Item ID Number	01394
Author	Beljan, John R.
Corporate Author	American Medical Association, Council on Scientific Affa
Report/Article Title	The Health Effects of "Agent Orange" and Polychlorinated Dioxin Contaminants: an Update, 1984, Technical Report
Journal/Book Title	
Year	1984
Month/Day	October 1
Color	☐
Number of Images	58
Description Notes	See items 483 and 1357 for earlier versions.

Reprinted by the Veterans Administration
with permission of AMA

THE HEALTH EFFECTS OF "AGENT ORANGE" AND POLYCHLORINATED DIOXIN CONTAMINANTS: AN UPDATE, 1984

American Medical Association
Chicago, Illinois

THE HEALTH EFFECTS OF "AGENT ORANGE" AND POLYCHLORINATED DIOXIN CONTAMINANTS: AN UPDATE, 1984

Technical Report

Prepared by the Council on Scientific Affairs'

Advisory Panel on Toxic Substances

John R. Beljan, MD, Chairman
Nelson S. Irey, MD
Wendell W. Kilgore, PhD
Kazuo Kimura, PhD, MD
Raymond R. Suskind, MD
Jaroslav J. Vostal, MD, PhD
R.H. Wheater, MS, Secretary

Environmental and Occupational Health Program

October 1, 1984

American Medical Association
Chicago, Illinois

Contents

The Health Effects of "Agent Orange" and Polychlorinated Dioxin Contaminants: Update, 1984

Preface

This report represents the second comprehensive study by the American Medical Association on the subject of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a topic that has been of considerable interest to the lay public and the medical community alike. In late 1980, the AMA's Council on Scientific Affairs directed its Advisory Panel on Toxic Substances to review and assess the available scientific information on Agent Orange and its associated contaminant, TCDD, and to develop objective information based on their findings. The outcome in October 1981 was a broadly accepted and widely distributed publication entitled, "The Health Effects of 'Agent Orange' and Polychlorinated Dioxin Contaminants." A summary version of the Panel's report also appeared in the 15 October 1982 issue of the Journal of the American Medical Association (JAMA).

In brief, The Panel found that,

> "In spite of the voluminous data on the biological effects of the phenoxy-type pesticides and their associated chlorinated dioxins, which oftentimes co-exist as contaminants of the pesticide formulations, there is still very little substantive evidence for many of the alleged claims that have been made against these compounds. The most serious of these allegations assert that Agent Orange, or compounds of a like nature, have caused malignant tumors, spontaneous abortions and birth defects. Although data from studies on experimental animals tend to support some of these claims, it is not certain that animal data are extrapolatable to man. No laboratory animal can fully substitute for man; we must, therefore, depend on the results of ongoing epidemiologic studies on persons who are known to have been exposed."

In the interim following publication, there has been a disquieting and continuing controversy both here and abroad over the long-term consequences of exposure to TCDD. The concern has not been limited to the military who served in Vietnam, but it has also extended to countless numbers of the general population who were believed to have been exposed to TCDD in the careless and oftentimes unwitting disposal of toxic wastes. Inasmuch as several important human epidemiologic studies have been underway since 1981, the AMA's House of Delegates called for an update of the Council's original report. Once again, the same Panel of experts was called upon to review the new studies, to consider the more recent and relevant scientific information, and to issue its conclusions and recommendations.

Acknowledgements

In the process of review, a number of principal investigators who were involved in ongoing human studies were invited to meet with the Panel and to brief it on the progress or results of their work. We are, therefore, indebted to the following persons and organizations for their cooperation:

Ralph R. Cook, MD (Dow Chemical USA); H. Denny Donnel, MD, MPH (Centers for Disease Control); Marilyn A. Fingerhut, PhD (NIOSH); William R. Gaffey, MD (Monsanto Co.); Sheila Hoar, ScD (National Cancer Institute); Richard E. Hoffman, MD, MPH (Missouri Division of Health); Renate Kimbrough, MD (Centers for Disease Control); George D. Lathrop, MD (US Air Force); Vern May, PhD (Dow

Chemical USA); D. Franco Merlo (Department of Epidemiology, National Cancer Research Institute, Genoa, Italy); Kenneth W. Sell, MD (National Institutes of Health); Barclay M. Shepard, MD (Veterans Administration); Robert F. Willis, PhD (Chairman, Joint Health and Welfare Canada/Environment Canada, Expert Advisory Committee on Dioxins); Alvin L. Young, PhD (White House Office of Technology Policy).

