

## IV. Synopsis and Discussion of Literature

### A. Overview

More than 20,000 scientific articles relating to the phenoxy herbicides have been published since the 1940's. Many of the articles cite herbicide-caused health effects in a variety of animal species, but most early studies used a myriad of herbicide formulations and unknowingly dealt with physically and chemically impure compounds, and the assay technology was far short of today's state-of-the-art. Many human studies have ascribed cause and effect relationships but have suffered from problems of clinical empiricism or questionable methodology. The only consistent chronic clinical finding associated with exposure to 2,4,5-T herbicide and TCDD has been chloracne, recognized by most workers as the herald sign of overexposure to the herbicide and other chloracneigens. It is now recognized that the chloracne was caused by the presence of TCDD rather than 2,4,5-T. Sequelae from chloracne, localized or systemic, appear to be unusual according to the preponderance of the literature. It is appropriate to note that sustained worldwide usage of herbicides for 30 years has not yet evoked a readily identifiable disease state. It is clear from the literature and the usage history of herbicides that if there are significant attributable long-term health effects, they are either reasonably rare, or of such nonspecific commonality that they blend unnoticeably into the symptoms, syndromes, or diseases associated with increasing age or other similar factors.

### B. Pharmacokinetics of 2,4-D, 2,4,5-T and TCDD

#### (1) 2,4-D

The pharmacokinetics of 2,4-D have been well studied in animals. 2,4-D is readily absorbed after oral administration, and is initially distributed in high concentrations to the central nervous system and liver. Eventually, all tissues are involved, with the kidneys accumulating twenty times the concentration of the other tissues. The plasma half-life of 2,4-D is approximately 3 to 12 hours, with elimination from the body through the kidneys at a dose-dependent rate. Generally, high doses or repeated lower doses result in tissue accumulation. The majority of 2,4-D is eliminated unmetabolized; however, esters of 2,4-D have been shown to undergo hydrolysis prior to excretion. Muscle and fat show the lowest accumulation of 2,4-D on repeated exposure, whereas the kidneys and liver show the highest accumulations. Within 24 hours of a single-dose administration of 2,4-D, 16.8% was present in the uterus, placenta, fetus and amniotic fluid in gravid rats. In addition, 2,4-D was found in the milk of lactating rats for up to six days following single-dose exposure.

#### (2) 2,4,5-T

The pharmacokinetics of 2,4,5-T have been well studied in animals. In all animals, 2,4,5-T has been shown to be readily absorbed upon oral administration. However, beyond this point, 2,4,5-T has shown marked variations in its pharmacokinetics depending on the species tested. These differences are thought to be due to variations in species, age, dosage levels, routes of administration

and chemical formulations used in the various studies. Generally, the distribution is ubiquitous throughout the body except in hamsters, which show no placental passage, and in mice, which show placental passage only in late gestation. Clearance from plasma and from the body varies greatly among species with rats showing faster clearance than dogs, mice and man. In addition, this clearance appears to be generally dose-dependent. The biological half-life of 2,4,5-T in rats, as estimated by tissue analyses and urinary clearance at administered dosages of 5 mg/kg, is 4.7 hours. However, at 200 mg/kg, the half-life in rats is prolonged to 25 hours. Excretion of 2,4,5-T is primarily via the kidneys. The elimination of 2,4,5-T at low doses is essentially achieved in an unmetabolized form. However, at higher or more chronic doses, elimination involves the liver in a more active role (i.e., conjugation). Higher doses and repeated lower doses appear to result in accumulation in animal tissues.

### (3) Phenoxy Herbicides in Humans

Relatively few studies have dealt with the pharmacokinetics of 2,4-D and 2,4,5-T in humans. Numerous reports of occupational exposures in industry and in commercial and private herbicide applications have supported percutaneous entry as a major route of exposure. Rapid absorption of 2,4-D and 2,4,5-T has been observed after oral administration. The primary mode of excretion of the phenoxy herbicides is via the urine with 74% of 2,4-D and 63%-72% of 2,4,5-T being cleared from the body within the first 96 hours. The majority of the herbicide is unmetabolized prior to excretion and the biological half-life of 2,4-D and 2,4,5-T in humans (as estimated by tissue analyses and urinary excretion) is 33 hours and 18 hours, respectively. Tissue analysis has revealed an ubiquitous distribution of the herbicides after absorption. Limited studies on the accumulation of the phenoxy herbicides following repeated doses suggest that such accumulation in humans is unlikely. This is in contrast to numerous animal studies on 2,4-D and 2,4,5-T which show that such accumulation does occur.

