 1979, Eriksson et al. reported the results of a similar study involving soft-tissue sarcoma patients who had worked in southern Sweden. The study included men in the age group of 25-75 years with soft-tissue sarcomas diagnosed between 1974-1978. Phenoxy acids used in the southern areas included 2,4-D, 2,4,5-T, MCPA, and phenoxypropionic acids. Control subjects were selected and exposure information was acquired in the same manner as by Hardell and Sandstrom (1978, 1979).

Ninety-nine patients were included in the analysis, 14 of whom reportedly were exposed to phenoxy acids. Five of 211 subjects in the control group had indicated a history of exposure. The relative risk for developing soft-tissue sarcomas was calculated to be 6.1. The risk was found to be greater for test subjects exposed for more than 30 days. This change in relative risk with respect to length of exposure was not found to be significant.

In an attempt to investigate the influence of TCDD contamination of the herbicides on relative risk for developing sarcomas, Eriksson et al. (1979) excluded patients with known exposure to 2,4,5-T from the calculations. When this was done, they reported that exposure to phenoxy acids not contaminated with TCDD led to a relative risk of 4.2, less than that reported for persons exposed to all phenoxy acids but still significantly higher than controls. The authors concluded that the data showed that exposure to phenoxy acids may

be a contributing factor in the development of soft tissue sarcomas and that the risk is not limited to phenoxy acids that may contain TCDD.

Eriksson et al. (1979) also presented data that failed to show any significant contributing effects from smoking histories or exposures to asbestos, glass fiber, power saw exhaust, other pesticides, or organic solvents. However, as with the reports by Hardell and Sandstrom (1978, 1979) it is impossible to derive any conclusions regarding exposure to any single agent as related to the risk of developing soft-tissue sarcomas.

In 1979, Hardell described a case in which a patient with a tumor in the soft tissue of the left femur was found to have had massive exposure to phenoxyacetic acids. Histological examination showed that the tumor was a malignant lymphoma. In a pilot study, Hardell (1979) reported that 14 of 17 lymphoma patients questioned about their occupations responded that they had been exposed to phenoxyacetic acids or chlorophenols. A more detailed survey of 149 lymphoma patients with either Hodgkins disease or non-Hodgkins lymphoma, of a similar design to the previous case-control studies described in this section, was reported by Hardell et al. (1980). Forty-one of the 149 patients were found to have had a history of past exposure to phenoxyacetic acids, as compared to 24 of 327 control subjects. The relative risk was found to be 4.8. No significant difference existed between cases and controls with respect to smoking history or exposure to a variety of other agents. The authors did note, however, that exposure to DDT and mercury-containing seed dressings did correlate with exposure to phenoxyacetic acids.

10.1.5 Cohort Studies of American Workers

Two cohort studies have been published examining the cancer mortality experience of American workers in 2,4,5-T or trichlorophenol production plants. In the first report, a cohort of 204 employees was selected based on company records showing that each member of the cohort had worked for at least one month at one of four jobs in which there was potential exposure to 2,4,5-T or TCP (Ott et al., 1980). Within the cohort, 157 had been involved with one or more of these jobs for less than one year. Production of 2,4,5-T began at the facility in 1950 and was shut down in 1971.

An industrial hygiene survey conducted at the plant in 1969 revealed that air concentrations of 2,4,5-T varied from less than 0.1 to 6.2 mg/m^3. Estimated time weighted average (TWA) concentrations of 2,4,5-T varied from 0.2 to 0.8 mg/m^3, depending upon the job. TWA concentrations of 1.6-9.7 mg/m^3 TCP were also reported. The product specifications for 2,4,5-T in 1966 called for a maximum of 1 ppm TCDD. Information on the TCDD content of 2,4,5-T prior to 1966 was not reported.

In addition to these exposures, the unit under which the process was organized was also responsible for the production of 2,4,5-trichlorophenoxypropionic acid, 2-methyl-4-chlorophenoxyacetic acid, and styrene-butadiene latex. Thus, many members of the cohort were potentially exposed to a variety of other substances during their employment.

None of the individuals within the cohort had ever been known to have had chloracne or porphyria cutanea tarda, which might have indicated excessive exposure to TCDD. The only death due to a malignancy was one case of respiratory cancer in a 63-year-old man who had been exposed to 2,4,5-T for eight years and who had been known to smoke up to two packs of cigarettes per day. Ott et al. (1980) will continue surveillance of persons with known exposure to 2,4,5-T because of the limited scope of this survey.

An incident that occurred at this facility in 1964 involved 49 TCP production workers who reported to the industrial medical department with skin conditions subsequently diagnosed as chloracne. A followup mortality survey of 61 employees known to have been working in the building during 1964 was reported by Cook et al. (1980). No information was supplied concerning exposures to TCDD. The vital status of the cohort through December 1978 was determined. According to Ott et al. (1980), the TCP production plant was separate from the 2,4,5-T production unit, so for this cohort there presumably was no exposure to 2,4,5-T or the other herbicides manufactured.

Of the four deaths reported for the cohort, three were due to malignant neoplasms: one adenocarcinoma (site unknown), one fibrosarcoma, and one glioma. None of these three decendents had any record of having developed chloracne while employed at the plant. All three were known smokers. After categorizing job classification into high and low potential exposure, it was determined that only the worker who died from adenocarcinoma had held a job in an area of high potential exposure. Cook et al. (1980) concluded that the data suggested that TCDD is not a potent human carcinogen, because of the lack of any apparent dose-response relationship, and because the latency period of 14 years was sufficient to allow the identification of a potent human carcinogen. The authors also recommended further research on other cohorts to determine whether TCDD has weak carcinogenic potential in humans.

In a letter to Lancet, Honchar and Halperin (1981) reviewed these two studies and the report by Zack and Suskind (1980--see Section 10.1.2). After combining the cohorts from these three studies and some additional unpublished data from Zack, Honchar and Halperin (1981) found that of 105 deaths among workers exposed to TCP or 2,4,5-T, three (2.9 percent) were due to soft-tissue sarcoma. They compared this to an 0.07 percent incidence of deaths in 1975 in American males between the ages of 20 and 84 years. Although realizing that none of the four cohorts showed an excess incidence of soft-tissue sarcoma, the authors concluded that a common pattern was suggested by the combined data.

10.1.6 Summary and Evaluation

Studies of the incidence of cancer mortality have been conducted on three major occupational groups for which there has been worker exposure to phenoxyacetic acids and/or trichlorophenol, both of which are known to be contaminated with TCDD. Generally, large excesses in the incidence of cancer mortality, which would suggest a strong carcinogenic effect, have not been demonstrated. From these studies, it is also impossible to implicate any one herbicide or TCDD as being responsible for the small number of cancer deaths documented, because of the confounding factor of mixed exposures.