Introduction

Historical Perspective

Phenoxy herbicides were introduced into commerce and agriculture in the mid-40s with the advent of 2,4-D, followed by 2,4,5-T in 1948. Both were used extensively and uneventfully in the control of woody and broadleaf vegetation in croplands, forests, rights-of-way, and turf until 1969, when public concern was raised over the human health hazards of 2,4,5-T and/or its contaminant, TCDD. As this concern mounted, the Secretary of Agriculture suspended applications of the herbicide in areas where humans might encounter greatest exposure to TCDD. In 1971, the Environmental Protection Agency (EPA) initiated cancellation proceedings against certain other uses of 2,4,5-T. This notice was subsequently withdrawn, and EPA initiated a plan to develop necessary data for their decision-making processes. In April 1978, EPA issued a notice of Rebuttable Presumption Against Registration (RPAR), an intensive benefit/risk review to ascertain the desirability of continued registration and unrestricted use of 2,4,5-T.

One year later, the RPAR process was interrupted by an emergency ban on the use of 2,4,5-T in pastures, forests, rights-of-way, home gardens, and for aquatic weeds and other applications, excluding its use on rangeland and rice fields. This suspension was prompted by reports of spontaneous abortions in humans in Alsea, Oregon attributed to use of herbicides by the US Forest Service. Two studies done by the EPA--Alsea I and Alsea II--were criticized severely for insufficient evidence to support the alleged claims. Inasmuch as the EPA believed that silvex (or 2-2(2,4,5-trichlorophenoxy)-propionic acid) was related closely to 2,4,5-T and also apt to be contaminated with TCDD, the Agency suspended silvex as well.

During the US involvement in Vietnam, herbicidal mixtures of 2,4-D, 2,4,5-T, picloram, and cacodylic acid were widely employed as defoliants; these mixtures were variously identified as Agents Orange, White, Blue, Purple, Pink, and Green. The most widely used was Agent Orange, a 50:50 mixture of 2,4-D and 2,4,5-T; early formulations of the agent were heavily contaminated with as much as 47 ppm (a weighted mean concentration of 1.98 ppm) of 2,3,7,8-TCDD (Young et al, 1978).

After termination of defoliation operations in Vietnam in 1970, two reports by Dr. Ton-That Tung (1971, 1973) linked Agent Orange with increases in liver cancer, abortion, and birth defects. Although hard scientific evidence for that conclusion was lacking in Tung's reports, a variety of other studies clearly demonstrated that TCDD was extremely toxic to laboratory animals.

A large number of US personnel in Vietnam who may have been exposed to herbicides are greatly concerned that Agent Orange could be responsible for the deleterious health effects that they and their offspring are experiencing. As a consequence, a class action suit was filed in 1979 against five herbicide manufacturers (Dow Chemical, Monsanto,

Hercules, Diamond Shamrock, and Thompson-Hayward); the suit was extended to "all American servicemen whose health has been damaged because of contact with Agent Orange." The companies, in turn, filed a third-party action against the US government, passing the responsibility for alleged harm to the government for its "negligent misuse" of the chemicals. A similar class action suit was filed in January 1981 against the Veterans Administration and the Department of Defense.