No specific data are available on the odor threshold of Herbicide Orange. Data are available however, on the odor threshold of a butyl ester formulation of 2,4,5-T. The odor threshold was found to be about 0.3 ppb (the taste threshold was 1.3 ppb). A Threshold Limit Value (TLV) of 10 mg/m<sup>3</sup> for both 2,4-D or 2,4,5-T has been adopted by the American Conference of Governmental Industrial Hygienists. The TLV is a time-weighted average concentration for a normal 8-hour workday/40-hour workweek to which workers may be repeatedly exposed, day after day, without adverse effect. Analysis of ambient air samples collected adjacent to and downwind from actual drumming operations involving Herbicide Orange were at least two orders of magnitude below the TLVs.

### (4) TCDD

Information on the absorption, distribution and excretion of TCDD has been mostly derived from animal models. Studies in rats, mice and guinea pigs generally show that intestinal absorption of TCDD is relatively complete, with a large proportion being stored unmetabolized in the liver. The majority of this TCDD is assumed to be localized in the liver microsomes (centrifugation

techniques). Initially, adipose tissue accumulates TCDD, followed later by accumulation in the liver, adrenals, kidneys and lungs. The level of TCDD in the liver and adipose tissue is about ten-fold greater than in other body tissues; however, significant species variability has been observed. The biological half-life of TCDD varies by species, but is reported to range from 12 to 50 days. The major route of excretion is via the feces with urinary excretion occurring at a much reduced rate.

### C. Proposed Cellular Mechanisms of Action for TCDD

TCDD has three proposed mechanisms of action by which its variety of effects, both documented and suspected, can be understood. All currently available information in this area is derived from animal, plant, and bacterial models. The few human studies dealing with mechanisms are limited to the clinical manifestation of chloracne.

#### (1) Microsomal Enzyme Induction

TCDD's ability to induce a variety of microsomal enzymes is well documented. The induction of aryl hydrocarbon hydroxylase, delta-aminolevulinic acid synthetase, and cytochrome P-448/P-450 associated enzymes has been implicated in the development of cutaneous porphyria. The induction of aryl hydrocarbon hydroxylase and other mixed function oxygenases/oxidases has been associated with carcinogenesis and tumorigenesis. In addition, TCDD has been shown to be a possible promoter or cocarcinogen of known carcinogens. In some nonhuman studies, TCDD produced a protective effect against endocrine tumors (e.g., pituitary, uterine, pancreatic, adrenal, and mammary tumors). TCDD's induction of UDP-glucuronyl transferase, an important enzyme in steroid metabolism, may explain this peculiar effect. The induction of DT-diaphorase and lysosomal acid proteinases has been implicated in TCDD's neuropathic effects. These and other biochemical alterations may account for TCDD's clinical manifestation of chloracne resulting from an over production of keratin in the sebaceous ducts.

#### (2) DNA/TCDD Interaction.

Alterations in the structure and fidelity of transcription of DNA due to TCDD have been indirectly demonstrated. TCDD, because of its planar ring structure, may "intercalate" with DNA causing "frame-shift" mutations in a manner similar to that seen with the acridine family of compounds. A few laboratory studies with bacterial systems (Escherichia coli and Salmonella typhimurium) and one plant system (the African Blood Lily) have implicated TCDD as being capable of producing chromosomal aberrations and perhaps a weak dominant lethal effect. This hypothesized DNA/TCDD interaction could explain the development of chloracne, as well as the suggested mutagenic and carcinogenic effects, if similar mechanisms occur in mammalian species.