A significant increase in tumor morbidity among a group of Swedish railway workers exposed to phenoxyacetic acids (Axelson and Sundell, 1977) was demonstrated only after reevaluating previous data (Sundell et al. 1973; Axelson and Sundell, 1974). A later unpublished report (Axelson et al., undated) on the same study group showed that the incidence of death from stomach cancer was significantly higher than national statistics would indicate (two actual cases versus 0.33 expected). The total incidence of deaths from cancer was not significantly high. Workers were known to have been exposed to other herbicides, and exposure levels of 2,4,-D, 2,4,5-T, and TCDD were not available.

Case-control studies have pointed to an association between exposure to phenoxy acids and/or chlorophenols used by Swedish forestry and agricultural workers, and the development of malignant mesenchymal soft-tissue sarcomas (Hardell, 1977; Hardell and Sandstrom, 1978; Hardell and Sandstrom, 1979; Eriksson, 1979). The authors suggested that the risk was not limited to phenoxy acids that contain TCDD (Eriksson et al., 1979).

Similar case-control studies have also revealed an increased relative risk of exposure to phenoxyacetic acids or chlorophenols and the development of malignant lymphoma (Hardell, 1979; Hardell et al., 1980). It is impossible to ascertain the contribution by 2,4-D, 2,4,5-T, or TCDD alone, as there was concomitant exposure to other herbicides and chemical agents in these study populations.

The third major group of persons studied that have been exposed to 2,4,5-T and TCDD were workers involved in trichlorophenol (TCP) or 2,4,5-T production. Two investigations were conducted on populations with known large exposures following plant accidents. In one study (Theiss and Frentzel-Beyne, 1977), a significantly higher incidence of death from stomach cancer was found among a group of men exposed to TCP and TCDD following the accident at the BASF facility in Germany in 1953. This finding was not supported in a study of a group of workers exposed to TCP and TCDD following the accident at Nitro, West Virginia (Zack and Suskind, 1980). A larger cohort and a longer observation time was available for the latter study.

Two cohort studies of workers exposed to 2,4,5-T and/or TCP, both containing TCDD, have not individually reported an excess incidence of cancer deaths (Ott et al., 1980; Cook et al., 1980). However, when these data were combined with the data of Zack and Suskind (1980) and unpublished data by Zack, a pattern was observed in which exposure to 2,4,5-T and/or TCP led to a greater-than-30-fold increase in the mortality incidence of soft-tissue sarcoma, compared to the background incidence of U.S. males (Honchar and Halperin, 1981). It is worth noting that of the three cases of soft-tissue sarcoma found, two were described in workers involved in TCP production where there was no known exposure to 2,4,5-T reported. The third case was of an employee engaged in 2,4,5-T synthesis. No cases of mortality from soft-tissue sarcoma were described in the only published study of a cohort of 2,4,5-T production workers (Ott et al., 1980).

The number of cases of soft-tissue sarcoma described in these studies is too small to make any firm conclusions regarding the risk from exposure to 2,4,5-T or TCDD. Although these findings do not contradict the Swedish case

control studies of forestry and agricultural workers, exposure to TCP is a confounding factor in the assessment of the carcinogenic risk of 2,4,5-T and TCDD. More information may become available following analysis of the mortality experience of a larger cohort currently being assembled by the National Institute of Occupational Safety and Health (Honchar and Halperin, 1981).

10.2 ANIMAL STUDIES

The following sections describe animal bioassays that have been conducted on 2,4-D, 2,4,5-T, and TCDD. Animal studies on picloram, monuron, and cacodylic acid, compounds for which no human studies are available, are also reviewed in the latter parts of the section.

10.2.1 2,4-D

10.2.1.1 Mice

Two strains of mice, C57BL/6 x C3HAnf and C57BL/6 x AKR, 18 of each sex, were fed 46.4 mg 2,4-D per kg by gavage until the mice were 4 weeks of age (Innes, 1969; Bionetics, 1968). Thereafter, the mice were fed 149 mg 2,4-D per kg of diet until they were killed for examination at approximately 18 months of age. Another group of C57BL/6 x AKR mice, 18 of each sex, were fed 2,4-D at higher doses of 100 mg/kg from day 7 to 28, and 323 mg/kg of diet from day 28 to 18 months. No significant increase in tumor incidence within the combined groups or compared to 338 untreated mice was reported at either level. Similar results were obtained in mice fed 2,4-D isopropyl, butyl, or isooctyl esters. IARC (1977) reported that the tumor incidence in individual groups was also comparable to controls.

Groups of these same two strains of mice were given 2,4-D by subcutaneous injection on the 28th day of life at doses of 215 or 464 mg/kg. Additional groups of both strains were given subcutaneous injections of 21.5 mg 2,4-D butyl ester per kg, 100 mg 2,4-D isopropyl ester per kg, or 21.5 mg 2,4-D isooctyl ester per kg. When tumor incidence was compared to 613 untreated animals, a significant excess of total tumors and reticulum cell sarcomas was found for the mice treated with 2,4-D isooctyl ester. No increase in tumor incidence was found in any other treated group.

Arkhipov and Kozlova (1974), using groups of 100 OSVA x S57/VL hybrid female mice, found no tumors in mice fed 2,4-D at one-tenth the LD_{50} (dose not stated) throughout the animals' lives. The same result was reported for a second group of animals following weekly applications of two drops of a 10 percent 2,4-D solution on the skin. However, a serious lack of detail in reporting the experimental protocol and results makes this study difficult to evaluate.

10.2.1.2 Rats

Groups of 25 male and 25 female Osborne-Mendel rats were maintained for two years on diets containing from 5 to 1,250 mg 2,4-D per kg diet (Hansen et al., 1971). Analysis of 2,4-D by gas chromatography did not detect TCDD or 2,7-dichlorodibenzo-p-dioxin (limit of detection was 1 ppm). For male rats receiving the highest dose, the number of animals with malignant tumors was found to be significantly greater than in controls (7/25 as compared to 3/25). No specific target organ was apparently affected and the authors noted that the tumors found in treated rats were of the type commonly seen in aging Osborne-Mendel rats. The authors concluded that their study, like that of Innes (1969), supported a lack of carcinogenic effect of 2,4-D in animals.

In contrast, Arkhipov and Kozlova (1974) found two rats with tumors among a group of 120 male and 45 female randomly bred rats fed 2,4-D at one-tenth the LD_{50} (dose not specified) for 2 years. The tumors found were a fibroadenoma of the mammary gland, which was also seen in one control rat, and a hemangioma of the mesentery. Lack of detail in the report make these results difficult to evaluate, but it is apparent that these results failed to demonstrate significant carcinogenic activity of 2,4-D in rats.

10.2.1.3 Summary and Evaluation

2,4-D has been tested in feeding studies using small numbers of mice and rats and has not been found to exert a strong carcinogenic effect (Innes, 1969; Bionetics, 1968; Hansen et al., 1971; Arkhipov and Kozlova, 1974). A significant increased incidence of tumors was found among mice given 2,4-D isooctyl ester by subcutaneous injection. IARC (1977) concluded that no evaluation of the carcinogenicity of 2,4-D could be made based on these studies due either to inadequate reporting or the small number of animals used in the tests.