Some 2.4 million Vietnam veterans, including 1,200 Operation Ranch Hand personnel, and 20 civilians involved in destroying surplus Agent Orange, are presumed to have been exposed. The news media have stated that potential "plaintiffs" will exceed 2.5 million people and suggest that 40,000 veterans may become ill or die; an additional 2,000 or more children also will suffer "catastrophic" birth defects. More than 1,250 lawyers from 150 law firms in the United States have been involved in the litigation as of 1980 (Elson, 1980). The suit was scheduled to go to trial in Brooklyn, New York in May 1984. The corporation defendants, however, came to an out-of-court agreement; without admitting culpability, they set aside $180 million for distribution to claimants and their respective heirs. An equitable means for assessing and awarding damages remains to be established. Many persons are not satisfied with the outcome and may still seek redress in the courts. Meanwhile, the veterans, their heirs, and the general public anxiously await medical evidence that will either support their allegations of harm from the chemical or prove convincingly that dioxin has no serious health consequence.

To date, at least 579 industrial workers are also known to have been exposed to TCDD. The first of several accidental releases of TCDD during the manufacture of 2,4,5-trichlorophenol (TCP) or 2,4,5-T occurred in 1949; a larger number of employees were also exposed to the manufacturing process from 1948-69. A suit has been filed against the Monsanto Chemical Company on behalf of former Monsanto employees and representatives of deceased persons who worked in the company's Nitro, West Virginia plant.

One of the first large-scale exposures of the general population to TCDD occurred near Seveso, Italy in July 1976; the compound was released accidentally at the ICMESA (Industrie Chimiche Meda Societa Anonima) trichlorophenol synthesis plant. Approximately 500 acres of surrounding countryside were contaminated to varying degrees. Some people in the most highly contaminated area became ill immediately, while others experienced chemical burns of the skin, abdominal pain, and internal hemorrhage. Although there were deaths of small animals and vegetation, there were no documented human deaths. Approximately 511 of the 736 evacuees were allowed to return to their homes within a year (Homberger, 1979). Meanwhile, cleanup of the contamination in Seveso has been completed. Seven-million cubic feet of the dirt were scraped from 115 acres and sealed in a reservoir outside the town. All houses in the immediate area were destroyed and the area planted with trees; former residents have been forbidden to return to their former homesites (Anon, 1984).

In the early 1970s, dioxin-contaminated waste oil was widely applied to certain areas of Missouri to suppress dust, most notably near the town of Times Beach and at three horse arenas in Lincoln County. Reports were to follow of animal deaths and complaints of human illness that sparked a national concern, which was further fueled by the news media: the town of Times Beach was purchased by the government, other extensively contaminated sites were discovered in eastern Missouri and elsewhere in the US, and new rule-making by the EPA was established for the disposal of dioxin-contaminated wastes.

Meanwhile, several reports from Scandinavia indicated a possible link between exposure to dioxin and cancer, especially soft tissue sarcomas. These reports were soon followed by discovery of a number of soft tissue sarcomas among an industrial population in the

US. An apparent increase in the number of deaths due to connective tissue cancers among white women was then perceived in the Midland, Michigan area; this news received wide publicity by environmental groups when dioxin contamination was discovered in the Tittabawassee and Saginaw Rivers, Saginaw Bay, and the grounds of Dow Chemical Company. The Michigan Department of Health investigated the situation, the outcome of which is presented below.

In May 1983, a representative action was brought by several Nova Scotian residents against a forest industry company that operated in their area. Plaintiffs were seeking an injunction that would prevent the company from further spraying of 2,4-D, 2,4,5-T, and mixtures thereof. A major part of the documentary evidence and testimony of environmentalists and medical experts related to the alleged adverse health effects of these compounds and dioxin. Quoting from the opinion of the trial judge (Nunn, 1983), who denied the injunction for lack of evidence that the spray operation constituted an unreasonable risk to health:

> "...on the whole of the evidence, where risk to health is claimed in any study, the circumstance has been one of massive exposure and such are not of significant probative value in light of the actual low possible exposure here. ...most of the more highly publicized studies in these situations are regarded in the wider scientific community as flawed...particularly...the Alsea II study. Where risk to health might be expected, for example, ...at Seveso, which caused a massive dioxin exposure, none have found. ...the evidence of risk assessments clearly indicates that any risk here in Nova Scotia, if, indeed, there is a risk at all, is infinitessimally small and many, many times less than one in a million...(whereas,) the risk of cancer to a smoker is 1 in 800, and for a non-smoker continuously in the same room with smokers it is 1 in 100,000...this court is of the opinion that these spraying operations can be carried out in safety and without risk to the health of the citizens of the province. (Nunn DM: Judicial decision in the trial of Palmer et al vs Stora Kopparbergs Bergslags Aktiebolag (or Nova Scotia Forest Industries), Supreme Court of Nova Scotia (15 Sep 1983)).