#### (3) Toxicity.

A nonspecific or as yet unspecified toxicity continues to serve as a reasonable mechanism for TCDD's hepatic and thymus toxicity. TCDD has been

described by some as "one of the most potent, low molecular weight toxins known", with extremely low concentrations producing severe liver damage and death in various animal studies. The immune suppression effect of TCDD has been shown to result specifically from its T-cell (thymus) toxicity.

If bioaccumulation and persistence of TCDD occur in human adipose tissue, it could be released into the circulation under situations of weight loss (e.g., life style modification, medical indications, or disease). Such hypothesized reemergence of the agent could result in low doses being either detectable and/or toxic at some later point in time. If TCDD's primary toxicity results from low doses (e.g., a mutagenic/carcinogenic effect) rather than high doses (e.g., cellular poisoning and cell death), then the deposition of TCDD in the adipose tissue may have greater significance with respect to delayed effects on the long-term health of the exposed individual. This possibility raises a theoretical dose-response paradox which might "explain" the prevailing preponderance of symptoms in populations which may have been exposed to relatively low doses of TCDD (see Section IV D). However, persistence of TCDD in humans has not been demonstrated. Attempts to measure TCDD in human tissue are limited by technical difficulty in differentiating between the 2,3,7,8 isomer found in 2,4,5-T and the other 21 isomers from non-herbicide sources. There is also no reasonable method to determine whether tissue TCDD is from an RVN exposure, or from a more recent environmental source.

#### D. Animal Studies

A comparison of animal toxicity studies is difficult due to variations in experimental designs which include differences in (1) the species, age, and sex of animals used; (2) the level, route, and length of exposure to chemicals; (3) the purity of the chemicals used; and (4) the criteria measured and the time sequence of data collection. Animals have shown a wide range of toxic effects, but this range may serve as a guide to anticipate the potential toxic effects in humans following exposure to Herbicide Orange.

A summarization of the literature is presented in Table A-1 of the Appendix, Section XV. It is apparent that the toxic effects of 2,4-D and 2,4,5-T are markedly different from the effects of TCDD. TCDD is approximately 1000 times more toxic in acute studies. In addition, the slower clearance time of TCDD may account for the significantly lower daily doses required to elicit chronic toxicity. A consistent finding in TCDD toxicity is depletion of the lymphoid tissues throughout the host. This is readily characterized by involution of the thymus in all species studied. In relation to the chronic maternal toxic dose, the embryotoxic dose is markedly lower for TCDD than for 2,4-D and 2,4,5-T. Both 2,4,5-T and 2,4-D appear to be very weak teratogens and/or carcinogens at best, but these evaluations are complicated by varying levels of contamination by various dibenzo-p-dioxins. TCDD appears to have significant teratogenic and carcinogenic potentials which appear to be species specific.

The most striking observation noted in the literature is a marked variation in response among species. Examples of these variations are in the areas of acute toxicity (TCDD's LD<sub>50</sub> in guinea pigs is 1 µg/kg compared to 1000 µg/kg

in dogs), excretion (2,4,5-T plasma half-life in rats in 4.7 hrs compared to 77 hrs in dogs), and oncogenicity. Even among strains of the same species (rats) variations in oncogenicity were noted following 2,4,5-T exposures. As noted earlier, this high variability between species is an important consideration in the designing of human studies.

A second area of interest noted in the literature is a hypothetical dose-response paradox in nonhuman primates (rhesus monkey) following exposure to TCDD. Animals in a chronic exposure study fed a low level of TCDD in feed [e.g., 50-500 parts per trillion (ppt)] have shown signs of disease only after several months when total TCDD consumption was approximately 1 µg/kg body weight. Unfortunately, animals receiving comparable amounts of TCDD in a single-dose acute toxicity studies (LD<sub>50</sub> determinations) have not been observed for the emergence of chronic effects. Therefore, it remains unclear whether the toxicity demonstrated in chronic exposure studies is dependent upon repetitive, cumulative exposure or whether similar toxicity would also be demonstrated following an equivalent single dose after a comparable observation period. Much concern has been raised over the potential of 2,4-D, 2,4,5-T or TCDD to induce genetic change in male animals which are subsequently passed on to the progeny of these exposed animals. In a recent experimental study by Lamb, Moore, and Marks, 150 male mice were exposed to various concentrations of the three chemicals in their food for eight weeks. Acute toxicity was evident with all dosages, as animals lost weight and had dose-related liver and thymus abnormalities, but these effects were reversed upon return to a normal diet. These exposures did not result in abnormalities in sperm concentration, motility or morphology. After the exposure period, the mice were mated, and no dose-related differences in mating frequency, fertility or reproductive success were evident between the chemically exposed mice and their 50 nonexposed controls.