10.2.2 2,4,5-T

10.2.2.1 Mice

Groups of 18 male and 18 female mice of each strain C57BL/6 x C3HAnf and C57BL/6 x AKR received commercial 2,4,5-T at a dose of 21.5 mg/kg daily from age 7 to 28 days (Innes, 1969; Bionetics, 1968). Thereafter each group was fed 60 mg 2,4,5-T per kg of diet until the mice were approximately 18 months of age, when they were killed and examined. When compared to 338 untreated animals, the combined group of treated animals did not show an excessive number of tumors. Similar groups of both strains were given single subcutaneous injections of 2,4,5-T on day 28 of life at a dose of 215 mg/kg. The tumor incidence in the combined group did not differ from a group of 613 untreated mice. IARC (1977) also reported that in both the feeding and injection study the tumor incidence within individual treated groups did not differ from controls. Although the 2,4,5-T was reported to be 98 percent pure (Innes, 1969; Bionetics, 1968), the TCDD content was not reported.

No increase in tumor incidence was reported among a group of 20 male and 19 female XVII/6 mice given 100 mg 2,4,5-T per liter in the drinking water for 2 months, followed by 80 mg 2,4,5-T per kg of diet for the animals' lifespan (Muranyi-Kovacs et al., 1976). However, when a group of 22 male and 35 female C3Hf mice were treated similarly, a significant increased total incidence of tumors was found among female mice. When tumor types were separated into incidental tumors (discovered in an animal which died from some other cause) and nonincidental tumors (diagnosed during life or causing the death of the animal), treated C3Hf males and females showed an increased incidence of non-incidental tumors. No difference was found between treated C3Hf and control mice in the occurrence of incidental tumors. Nonincidental tumor types included leukemias, cutaneous tumors, a variety of sarcomas, and hepatomas diagnosed in living animals. Occasional rare tumor types were found among treated C3Hf mice; these included two cutaneous squamous-cell carcinomas and one osteosarcoma. The authors attributed the increase in tumorigenesis in C3Hf mice to 2,4,5-T, since their sample was reported to contain only between 0.02 and 0.03 ppm TCDD. However, they suggested that the effect produced by 2,4,5-T in C3Hf mice may have been dependent upon the species of animal tested since the carcinogenicity of 2,4,5-T could not be replicated in mice of strain XVII/6. Further testing in other animal strains was recommended.

10.2.2.2 Rats

Kociba et al. (1976) examined the carcinogenic potential of 2,4,5-T in Sprague-Dawley rats in one well-designed study. Groups of 100 rats (50 of each sex) were fed 3, 10, or 30 mg 2,4,5-T per kg mixed in the diet for two years. The 2,4,5-T was reported to contain less than 0.33 ppm TCDD. The body weights and food consumption of randomly selected rats were checked periodically to confirm the dosage. The highest dose level was associated with some degree of toxicity, including increases in relative kidney weight, urinary excretion of coproporphyrin and uroporphyrin, and slight morphological changes in the kidney, liver, and lungs. Death rate and food consumption were not changed as compared to 86 male and 86 female control rats.

At the end of the feeding period, rats were killed and all major organs were examined for gross and histological changes. Female rats fed the lowest dose were found to have a higher incidence than controls of interfollicular C-cell adenoma of the thyroid, but this was attributed to an unusually lower incidence of this lesion in controls than would be expected historically in this strain. No other increased oncogenic response could be demonstrated in male rats or female rats fed the two highest doses. The authors concluded that 2,4,5-T was not carcinogenic in Sprague-Dawley rats in doses high enough to induce toxic changes.

10.2.2.3 Summary and Evaluation

2,4,5-T has been tested in chronic feeding studies using four strains of mice and one strain of rat (Innes, 1969; Bionetics, 1968; Muranyi-Kovacs et al., 1976; Kociba et al., 1979b), and by single subcutaneous injection in two strains of mice (Innes, 1969; Bionetics, 1968). An excess number of tumors was found in only one of the strains of mice tested, indicating that a species

specific effect was observed. Muranyi-Kovacs et al. (1976) supported this idea by citing evidence showing that the rate of metabolism of 2,4,5-T differs among animal species. Kociba et al. (1979b) questioned the validity of the finding of a positive carcinogenic effect of 2,4,5-T in one mouse strain because no specific target organ was involved, an observation that they reported is usually seen in animal bioassays of carcinogenic substances.

IARC (1977), based on the mouse studies of Innes (1969) and Muranyi-Kovacs (1976), concluded that no evaluation of the carcinogenicity of 2,4,5-T could be made due to the small number of animals used in the tests. The data presented by Kociba et al. (1979b), which appeared after the IARC review, supports the previous findings of a lack of a strong carcinogenic effect by 2,4,5-T.

10.2.3 TCDD

10.2.3.1 Mice

The carcinogenic effects of TCDD were examined in Swiss-H/Riop mice. Preliminary data were reported in Toth et al. (1978), and completed data appeared in Toth et al. (1979) and Sugar et al. (1979). In this bioassay, five groups of 100 male and female mice were given various combinations of 2,4,5-trichlorophenoxyethanol (TCPE) and TCDD by gastric intubation once per week for one year. Two groups of 200 mice served as controls. The dose of TCPE varied from 0.7 to 70 mg/kg while the dose of TCDD varied from 0.00007 to 0.112 mg/kg. The purity of the TCPE or TCDD was not stated.

Similarly, three groups of 45 male Swiss-H/Riop mice were given TCDD in doses of 0.007, 0.7, and 7.0 mg/kg (Toth et al., 1979). Forty-five male mice served as controls. After the one-year exposure period, all animals were kept for their lifetimes. At death all major organs were examined histologically.

A relatively high incidence of hepatomas and hepatocellular carcinomas was seen in male control animals, indicating the sensitivity of this strain to developing liver tumors. The incidence of liver tumors was approximately doubled among the two groups of male mice receiving either 67 or 70 mg TCPE per kg, which contained 0.112 and 0.007 mg TCDD per kg, respectively. The difference was statistically significant compared to controls. No increase in tumor incidence was observed for any of the female mice or the male mice receiving lower doses of TCPE. However, in one of these lower TCPE dose groups, the amount of TCDD administered was 0.07 mg/kg, 10 times higher than in one of the two groups with tumors. Toth et al. (1979) and Sugar et al. (1979) concluded that the carcinogenic effect observed was due to TCPE and that TCDD did not have a tumor-enhancing effect at doses of 0.07 mg/kg or less.

When TCDD was administered alone to three groups of male mice, a significant increase in liver tumors was found in the group receiving 0.7 mg/kg, but not in the groups given 0.007 or 7.0 mg/kg (Toth et al., 1979). No excess of deaths occurred in any group. Thus, a dose-response relationship was not observed. At the two highest doses, about one-half of the animals

showed skin lesions caused by TCDD. This was the only other toxic effect reported. The authors concluded that TCDD had a liver tumor enhancing effect in Swiss mice at a threshold dose of 0.7 mg/kg, but this does not explain the smaller incidence at 7.0 mg/kg.