The general public continues to be concerned about the possible adverse health effects of dioxin, especially those of a long-term nature; eg, cancer, birth defects and reproductive abnormalities. However, this concern is best addressed by scientific facts, not fanciful conjecture.

Summary of AMA's 1981 Report

Before discussing newer developments, it would be well to review the salient points of the 1983 report.

* Agent Orange, as used in Vietnam, was a 50:50 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), together with a minor amount of the free acid 2,4,5-T (1% of the total mixture) and varying amounts of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

* Commercial formulations of 2,4,5-T, as well as silvex (or 2-2(2,4,5-trichlorophenoxy)propionic acid) may contain TCDD as a contaminant. At one time, the amount of TCDD was as much as 70 ppm; present methods routinely produce levels below 0.01 ppm. TCDD may form as a by-product in the synthesis of 2,4,5-trichlorophenol (a precursor of 2,4,5-T). It may also be produced by the pyrolysis of certain chlorinated compounds in industrial or municipal wastes and

by the burning of vegetation that has been sprayed with the chlorinated phenoxy acid herbicides. Note that 2,4-D, a phenoxy herbicide closely related to 2,4,5-T, is not normally contaminated with TCDD.

* In addition to the marked variations in the sensitivity and susceptibility of animal species to all toxic substances, there are significant differences between some of the toxic effects of TCDD in experimental animals and the human experience; thus, the animal data cannot be translated directly to man.

* One of the more pronounced biological effects of TCDD, as well as a number of other chlorinated aromatic compounds, is a tendency to cause chloracne in certain animals and man. This skin disease is, in fact, regarded as the clinical marker for TCDD exposure. Systemic disorders in man from exposures to TCDD are unlikely to occur in the absence of chloracne. Such signs and symptoms as impaired liver function, nephropathy, gastroenteritis, myopathy, neuropathy, and central nervous system manifestations have been reported after exposure to large amounts of TCDD; however, they have not been progressive and have always cleared with time (the nephropathy and myopathy have not been proven).

* Other toxic effects of TCDD in experimental animals appear as pathological effects in the liver, peripheral nerves, and hematopoietic and reticuloendothelial systems. TCDD is a very powerful enzyme inducer. In addition to altering normal enzyme activity, it may potentiate the harmful action of other toxins or even render an otherwise innocuous agent toxic.

* TCDD promotes carcinogenesis or induces cancer in some strains of rats and mice. In contrast to other chemical carcinogens, however, this carcinogenicity is always accompanied by considerable systemic toxicity. By itself, 2,4,5-T does not presently appear to be a carcinogen, nor has it been shown to cause genetic changes in any animal species.

* A consensus of studies utilizing high prenatal doses of 2,4,5-T with 0.1 ppm or less of TCDD showed cleft palate in mice (but no other species) and embryotoxicity in the mouse, rat, hamster, sheep, monkey, and rabbit. TCDD does induce genetic changes by the Ames test with S. typhimurium and E. coli. However, dominant lethal and cytogenetic evaluations in rodents do not confirm that such changes also occur in other animals, especially man.

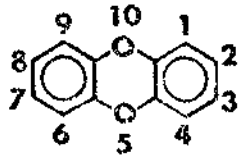
* While 2,4,5-T and 2,4-D pesticides have been used in agriculture, forest management, and commercial and residential landscaping for over 30 years, there is still no conclusive evidence that they and/or TCDD are mutagenic, carcinogenic, or teratogenic in man, nor that they have caused reproductive difficulties in man.

* Both 2,4-D and 2,4,5-T undergo rapid decomposition in the soil and are, therefore, of little environmental concern. TCDD does persist in soil longer than 2,4,5-T but, in general, ... in the presence of ultraviolet light, it breaks down rapidly when present as a thin film on plants, water, and the surface of soil. (Its half-life in deep soil is now known to be much longer than one year.)