#### E. Case Reports

Much of the medical literature on 2,4-D, 2,4,5-T and TCDD exposures in humans is based on individual case reports following acute exposures. Since most of the patients discussed in these reports were exposed to multiple chemical agents, it is difficult to determine which agents were responsible for specific symptoms. Nevertheless, the general areas of dermatologic and neuropsychiatric disease have been of primary interest in most investigations. Since the neuropsychiatric symptoms of herbicide exposure are numerous and largely subjective in nature, they have been extremely difficult to assess from a clinical standpoint. In addition, hepatic dysfunction, and renal, gastrointestinal and cardiac disturbances have been "linked" to exposures to these chlorophenolic compounds.

##### (1) 2,4-D

A multitude of symptoms have been attributed to 2,4-D and the ones reported most consistently are listed in the Appendix, Table A-2. Components of some of these selected symptoms/signs are described in Table A-3 of the Appendix. The asthenic syndrome, peripheral neuropathy, and hepatic dysfunction are

of particular interest. Other symptoms of acute systemic toxicity occur, but with 2,4-D exposure has been extensively described. It has an early onset, causes prolonged disability of variable degree, and recovery has been incomplete in many cases. Electromyography in some patients has demonstrated denervation, and some studies have detected decreases in nerve conduction velocities. One autopsy study demonstrated a demyelination process within the brain of a 76-year-old male who committed suicide by ingesting 2,4-D in kerosene.

## (2) 2,4,5-T/TCDD

The human effects of 2,4,5-T are difficult to evaluate since the chemical is contaminated with TCDD in the manufacturing process. The effects of TCDD itself have been determined from studies of trichlorophenol workers, and from laboratory workers using TCDD. Symptom/sign complexes attributable to exposure to 2,4,5-T and TCDD are listed in Tables A-2 and A-3 of the Appendix. Chloracne usually begins in the zygomatic/temporal region and is often found on and behind the pinna of the ear. This is an oily acne-like skin condition characterized by comedones and inclusion cysts which may result in extensive scarring. In severe cases following heavy exposure, spread of lesions to the throat, back and inguinal areas has been noted. This skin condition is frequently preceded by erythema and blepharoconjunctivitis. Active lesions usually disappear within two years, but have been found 30 years after exposure. Porphyrin cutanea tarda and hypothyroidism have also been linked to 2,4,5-T/TCDD exposure. Other symptoms such as asthenia, liver and renal dysfunction, neuropathy, and gastrointestinal and cardiac disturbances are probably due to mechanisms similar or identical to those of 2,4-D. With the exception of chloracne and possible disorders of porphyrin metabolism, all of these effects have been acute or subacute in nature.

Numerous instances of alleged disease due to 2,4-D/2,4,5-T exposure have been the subject of heavy media attention, particularly an episode of alleged 2,4,5-T exposure in Globe, Arizona, in 1969. Despite extensive scientific review and analysis with negative findings, the Globe incident continues to be cited in news media presentations. An incident in Missouri in 1971 in which six children, two adults and numerous animals were exposed to TCDD-contaminated oil is frequently described as well. Many of the animals died and the humans developed chloracne and other acute toxic effects; however, all humans were healthy after five years of follow-up study. A final prospective assessment of fertility, teratogenesis and carcinogenesis, in these individuals will probably be made in the future.

## F. Veteran Concerns

The Veterans Administration provided the USAF with data on 46,771 patients participating in the Herbicide Registry. Numerous media presentations emphasizing both military and civilian herbicide exposures have described a remarkably wide spectrum of health effects being claimed by the veterans. Three compensation claims have been allowed for service-connected acneiform skin lesions (but not chloracne), 16 claims for other skin conditions, and an additional three claims for other diagnoses. A direct causal relationship between a disease and a specific exposure is not necessary to receive compensation.