10.2.3.2 Rats

Nine groups of 10 male Sprague-Dawley rats were placed on a diet containing from .001 to 1,000 ppb TCDD (Van Miller et al., 1977) for 78 weeks. The purity of the TCDD used was not reported. All surviving animals were killed after an additional 17 weeks and their major organs examined histologically.

At the three highest doses (50, 500, and 1000 ppb), all rats died between the second and fourth week of the experiment. The food intake for the six remaining groups was comparable to controls. Only one animal in these groups died before the 30th week. All of the animals fed 1 and 5 ppb TCDD died by the 90th week. By the end of the experiment, 60 percent of the control rats had died, a figure higher than that among groups fed from .001 to 0.5 ppb.

The overall incidence of neoplasms in the six treated groups was 38 percent, with no neoplasms seen among control rats or rats fed the lowest TCDD dose (0.001 ppb). All 10 rats fed the highest dose (5 ppb) had neoplasms, but no other dose-effect relationship was observed among the remaining groups and no statistical analysis of the data was provided. There was a variety of neoplasms reported with no particular target organs affected.

Van Miller et al. (1977) concluded that while this study did not prove that TCDD was carcinogenic, the possibility exists that TCDD enhanced the development of neoplastic changes that were induced by an unknown agent. He based this idea on the observations that few tumors were found in the liver, which is the site of TCDD localization, and that the wide variety of neoplasms found is not consistent with the findings of many other known chemical carcinogens.

In another study using Sprague-Dawley rats, Kociba et al. (1978, 1979a) maintained rats on diets of 0.1, 0.01, and 0.001 mg TCDD (99 percent pure) per kg daily for two years. Analysis of the diets showed that the animals were ingesting a diet containing an average concentration of 2.193, 0.208, and 0.022 ppb, respectively. Fifty rats of each sex were used per group. At the end of the two-year feeding period, all rats were killed and tissues examined.

A variety of non-neoplastic changes were found in the liver, lymphoid tissue, and respiratory organs in rats fed the highest dose of TCDD (0.1 mg/kg). The mortality rate was significantly increased in female rats given this dose. At 0.01 mg/kg, the body weights of females were significantly decreased, and non-neoplastic liver and lung changes were observed in both males and females. At the lowest TCDD dose, no general toxic changes were noted, but females had a higher incidence of local hepatocyte swelling.

At the highest dose level (0.1 mg/kg), the incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate, nasal turbinates, and tongue was significantly increased. The incidence of a variety of

other tumor types in treated rats was decreased compared to controls. In rats fed 0.01 mg/kg, the incidence of liver nodules was increased in females only; no other tumor-like lesions were observed in low-dose females or males given the two lowest doses. Kociba et al. (1978, 1979a) concluded from these data that TCDD, at doses high enough to induce toxicity and non-neoplastic tissue changes, could alter the incidence of tumors in rats.

10.2.3.3 Studies of TCDD Cocarcinogenesis and Tumor Promotion and Initiation in Mice

The general lack of organ specificity reported for TCDD-induced tumorigenesis, and the observation that TCDD has been found to increase tumor incidence in animals only at toxic doses, has led some investigators to speculate that TCDD promotes tumorigenesis, but is not necessarily by itself carcinogenic (Van Miller et al., 1977; Rappe, 1979). A number of studies have been undertaken in two-stage animal models to investigate the tumor-promoting ability of TCDD. In these studies, a subthreshold dose of a known carcinogen is applied to the skin of an animal (initiation phase). This is followed by repetitive application of a noncarcinogenic tumor promoter (promotion phase). In the studies reviewed in this section, TCDD has been tested as a tumor initiator as well as promoter.

The first such study of TCDD as a tumor initiator was reported by DiGiovanni et al. (1977). Using groups of 30 female CD-1 mice, 2 ug of TCDD was applied to a shaven area of the back. One week after initiation, 5 ug of the known tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied to the same area twice weekly for 32 weeks. A group of positive control animals received the known tumor initiator 7,12-dimethylbenz(a)-anthracene (DMBA) followed by TPA. The incidence of papillomas and carcinomas was observed weekly.

The dose of TCDD used was sufficient to kill one-third of the animals by 32 weeks. TCDD showed a weak tumor initiating ability, producing an average of 0.1 papillomas per mouse. The average number of papillomas in positive control animals was not stated; however, in an experiment using TCDD and DMBA simultaneously as initiators, it was reported that the effect was approximately additive and gave rise to an average of 2.2 papillomas per mouse. The authors concluded that TCDD is a weak tumor initiator in this system and does not greatly influence the effect of DMBA, a known tumor initiator.

Berry et al. (1978) showed that TCDD did not promote papilloma formation when it was applied twice weekly following DMBA initiation. The dose used was 1 ug per mouse for each application. Positive contol mice given DMBA followed by TPA promotion had an average of 8.1 papillomas per mouse. No papillomas were observed on TCDD promoted mice. A group of mice receiving 1 ug TCDD twice weekly but without DMBA initiation also failed to develop papillomas. The authors also reported that the dose of TCDD used was the maximum tolerated dose; a higher dose could not be used because of excessive mortality.

In a continuing investigation using the same system described above, Berry et al. (1979) tested the effect of TCDD on DMBA-induced tumorigenesis. The same results are also reported in DiGiovanni et al. (1979). Groups of

30 female CDI mice were treated once with DMBA and twice weekly with the tumor promoter TPA. However, three groups were pretreated once with 1.0 ug TCDD 1, 3, or 5 days before DMBA was applied. It was found that TCDD did not enhance, and in fact inhibited, DMBA-initiated tumorigenesis. A similar result was achieved when benzo(a)pyrene instead of DMBA was used an an initiator (DiGiovanni et al., 1979). The extent of inhibition of DMBA-induced tumorigenesis by TCDD was found to increase with increasing dose and pretreatment time.

Examination of DMBA metabolic products found in TCDD-treated mice revealed that TCDD was a potent inducer of the monooxygenase system which converts DMBA to its noncarcinogenic hydroxylated products. The authors concluded that nontoxic doses of TCDD inhibited skin carcinogenesis induced by polyaromatic hydrocarbons, whereas TCDD itself failed to promote skin tumors at near-toxic doses. A similar finding in a different species was presented by Cohen et al. (1979), who reported that TCDD inhibited skin tumorigenesis of DMBA and benzo(a)pyrene in Sencar mice.

A second effect of TCDD on polyaromatic hydrocarbon induced tumorigenesis was described in a report by Kouri et al. (1978), who found that a subcutaneous injection of 100 ug TCDD per kg given simultaneously with a subcutaneous injection of 3-methylcholanthrene (3-MC) markedly increased the incidence of tumors, characterized as injection-site fibrosarcomas, in DBA/2 mice. When TCDD was given intraperitoneally 2 days prior to or simultaneously with 3-MC, the carcinogenic incidence increased only slightly or remained unchanged compared to mice given 3-MC alone. Identical testing of C57BL/6 mice, using only the intraperitoneal dose route for TCDD, did not affect 3-MC-induced carcinogenesis. The subcutaneous route of TCDD application, which was tumor enhancing in DBA/2 mice, was not tested in C57BL/6. TCDD itself, at a dose of 100 ug/kg injected once intraperitoneally, did not induce tumors in either C57BL/6 or DBA/2 mice. This dose was sufficient to kill 30-70 percent of the animals within the 36-week observation period.