Chemistry of Dioxins

General Structure

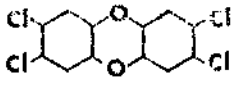
Of late, the term "dioxin" has been applied--though incorrectly--to any of a number of polychlorinated dibenzo-p-dioxins and more specifically to 2,3,7,8-tetrachlorodibenzo-p-dioxin (or "TCDD" as it is usually but inappropriately called for brevity). More precisely, a dioxin is any of a group of dibenzo-p-dioxins whose structure consists of two benzene rings interconnected by two oxygen atoms as shown below:



Dioxins differ only in the nature and position of their substituents; ring positions 1 through 4 and 6 through 9 can be occupied by hydrogen or halogen atoms, or various organic radicals. Theoretically, there can be 75 different chlorinated dioxins; 40 different chloro- isomers have been prepared and identified to date (Buser, 1975; Buser et al, 1978; Poland et al, 1972; Bolton, 1978), while five others have been identified but not separated from their accompanying compounds (Buser, 1975; Buser et al, 1978; Rappe, 1978). Dow Chemical Company has synthesized all 22 tetrachloro (TCDD) isomers (US EPA 1980).

2,3,7,8-tetrachlorodibenzo-p-dioxin

Of the 22 tetrachlorodibenzo-p-dioxins, the 2,3,7,8- isomer--wherein chlorine has been substituted for hydrogen in the 2,3,7 and 8 positions in the dibenzo-p-dioxin structure shown above--has received the most attention because of its adverse health effects in experimental animals and its potential hazard to humans. It is structurally represented as:



TCDD

2,3,7,8-TCDD is a colorless, crystalline solid at room temperature, chemically stable and extremely lipophilic. It is only sparingly soluble in water and most organic liquids. Some of its physical properties, along with those of 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (OCDD), another chlorinated dioxin with twofold symmetry, are listed in Table 1 (Crummett et al, 1973; International Agency for Research on Cancer, 1977).

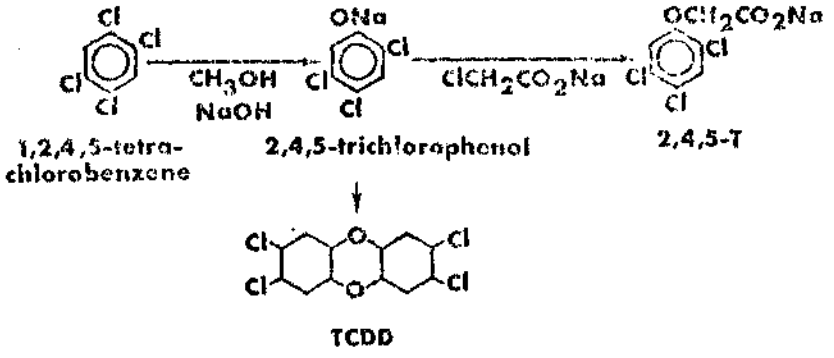
Table 1 -- Physical Properties of Two Chlorinated Dioxins, 2,3,7,8-TCDD and 1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (OCDD)

Property		2,3,7,8-TCDD	OCDD
Empirical formula		$C_{12}H_4Cl_4O_2$	$C_{12}Cl_8O_2$
Percent by weight	C	44.7	31.3
	O	9.95	7.0
	H	1.25	----
	Cl	44.1	61.7
Molecular weight		322	459.8
Melting point		305°C	130°C
Decomposition temperature		Above 700°C	Above 700°C
Solubility			
o-Dichlorobenzene		1.4 g/liter	1.83 g/liter
Chlorobenzene		0.72	- - -
Anisole		- - -	1.73
Xylene		- - -	3.58
Benzene		0.57	- - -
Chloroform		0.37	0.56
n-Octanol		0.048	- - -
Methanol		0.01	- - -
Acetone		0.11	- - -
Dioxane		- - -	0.38
Water		0.2 ppb	- - -

Adapted from the US Environmental Protection Agency (1980).