If the condition is shown to have occurred during active duty or within a reasonable time after separation, it is compensable, regardless of cause. Current Veterans Administration guidelines state that the only chronic residual of defoliant exposure has been chloracne. Table 2 summarizes the descriptive characteristics of 46,771 patients in the VA Herbicide Registry as of 31 August 1980. Table 3 summarizes symptoms from these patients by category.

Table 2

SUMMARY OF DESCRIPTIVE CHARACTERISTICS OF PATIENTS IN  
THE VA HERBICIDE REGISTRY, AS OF 10 FEBRUARY 1981

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Total Number of Registered Patients: 46,771  
Branch of Service of Registered Patients:

Army	66.3%
Marine Corps	18.9%
Air Force	7.3%
Navy	5.9%
Other	1.6%

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Table 3

VA HERBICIDE REGISTRY SYMPTOM REPORTING

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Number of Registered Patients: 46,771  
Number of Symptomatic Patients: 34,145 (73%)  
Mean Number of Symptoms per Symptomatic Patient: 2.6

<u>Symptom Category</u>	<u>Number of Patients</u>	<u>Percent of Registered Patients</u>	<u>Percent of Symptomatic Patients</u>
Dermatologic	18,675	39.9	54.7
Psychiatric/Psychological	11,745	25.1	34.4
Headache	6,021	12.9	17.6
Peripheral Neuropathy	5,729	12.3	16.8
Asthenia	5,637	12.0	16.5
Gastrointestinal	5,454	11.7	16.0
Sexual Dysfunction	2,105	4.5	6.2
Other	20,702	44.3	--
No symptoms	12,626	--	27.0

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Study design implications that can be drawn from these data are limited because registered veterans may not be truly representative of the exposed population. The demonstrated lack of an easily identifiable symptom complex on review of the registry data clearly substantiates the need for a comprehensive evaluation of individual patients.

#### G. Epidemiologic Studies

Epidemiologic studies of occupational groups have validated links between exposure to TCDD and the development of chloracne. Associations between TCDD and psychological abnormalities have also been suggested. A series of studies published from 1978-1980 by Hardell, Sandstrom, Axelson, and others in Sweden evaluated occupational exposure to chlorophenolic compounds in cancer patients. They found an association between cancer and exposure, but were unable to assess causality due to methodological limitations. Preliminary results of a case-control study of soft tissue sarcoma in New Zealand (Smith) did not detect any unusual clustering of occupations among the sarcoma cases.

Tung (1973) reported an abnormal increase in the occurrence of primary carcinoma of the liver in Vietnam (26 cases per year during 1955-1961 versus 144 cases per year during 1962-1968). He attributed the increase to a suspected carcinogenic effect of TCDD. His published study, however, has been criticized for failure to contain sufficient data and descriptions of methodology to verify his conclusions, and the role of aflatoxin as an alternative cause of liver cancer was not addressed. His study is generally considered to be an empiric clinical observation. A study sponsored by the EPA in 1979 in Alsea, Oregon, found a statistically significant increase in spontaneous abortion in areas where 2,4,5-T herbicide was routinely used in reforestation programs. The EPA concluded that "for all its complexity, this analysis is a correlation analysis, and correlation does not necessarily mean causation." Nevertheless, this study was used by the EPA to institute the ban on most uses of 2,4,5-T containing products. This report has been the subject of intense scientific criticism. Differences in the availability of specialty obstetrical care and in the patterns of health care delivery existed between the exposed and control areas; these differences were not taken into consideration by the researchers. Variations in the ascertainment of spontaneous abortions in each of the areas severely limited the validity of the data, and of the conclusions derived from them. A recent study conducted in Australia (1978) was unable to find an association between neural tube birth defects and the use of 2,4,5-T herbicide. A reproductive study of the wives of 370 2,4,5-T/TCDD exposed workers at the Dow Chemical Company in Midland, Michigan was recently completed (Cook and Bodner). No differences in fertility patterns, fetal wastage, or birth defects were detected.