Kouri et al. (1978) concluded from this study that TCDD, at a dose causing death in 30 to 70 percent of treated mice, did not appear to be carcinogenic in the survivors examined 36 weeks later. However, TCDD at the same dose injected subcutaneously, which was less lethal, did enhance carcinogenicity of 3-MC in DBA/2 mice and is, therefore, a cocarcinogen. They suggested that when TCDD was given together with 3-MC, metabolism of 3-MC to the ultimate carcinogen was made more efficient by TCDD induction of aryl-hydrocarbon hydroxylase (AHH) or other monooxygenases. The DBA/2 strain, known as an "unresponsive" strain, was shown to be relatively resistant to the carcinogenic effect of 3-MC due to poor induction of AHH by polyaromatic hydrocarbons. Thus, the TCDD was believed to aid in the induction of enzymes required to convert 3-MC to its carcinogenic metabolites. The C57BL/6 strain, on the other hand, is very responsive and sensitive to 3-MC. It was believed by the authors that TCDD induction of AHH had little effect on enhancing 3-MC metabolism since this strain of mouse inherently exhibited the maximal limit for 3-MC-induced tumorigenesis in this system.

Berry et al. (1979) disagreed with this suggestion of Kouri et al. (1978), claiming that by using simultaneous administration of TCDD and 3-MC, not enough time was available for TCDD to induce AHH. In the study by Berry et al. (1979), time was a necessary factor in the successful inhibition of

DMBA tumorigenesis by TCDD, which presumably was caused by TCDD induction of the monooxygenease system responsible for DMBA detoxification. Other possible mechanisms of cocarcinogenesis were not ruled out in the experiments of Kouri et al. (1978).

It appears, then, that TCDD is capable of exerting two different effects on polyaromatic hydrocarbon induced tumorigenesis. Both effects are dependent upon the species and carcinogen tested, the time between TCDD pretreatment and application of the carcinogen, and the dose route used. It is highly unlikely that these types of effects will ever be demonstrated in humans. However, as recommended by Berry et al. (1979), more work is needed to elucidate the mechanisms of TCDD inhibition or enhancement of chemically induced carcinogenesis to permit extrapolation of these effects to humans.

10.2.3.4 Summary and Evaluation

TCDD has been shown to enhance the incidence of liver tumors in one strain of mice (Toth et al., 1979), but only at doses high enough to induce skin lesions. A dose-response relationship was not observed.

Two studies were published in which TCDD was tested in Sprague-Dawley rats (Van Miller et al., 1977; Kociba et al., 1978, 1979a). In both, an excessive incidence of tumors was demonstrated using only toxic doses of TCDD. No specific target organs were affected and in only one of the studies (Kociba et al., 1978, 1979a) could a dose-response relationship be derived. Although the high toxicity of TCDD has complicated investigations into TCDD-induced carcinogenesis, nontoxic doses have been used in both the mouse and rat studies and have failed to induce carcinogenesis.

The observations that TCDD can induce tumors only at toxic doses and without affecting specific target organs have led to the hypothesis that TCDD may be a tumor promoter. This has been studied in a number of systems. TCDD has been found to be a very weak tumor initiator when used in combination with a noncarcinogenic tumor promoter (DiGiovanni et al., 1977). It has not been shown to be a tumor promoter when applied in combination with carcinogenic polyaromatic hydrocarbons such as DMBA or benzo(a)pyrene (Berry et al., 1978, 1979; DiGiovanni et al., 1979), and can, in some systems, inhibit tumorigenesis caused by polyaromatic hydrocarbons. It has been suggested that the latter may be the result of the induction of detoxification pathways by TCDD.

TCDD has been shown to act as a cocarcinogen with 3-methylcholanthrene in one mouse system. However, this effect was specific for the species of mouse tested and the route of TCDD administration used. More studies are needed on TCDD enhancement of carcinogenicity induced by other chemicals before any conclusive statements can be made regarding humans. As of April 1981, final reports of two NCI bioassays of TCDD were in review and a decision has been made to begin a third test.

10.2.4 Picloram

10.2.4.1 Mice

Two groups of B6C3F1 hybrid mice, containing 50 of each sex per group, were fed picloram (90 percent pure) in the diet in time weighted average doses of 5,062 and 2,431 ppm for 80 weeks (NCI, 1978). Mice were observed an additional 33 weeks, were killed, and all organs were examined for gross and histological changes. The incidence of lesions in the treated mice were compared to matched and pooled control groups.

At the end of 17 weeks, one low-dose and five high-dose females had general body tremors. During the first year of feeding, the condition of the mice was reported to be comparable with controls. During the second year of feeding, clinical signs of toxicity became increasingly evident among treated mice. Slight hyperactivity, the presence of rough hair coats, and abdominal distention were noted in treated animals. There was no significant excess mortality among treated mice as compared to controls. At the termination of the study, no significant difference between treated animals and controls was apparent in the incidence of neoplastic and nonneoplastic lesions.

10.2.4.2 Rats

Two groups of Osborne-Mendel rats, 50 of each sex per group, were fed time weighted average concentrations of 14,875 and 7,437 ppm picloram in the diet for 80 weeks (NCI, 1978). The rats were observed for an additional 10 weeks, after which they were killed and examined for gross tissue and histological changes. The incidence of lesions was compared to matched and pooled control groups.

During the second 6 months of feeding, treated rats were observed to have a moderate incidence of diarrhea, hematuria, and rough hair coats. Clinical signs of toxicity became more apparent in the test animals during the second year of feeding and included dermatitis, tachypnea, dark urine, diarrhea, and vaginal bleeding. Upon examination of tissues, feci of cellular alteration and neoplastic nodules of the liver were seen in treated rats. The increased incidence of neoplastic nodules in the liver was significant only for females receiving the higher dose of picloram. Statistical analysis showed a significant dose-related trend among female rats towards increasing liver nodule incidence with increasing dose. These nodules were interpreted to be benign tumors by the examining pathologist. Heptatocellular carcinoma was observed in one low-dose male and one high-dose female; no hepatocellular carcinoma was observed among matched controls. This finding was reported to be statistically insignificant. A high incidence of follicular and C-cell hyperplasia and neoplasia of the thyroid gland was observed among low-dose male and high-dose female rats, but this finding was not significant.

10.2.4.3 Summary and Evaluation

In the only well-controlled study found on picloram, female rats fed picloram at doses high enough to induce clinical signs of toxicity developed a significant excess of neoplastic nodules in the liver (NCI, 1978). No other carcinogenic lesions were found in treated rats or mice. The investigating pathologist concluded that picloram is not carcinogenic in these strains of mice and rats, but could induce benign liver tumors in female Osborne-Mendel rats.