Formation of Chlorinated Dioxins

One route to the formation of chlorinated dioxins begins with certain chlorinated organic chemicals. 2,4,5-Trichlorophenol, for example, can condense to form a phenoxyphenate or substituted diphenyl ether (a "predioxin") (Buser, 1978; Jensen et al, 1972; Moore, 1979; Nilsson et al, 1974) as shown here:



"Predioxins" have been found in waste sludges and commercial products, as well as in some products of laboratory scale reactions (Jensen et al, 1972, 1973; Arensault, 1976).

Chlorinated dioxins also can be formed during the combustion of various substances. For example, Olie et al (1977) reported the occurrence of chlorinated dioxins in the fly ash and flue gases of municipal incinerators in the Netherlands, while Buser et al (1978) found chlorinated dioxins in fly ash from a municipal incinerator and in an industrial heating facility in Switzerland; the levels ranged from 0.2 ug/g to 0.6 ug/g of ash. Buser (1979) also reported that chlorinated dioxins were formed by the pyrolysis of chlorobenzenes in the presence of air. Other studies have shown that chlorinated dioxins are to be found in cigarette smoke, fireplace soot, and in the burning of wood (Dow Chemical Company, 1978; Nestrick et al, 1982).

Occurrence of Chlorinated Dioxins

In 2,4,5-T (2,4,5-trichlorophenoxy acetic acid)

The levels of TCDD in drums of Herbicide Orange placed in storage in the US and in the Pacific before 1970 varied from 0.1 to 47 ug/g (Firestone, 1978). Since Herbicide Orange was formulated as a 1:1 mixture of the butyl esters of 2,4-D and 2,4,5-T, it is conceivable that the levels of TCDD in individual 2,4,5-T formulations for agricultural and residential use in the 1960s could have been as high as 100 ug/g. After the enactment of government regulations, the amounts of TCDD in 2,4,5-T formulations should be less than 0.1 ug/g.

In Chlorophenols

The most important use for chlorinated phenols, such as pentachlorophenol, 2,3,4,6-tetra-, and 2,4,6-trichlorophenols, is as a preservative of wood. These chlorophenols can contain a variety of contaminants, including polychlorinated dibenzo-p-dioxins (PCDDs) as well as dibenzofurans (PCDFs) (Nilsson et al, 1978). There can be several positional isomers of PCDDs and PCDFs, and since the relative ratios of PCDF to PCDD depend on the particular route of synthesis for the chlorophenol in question, the ratio of PCDFs/PCDDs can range from about 1 to greater than 20. Usually the level of 2,3,7,8-TCDD is relatively low, although a Scandinavian preparation of 2,4,6-trichlorophenol contained 0.5 ppm of 2,3,7,8-TCDD (Rappe et al, 1979).

In Hexachlorophene

Hexachlorophene is a bactericide and, like 2,4,5-T, is synthesized from 2,4,5-trichlorophenol. However, after extensive purification, the amount of 2,3,7,8-TCDD in hexachlorophene is usually less than 0.03 ug/kg (Rappe et al, 1979).

Decomposition of Chlorinated Dioxins

Photodegradation, or photolysis, is the process whereby chemical bonds are broken by means of photons of light. As applied to chlorinated aromatics, such as chlorinated dioxins, photodegradation usually proceeds by progressive loss of chlorine to create a free-radical, or a loss of chlorine through a nucleophilic displacement in the presence of a solvent or substrate molecule. According to Crosby (1982), three requirements must be met if degradation is to proceed: 1) light energy must be absorbed by the compound, 2) light of the appropriate wavelength must be available at a meaningful intensity, and 3) a source of excess reducing agent (proton donor) must be present, inasmuch as the reaction involves reductive dechlorination.