Epidemiologic studies are continuing in Seveso, Italy, where a population of 220,000 was potentially exposed to TCDD following an industrial accident in July 1976. These studies have involved investigations of more than 30,000 children, and detailed clinical examinations of 1,024 persons, including the most severely exposed children and adults. Recent data (Hombarger, et al.,

1979) indicated that most cases of chloracne from this incident cleared rapidly. To date, the growth and development of newborn infants and children, immunological response, chromosomal aberrations, the response to the challenges of infectious diseases, and the morbidity and mortality patterns of the study population have not been significantly altered by TCDD exposure. Thirty-eight cases of birth defects were reported in early 1977, approximately 6-8 months after the industrial accident. However, the authors ascribe this increase to an artifact of surveillance. Analysis of surveillance data on the occurrence of spontaneous abortions after July 1976 is compromised by the lack of valid baselines for the pre-accident period. The social pressures operating in the Seveso population prior to the accident fostered underreporting of birth defects, while the atmosphere after the accident made the occurrence of a birth defect more socially acceptable. The post accident congenital malformation rate is not significantly different than the rate in similar areas of Central Europe.

Another progress report on the aftermath of the Seveso accident (Pocchiari, et al. 1979) has revealed: (1) a decrease in the prevalence and severity of chloracne in the exposed population; (2) an increase in clinical and subclinical neurologic disease as demonstrated by delayed peripheral nerve conduction velocities; and (3) increases in the prevalence of hepatomegaly (8%) and alterations in liver function tests, which returned to normal over an 18 month period of follow-up. Thus far, immunologic, cytogenetic, and embryomorphologic analyses have been unable to detect significant differences between exposed and non-exposed individuals.

A 2,4,5-T Dispute Resolution Conference was held in Arlington, Virginia, from 3 to 7 June 1979. Fifty-six recognized experts from the United States and seven foreign nations were actively involved in the deliberations of the conference. Human Exposure, Carcinogenicity/Mutagenicity, and Teratogenicity Working Groups independently reached the conclusions that there was no valid scientific evidence linking fetotoxicity, teratogenicity or carcinogenicity in humans in a cause and effect relationship to 2,4,5-T/TCDD exposures. The Human Exposure Working Group also concluded that there were no epidemiologic data associating TCDD with any long-term health effects in humans other than persistent chloracne. While they did not find evidence of serious long-term health effects, neither could they find strong evidence for lack of effect. Most previous epidemiologic studies have not had sufficient statistical power to detect increased risks of low incidence/prevalence conditions in the observed populations, and the period of observation in many prospective studies has been less than ideal.

Several potentially valuable epidemiologic studies are currently in progress. Two independent and comprehensive studies of workers exposed to TCDD at a Monsanto manufacturing plant in Nitro, West Virginia, are currently being conducted (Mt. Sinai Medical Center, New York, and the Kettering Laboratory, University of Cincinnati, Ohio). These chemical industry workers were exposed over long periods of time, and were previously evaluated in 1953 and 1956, following an industrial accident which occurred in 1949. Zack and Suskind of the Kettering Laboratory have reported a follow-up study of 122 workers, 28 years

after heavy exposures to TCDD. There were 32 deaths in the group, and the relative risks of death were 0.69 for all causes, and 1.0 for malignancy; however, no firm conclusions can be drawn due to the small numbers involved. A Czechoslovakian study involving a 10 year followup of TCDD exposed workers, and a US National Cancer Institute (NCI) mortality study of 4,400 structural pest control workers are also underway. Preliminary results of a larger study of long-term morbidity by Suskind at the Nitro site have failed to reveal significant abnormalities other than persistent mild chloracne and decreased nerve conduction velocities, possibly associated with alcohol intake.

These new studies, and the continuing evaluations of the Seveso, Italy, population, should continue to provide valuable data. The large study groups involved in the Seveso and NCI studies should provide good statistical power, and the Nitro, West Virginia, and Czechoslovakian efforts will evaluate the effects of exposure after prolonged periods of time (10-30 years). The results of these studies should fill major gaps in the knowledge of 2,4,5-T/TCDD epidemiology, and should prove to be useful in evaluating the long-term effects of these compounds on health and reproductive outcomes.