However, after calculating the upper and lower confidence limits of the relative risk interval, it was found that the upper limit of relative risk was greater than 1. The result of this statistical test was interpreted as indicating that the bioassay design could have failed to reveal a positive carcinogenic effect. The statistical analysis of relative risk suggests that more studies be done before any definite conclusions are made regarding the possible carcinogenicity of picloram.

10.2.5 Monuron

10.2.5.1 Mice

Male and female mice of two hybrid strains, C57BL/6 x CBH/Anf and C57BL/6 x AKR, were fed 215 mg commercial monuron/kg by gavage daily until the mice were 4 weeks of age (Innes, 1969; Bionetics, 1968). Thereafter, the mice were given 517 mg monuron per kg of diet until they were killed for examination at approximately 18 months of age. The authors reported that a significantly increased incidence of pulmonary adenomas occurred among the treated animals when the combined groups of treated animals were compared with untreated controls (10/66 versus 20/338). The incidence of total tumor types, hepatomas, and reticulum cell carcinomas was not significantly different between control and treated animals. However, IARC (1976) reported that tumor incidence reported in this study was significant only for lung adenomas in males of one strain (6/16 versus 9/90). It is not clear from examining the original report (Innes, 1969; Bionetics, 1968), which did not contain a statistical comparison of test and control groups by sex and strain, how the conclusion made by IARC (1976) was formulated.

Rubenchik et al. (1970) fed 50 mixed-breed and 45 C57Bl mice 6 mg monuron in milk once per week for 15 weeks. The mice were held for 27 months. The number and survival rates of the control mice were not specified. The first tumor observed in the mixed-breed mice was found after 16 weeks, and in C57Bl the first tumor was discovered after four weeks. At these times, 23 (46 percent) mixed breed mice and 26 (58 percent) C57Bl mice had survived. A variety of "reactive changes" reportedly was found early in the mice, including lymphocytic infiltrates, catarrhal inflammation, proliferation of epithelium of the bronchi, and focal necrosis of the liver. It was not stated whether these changes occurred in control or treated animals or at which point during the investigation these changes were noted. A total of 13 tumors was reported in the mixed-breed mice and seven tumors occurred in the C57Bl strain. Most of the tumors consisted of benign hepatomas, hepatocellular

carinoma, alveolar carcinoma, and kidney cancer. The survival rate of the treated mice at the end of the experiment was not reported and no statistical analysis of the data was provided.

Separate groups of both sexes of two hybrid strains of mice were given single subcutaneous injections of 10 mg monuron per kg on the 28th day of life and observed until they were approximately 18 months of age (Innes, 1969; Bionetics, 1968). No significant increase in tumors was noted. IARC (1976) commented that a single injection may not be an adequate basis for discounting monuron-induced carcinogenesis.

10.2.5.2 Rats

Hodge et al. (1958) maintained groups of 30 male and 30 female Rochester albino rats on diets containing 0.0025, 0.025, and 0.25 percent monuron for 2 years. Because of a respiratory infection, 70-90 percent of all groups, including controls, died. Of the remaining animals, gross and microscopic examination of all major tissues reveaed no carcinogenic lesions. IARC (1976) noted that a lack of detail in reporting data made this study difficult to evaluate.

Fifty random-bred male rats received 450 mg monuron per kg daily in their diet for 18 months and were observed for an additional 9 months (Rubenchick et al., 1980). The first tumor was discovered after 18 weeks, at which time 32 rats were still alive. The number of rats surviving for the 27-month experimental and observation period was not stated. Fifteen rats were found to have tumors with no specific target organ excessively affected. No tumors were found in any of the 30 control rats, which IARC (1976) found unusual.

10.2.5.3 Summary and Evaluation

Two studies in which mice of different strains were fed monuron were able to demonstrate the induction of tumors, with the lungs and liver being the major target organs (Innes, 1969; Rubenchick et al., 1970). One of these studies (Rubenchick et al., 1970) also showed that mixed breed rats fed monuron developed tumors, but no specific target organ was apparent. The report by Innes, 1969 (details of which are provided in Bionetics, 1968) used a small number of animals in test groups. Innes (1969) recommended that monuron be subjected to further study. Serious reporting deficiencies are evident in the publication by Rubenchick (1970), especially with regard to data on control animals, which make evaluation of the study difficult.

Based on the information presented for monuron and in the absence of any human data, IARC (1976) concluded that the data suggest that monuron is carcinogenic. In light of the deficiencies of the data presented here, final conclusions should await publication of a chronic feeding study in mice and rats that is currently underway by the National Cancer Institute.

10.2.6 Cacodylic Acid

Two strains of mice, C57BL/6 x C3H/Anf and C57BL/6 x AKR, 18 of each sex, were fed 464 mg cacodylic acid/kg by gavage daily until the mice were 4 weeks of age (Innes, 1969; Bionetics, 1968). Thereafter, mice were fed 121 mg cacodylic acid per kg of diet until they were killed for examination at approximately 18 months of age. In a parallel experiment, 18 of each sex of the same two hybrid strains were given 464 mg cacodylic acid per kg by subcutaneous injection on the 28th day of life and observed until they were approximately 18 months of age. No significant increase in tumor incidence, as compared to 338 untreated mice, was reported from use of either dose route. The number of animals used in this test was relatively small, so it is difficult to draw any firm conclusions regarding the lack of carcinogenic potential of cacodylic acid.

10.3 SUMMARY OF CARCINOGENIC POTENTIAL OF HERBICIDES

There is no evidence available to suggest the presence of a potent carcinogenic risk to humans exposed to 2,4-D, 2,4,5-T, and TCDD. Some studies have indicated that a causal relationship may exist between exposure to phenoxyacetic acids and the development of soft-tissue sarcomas. This has come primarily from case-control studies of Swedish forestry and agricultural workers, and cohort studies of workers exposed to trichlorophenol, 2,4,5-T, and TCDD during herbicide production. However, confounding factors exist which make it impossible to implicate 2,4-D, 2,4,5-T, or TCDD specifically.

In the case of the Swedish studies, a relatively high proportion of patients with soft-tissue sarcomas were found to have been exposed to phenoxyy acids in forestry and agricultural work. In addition to exposure to 2,4-D and 2,4,5-T, these patients had known exposure to other phenoxy acids and chemical agents. Studies of production workers, which included employees exposed at high levels following accidents as well as employees exposed during normal production operations, are not conclusive because all workers were known to be exposed to trichlorophenol, the precursor of 2,4,5-T. In these cohort studies, a total of only three deaths from soft-tissue sarcoma has been reported, two of which did not involve exposure to 2,4,5-T. Studies of these cohorts is continuing, and the National Institute of Occupational Safety and Health is currently developing a registry of trichlorophenol and 2,4,5-T production workers so additional cohorts may be available in the future. However, from the viewpoint of 2,4,5-T and TCDD carcinogenicity in humans, any future study of production workers will always have the confounding variable of trichlorophenol exposure. Additional study populations, relatively free of trichlorophenol exposure, should be identified for investigation.