There is clear experimental evidence to show that dioxins may be photodegraded in the environment by natural sunlight. The maximum absorption of ultraviolet light for chlorodioxins is in the range of 290 to 320 nm; 2,3,7,8-TCDD absorbs maximally at 307 nm. Crosby et al (1971) studied photolysis rates of 2,3,7,8-TCDD dissolved in methanol using both natural and artificial sunlight of 100 mW/cm^2 intensity and at the absorption maximum of 2,3,7,8-TCDD. Complete photolysis to less chlorinated dioxin isomers occurred in 24 hours under natural sunlight, while 6 hours of artificial light destroyed almost 50% of the original 2,3,7,8-TCDD.

Irradiation had no effect on a crystalline water suspension of 2,3,7,8-TCDD. Apparently the crystalline state may prevent the loss of chlorine or abstraction of hydrogen atoms (Plimmer, 1978). On the other hand, irradiation reduced the dioxin content of a benzene solution of 2,3,7,8-TCDD added to water and stabilized with a surfactant (Plimmer et al, 1973). Crosby (1971) also found that, when 2,3,7,8-TCDD was applied to dry or moist soil, irradiation caused no change after 96 hours. In addition, no degradation occurred when the substance was applied to a glass plate and irradiated for up to 14 days.

When Crosby et al (1977) exposed on glass petri dishes thin layers of Herbicide Orange containing 15 ppm of 2,3,7,8-TCDD to summer sunlight, approximately 60% of the TCDD disappeared after 6 hours. A commercial herbicide composed of butyl esters of 2,4-D and 2,4,5-T and 10 ppm 2,3,7,8-TCDD was exposed in the same manner; after 6 hours, only about 30% of the initial TCDD content remained. Herbicide Orange was also applied to excised rubber plant leaves and to the surface of Sacramento loam soil and then exposed to sunlight; no TCDD was detected on the leaves after 6 hours at an application rate of 6.7 mg/cm^2 of leaf surface, while about 30% remained after 6 hours when applied at 1.3 mg/cm^2. About 90% of the TCDD remained after 6 hours when the dioxin was applied to soil at a rate of 10 mg/cm^2. According to the authors, partial shading of the lower layers by soil particles accounted for the lesser degree of photolysis.

Analysis

Extraction

Since 2,3,7,8-TCDD has been found in a variety of substances, a number of different extraction procedures have been developed to separate the compound from its substrate and to reduce the sample volume to a manageable size. Generally, these procedures take place in either a neutral or highly basic (alkaline) medium. Contaminants, such as polychlorinated biphenyls (PCBs) and dichlorodiphenylethane (DDE) isomers, are frequently present in the extracts in relatively high amounts compared to the likely amount of 2,3,7,8-TCDD. It is now possible to isolate and quantitatively determine 2,3,7,8-TCDD in the presence of the other twenty-one tetrachloro- isomers.

The basic extraction procedures were developed first for the detection of 2,3,7,8-TCDD in environmental samples (Baughman et al, 1973 a,b; Crummett et al, 1973). After digestion with alcohol and a strong base, a sequence of extractions with various organic solvents (eg, ethanol, petroleum ether, methylene chloride, hexane) separates the TCDDs from the alkaline mixture. The resulting extracts are combined, washed with distilled water and a strong acid, then treated to remove any traces of water and passed through one or more chromatographic columns for the partial removal of contaminants.

The neutral extraction procedure was developed primarily to avoid a possible base-catalyzed formation of chlorodioxins within the mass spectrometer. In this process, developed by O'Keefe et al (1978) and modified by Albro et al (1977), the sample is first

extracted with hexane; the extract is then passed through a series of chromatographic columns--the first being magnesia-Celite 545, followed by an alumina minicolumn, and finally a Florisil minicolumn. The Florisil column is eluted with methylene chloride and the product then condensed for analysis. This technique is particularly effective for fish tissues and human milk.

Detection

Technological advances in analytical chemistry permit the detection of dioxin concentrations at least as low as one part per trillion (1 ppt). However, relatively few analytical laboratories are capable of identifying, much less quantifying, individual isomers at such a low level. Then too, regulatory agencies can only speculate as to the safest levels that should be allowed for human exposures.