It is generally suspected that TCDD, a contaminant of 2,4,5-T and trichlorophenol, may have been a contributing factor in the development of the soft-tissue sarcomas. This idea is supported by animal studies, one in which male mice fed combinations of trichlorophenoxyethanol and TCDD showed a significantly higher incidence of liver tumors than control mice. However, tumor incidence was related to the dosage of trichlorophenoxyethanol, and not TCDD. This study and others in which mice and rats have been fed TCDD have shown that TCDD can increase tumor incidence but only at doses sufficient to

induce other toxic effects. This observation, along with the wide variety of tumor types found in animal studies, led to the hypothesis that TCDD may be a tumor promoter rather than a chemical carcinogen; that is, a second biochemical event would be required before TCDD could promote carcinogenesis.

This hypothesis has been tested in two-stage animal models. TCDD has been shown to be a weak tumor initiator in mice exposed to TCDD and a noncarcinogenic polyaromatic hydrocarbon. TCDD has not been shown to be a tumor promoter in these systems and, in fact, can inhibit tumorigenesis by polyaromatic hydrocarbons. Evidence has suggested that the mechanism for inhibition involves the induction by TCDD of metabolic pathways that detoxify polyaromatic hydrocarbons. In one instance, TCDD was shown to enhance the tumorigenicity of 3-methylcholanthrene, but this effect was reported to be specific for only one mouse strain and dose route.

2,4-D and 2,4,5-T, when tested in animal bioassays, have consistently failed to induce tumors in mice and rats. In one instance, one strain of mouse fed 2,4,5-T showed a high tumor incidence but no specific target organ was affected. Three other strains similarly tested were not susceptible, indicating that a species-specific effect may have been involved. The small number of animals used in these bioassays prohibits making a definitive conclusion that 2,4-D and 2,4,5-T are not carcinogenic under any circumstances, but it does not appear that these compounds are potent animal carcinogens.

A similar conclusion may be made with regards to picloram and cacodylic acid, but only one study for each was available. Two studies have suggested that monuron is carcinogenic in mice and rats; however, one study was of limited design and the other had serious reporting deficiencies. Monuron is currently being tested by NCI, and additional studies on picloram and cacodylic acid are needed.

CHAPTER 10.

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APPENDIX

ONGOING EPIDEMIOLOGIC RESEARCH

In this appendix, summary information on five epidemiologic investigations of Vietnam exposure to Herbicide Orange are presented. These five studies are planned to begin shortly or are currently underway, and include:

- Ranch Hand II: U.S. Air Force
- Birth Defects and Military Service in Vietnam: Center for Disease Control, USPHS
- Epidemiologic Studies of Agent Orange: Veterans Administration
- Proportional Mortality of Vietnam Veterans, Other Veterans and Matched Controls in New York State, Exclusive of New York City: State of New York
- Epidemiological Study of Soft-Tissue Sarcoma: State of New York.

It is likely that additional epidemiologic research on Vietnam veterans will be initiated at the Federal and State levels. This appendix, therefore, should be considered only an indication of the kinds of studies already planned or underway, rather than an exhaustive listing of all epidemiologic research on Vietnam veterans currently being considered.

In the following sections, each of these five studies is addressed.

A.1 RANCH HAND II: U.S. Air Force

RANCH HAND II was developed by the U.S. Air Force to determine whether long-term health effects exist and can be attributed to occupational exposure to Herbicide Orange.

Project RANCH HAND II uses a nonconcurrent prospective design entailing mortality, morbidity, and followup studies. The study population to be investigated are Air Force personnel involved in the RANCH HAND Organization, the Air Force unit that flew C-123 spray missions during the Vietnam Conflict. Data will be obtained from Air Force personnel records. The exposed cohort consists of approximately 1,200 individuals. The control population of 12,000 will be selected from a control universe of 25,000 crew members and support personnel from other units assigned to duty in Southeast Asia.

Ten statistically equivalent control individuals will be matched to RANCH HAND personnel for the variables of age, type of job, and race. The mortality analysis will utilize a one-to-five ratio design of one RANCH HAND subject to fifty percent of the randomly selected controls. The study population will be followed yearly over a 20-year period.

The morbidity design will utilize a one-to-one ratio, with the first of the randomized mortality controls entered into the questionnaire and physical examination phase of the study.

Questionnaire data will be used to reconstruct occupational, social, and medical histories to quantitate morbidity endpoints and confounding factors. Comprehensive physical examinations will be performed, emphasizing dermatologic, neuropsychiatric, hepatic, immunologic, reproductive, and neoplasic conditions. Questionnaires will be administered using personal and telephone interviews. Blind assessment protocols will be used to avoid bias and limit data variability. Physical examinations and questionnaires will be developed for followup in years 3, 5, 10, 15, and 20 of the study.

Inferences about disease state will be developed by identifying symptom complexes on physical findings. Comparison of these symptoms between groups will be utilized to calculate relative risks from baseline data which, if appropriate, will be used in the followup analyses.

Statistical methodologies to be used include combinational and correlational analyses to provide statements of probability of disease state, subclinical state, and to correct for over-reporting.

Regression techniques will be applied to a normalized exposure index among exposed individuals exhibiting symptoms and/or signs to clarify disease state or syndrome. Mortality data will be analyzed using several different approaches, including age, age-disease specific rates, standardized mortality rates, and modified life table approaches, as well as logistic and multiplicative models. Questionnaires and physical examinations will be analyzed using log-linear models for dichotomous or polytomous data to verify the appropriateness of the standard statistical methodologies. Continuous variables will undergo covariance analysis to remove non-controlled effects, followed by the use of a paired difference statistic. Group scoring techniques will be used as appropriate.

A.2 BIRTH DEFECTS AND MILITARY SERVICE IN VIETNAM: Center for Disease Control, USPHS

The Birth Defects Branch of the Center for Disease Control (CDC) is presently conducting a study of Birth Defects and Military Service in Vietnam. The study uses a case-control design. The case population is composed of approximately 7,000 to 8,000 selected babies born with one or more serious birth defects during the years 1968-1980 registered in CDC's Metropolitan Atlanta Congenital Defects Surveillance Program (MSCDSP). The control population will be selected from live births in the metropolitan Atlanta area during the same time-period, between 1968-1980. Criteria for case-matching include race, month and year of birth, and hospital of birth. The ratio of cases to control is two to one.

The sample size of 3,000 randomly selected control families was selected on the basis that it would give fairly good sensitivity to a small increase in risk for all malformations identified. Case and control population candidates

identified are traced, contacted, and secured for participation. Participants are interviewed using telephone and mail questionnaires. Family members of case and control groups interviewed are the mother, father, and grandmothers of the infant.

Factors identified as possible sources of bias include: sociodemographic characteristics, age at pregnancy, familial history of birth defects, major organic diseases in relatives, and other environmental exposures.

Analysis is being performed upon four major groupings:

- All defects combined
- Individual category of defects
- Groups of babies affected with similar patterns of multiple defects
- All babies born with defects which may have been caused by a fresh dominant mutation.

The analysis proposed is a combination of the search for confounding and using the Mantel-Haenszel approach multidimensional contingency table analysis based upon the log-linear model. For all defects combined and some of the larger specific categories, the log-linear approach will be used. For smaller defect categories only the Mantel-Haenszel approach will be used, although the log-linear analysis done for all defects combined may provide some guidance as to possible higher order interactions. For very small categories of defects, not much searching for confounding will be done.

A.3 EPIDEMIOLOGIC STUDIES OF AGENT ORANGE: Veterans Administration

The draft protocol for "Epidemiologic Studies of Agent Orange" was developed for the Veterans Administration by the Division of Epidemiology, School of Public Health, University of California at Los Angeles. The purpose of the proposed studies is to determine whether exposure of ground troops to Herbicide Orange resulted in health effects.

The draft protocol uses a historical cohort study design, with cohort groups defined using Army and possibly Marine Corps records for the period 1965 through 1971, the period of heaviest herbicide application.

Casualties, both immediate and delayed, will be excluded from the cohort. The study will be also limited to draftees and one-term enlisted men.

Estimation of exposure for cohorts will be defined on two or more levels to observe dose-response effects. Estimated company exposure levels will be constructed using the HERBS tape and hand abstracted Army Records. These data will be used to generate time-place-company exposure grids by computer using mapping data and algorithms.

Companies which represent the maximum range of exposure to Herbicide Orange from high to low will be selected. All individuals serving in identified companies wil be initially included in the cohort. These troops will then be checked through personnel records in St. Louis for other Vietnam service time, discharge status, and most recent whereabouts.

All members of the cohort will then be traced to determine their vital status. All living cohort participants will be examined using standardized protocol procedures including questionnaire, laboratory testing, and physical examination.

The sample size of the study groups have not been given at this time. The sample size estimates to be developed will be based upon the expected disease frequencies, the expected variances of the measurements and the population size available to the study in each exposure group.

The details of the analysis will not be specified until the details of the data to be collected are known; however, it is expected that the analysis will begin with simple descriptive statistics and later proceed to testing specific hypotheses.

Preliminary to the historical cohort study, UCLA proposed three mortality studies. The first is a proportionate mortality study to attempt to determine if there are unusual causes of death or patterns of death among Vietnam veterans or a specific subgroup of Vietnam veterans. The second study is designated to estimate actual death rates of Vietnam veterans and Vietnam-era veterans who did not serve in Vietnam. The third study is a case-control study utilizing death as an outcome measure for case selection to examine subgroups of Vietnam veterans for evidence of higher risk of death. UCLA also proposed morbidity studies to address the question of whether there is an unusual morbidity experience among Vietnam veterans as compared to nonveterans or among subgroups of Vietnam veterans.

The first study proposes using information from the Agent Orange registry to determine the frequency distribution of complaints. The second study proposes to examine veterans claims files for two time periods and to compare the morbidity experience of Vietnam and non-Vietnam veterans, Vietnam veterans within combat and noncombat units, and within areas exposed and unexposed to Herbicide Orange.

To determine possible differences in patterns of claims following Vietnam to a similar group of males, UCLA proposes to sample claims from Korean War veterans for comparison with the Vietnam veterans.

A.4 PROPORTIONAL MORTALITY OF VIETNAM VETERANS, OTHER VETERANS, AND MATCHED CONTROLS IN NEW YORK STATE, EXCLUSIVE OF NEW YORK CITY: State of New York

The protocol, "Proportional Mortality of Vietnam Veterans, Other Veterans, and Matched Controls in New York State, Exclusive of New York City," was developed by the New York State Department of Health, Division of Epidemiology and Office of Biostatistics. The purpose of the study is to determine

whether post-war mortality among Vietnam veterans differs from other veterans or other Upstate New York men, and to identify which disease categories merit further study.

The mortalities of Upstate New York men 18 to 29 years old at any time during the period 1962 through 1971 will be sampled and followed through 1979. The deceased men of this cohort will be identified through New York State death certificate files. Veterans status will be obtained from information on death certificates and from the Veterans Administration.

The proportional mortality rate (PMR) is the ratio of deaths from a specific cause to all deaths reported in that population. The adjusted proportional mortality rates will be calculated for Vietnam veterans, non-Vietnam veterans and nonveterans.

Comparison will be of veterans serving in Vietnam to other veterans matched on age, race, educational level, and marital status, or adjusted for these factors. This matching is necessary in that these factors may affect the selection of individuals for military service and, in addition, who is at risk for the various causes of death under study.

The expected number of deaths for each cause (age adjusted by the indirect method) and the significance of its deviation from the observed number will be determined by an adaptation of the Mantel-Haenszel procedure using age-specific contingency tables and computation of a continuity-corrected chi-square.

In addition to the PMR analysis, standard mortality ratios may be calculated for Vietnam era veterans, if the denominator population of veterans of New York State, exclusive of New York City, can be approximated using census data.

A.5 EPIDEMIOLOGICAL STUDY OF SOFT-TISSUE SARCOMA: State of New York

The State of New York, Department of Health, Office of Public Health, Division of Epidemiology is also conducting an "Epidemiological Study of Soft-Tissue Sarcoma."

The study uses a case-control design. The case group is composed of male residents of New York State, exclusive of New York City, who were in the age group 18 to 29 years during the period 1962-1971, reported to the N.Y. State Cancer Registry as having soft-tissue sarcoma first diagnosed through December 31, 1980.

Each case will have two overlapping controls, with each group having a control-to-case ratio of one-to-one. The first control group will be made up of live males selected from drivers license files matched on 5-year age group and zip code. Alternate controls will be selected and stratified for race during analysis. Case group members will be matched against the license files to determine their representativeness on the basis of having a drivers license.

The second control group will include nonliving males deceased for all cases except cancer. For each dead case, two death certificates will be selected for men of the same five-year age group, years of education, race, and health systems area (a ten-county area). In addition to the negative control, a positive control will be attempted to be obtained. Positive controls will be aged-matched individuals who served in Vietnam.

Pathology reports and slides will be reviewed using a standard classification system. Reviewers will be blinded as to military history. Distribution of pathology data will compare Vietnam veterans to non-Vietnam veterans and nonveterans. Data on cases and controls will be collected using primarily telephone survey techniques with standardized questionnaires. Personal interviews will also be conducted.

Data collected will include smoking history, alcoholism, occupation, and other pertinent exposure factors. Data collected will be checked against records of the Veterans Administration. Data will be analyzed using multivariate statistical techniques, such as the linear logistic model for matched analysis.

Special attention will be made to correct for: systematic bias due to nonresponse; preferential recall by case and control subjects; systematic differences of access to medical care between cases and controls; eligibility variance for military service; and overall confounding.

The Part II study will analyze data on occupation and industry reported on death certificates. Subjects will include all males of New York State, with the exception of New York City residents, listed on death certificates as dying of soft-tissue sarcomas during the period January 1, 1970 to December 31, 1979. Controls will be selected from death certificate files, matched on dates of birth, years of education, and health systems area.

Occupation and industry data from the death certificate will be analyzed to determine if specific occupations or industries are represented in the soft-tissure sarcoma group. Findings of Part II will be used to generate hypotheses for further study.