Most of the current methods for the detection of 2,3,7,8-TCDD employ gas chromatography (GC) and/or mass spectrometry (MS) and even tandem mass spectrometry (MS/MS). Other procedures--electron spin resonance spectroscopy (ESR), low-temperature phosphorescence emission spectroscopy (LTE), and ultraviolet spectroscopy (UV)--have been used, but their usefulness is limited by poor selectivity and sensitivity.

For the present, a combination of gas chromatography and mass spectrometry (GC-MS)--both low- and high-resolution--is used almost exclusively for the detection and quantitative analysis of TCDDs. In the low-resolution scheme, fragment ions at nominal mass: energy ratios (m/e) of 320 and 322 are monitored and measured as the TCDDs emerge from the gas chromatograph. To differentiate these compounds from those with nearly identical mass, such as pentachlorinated biphenyls, high-resolution mass spectrometry can distinguish the dioxin component with an exact mass of 321.8936.

Toxicological Evidence of a Health Hazard in Animals

General Discussion

2,3,7,8-Tetrachloro-dibenzo-p-dioxin is still considered to be one of the most powerful poisons for lower animals and the most toxic of all tetrachloro- isomers. The large differences in acute toxicity among animal species have not been satisfactorily explained; toxicity levels for higher animals, such as the dog, are estimated to be between 300 and 3000 ug/kg of body weight (BW) (Schwetz et al, 1973). Acute toxicity symptoms develop after a latent period of 7 to 10 days; the animals experience a rapid loss in body weight and depletion of adipose tissue. The loss of body mass is only partially related to the reduced food intake--"wasting syndrome." The final mechanism responsible for death is unknown, but the general clinical appearance of the animals suggests general exhaustion of energy reserves (McConnell et al, 1978).

Significant reduction in the size of the thymus dominates the postmortem findings in all animal species (Buu-Hoi et al, 1972; Gupta et al, 1973; Vos et al, 1973), but the animals do not die from infection secondary to immune suppression (Greig et al, 1973). Pathological changes in other organs are characteristic for a given species; eg, hepatic lesions are striking in mice, rats and rabbits but relatively minor in guinea pigs, monkeys, cattle and horses. The organ manifestation may be related to the distribution of the compound in the body; mice and rats retain several times more of the compound in the liver, while guinea pigs have much lower concentrations in this organ (van Miller, 1976).

Mucosal changes, particularly cellular hyperplasia or metaplasia, have been observed in the gastric, intestinal, and urinary tracts of primates and cattle but were not reported in

the rat, mouse, and guinea pig.

Similar hyperplastic epidermal changes--ie, chloracne--are the most characteristic lesions observed in exposed human populations. Corresponding lesions do not, however, appear in many laboratory species; ie, rats, hamsters, guinea pigs. In mice, characteristic epidermal changes occurred in hairless strains but not in the ordinary laboratory or wild types of animals (Inagami et al, 1969; Knutson et al, 1982). It is interesting to note that the absence of the hair follicle is not a prerequisite for the skin changes; the symptoms were not produced by TCDD directly applied to areas without hair (ear) in laboratory mice (Poland et al, 1982). Local application of TCDD did produce characteristic changes in the ear of the rabbit (Kimmia et al, 1957; Vos et al, 1971). Specific epidermal changes also have been observed after systemic administration in rabbits and primates (Allen et al, 1977; McNulty, 1977; McConnell et al, 1978; McNulty et al, 1980, 1981).

The presence of TCDD increases keratinization in epidermal cell tissue cultures in spite of the simultaneous partial inhibition of DNA synthesis. This observation suggests that TCDD can stimulate differentiation of human epidermal cells in culture (Osborne et al, 1984).

The exact mechanisms by which TCDD produces its toxic action are still unknown. Biochemical studies indicate that TCDD is a very potent inducer of aryl hydrocarbon hydroxylase (AHH), as well as the mixed-function oxidases and the cytochrome P_1-450 (P-448) enzymes of the liver, lung, placenta, and kidney of the mouse and rat. However, Poland et al (1982) do not believe that the induction process is directly responsible for TCDD's toxicity; they attribute the pleiomorphic clinical symptomatology to the presence of an intracellular receptor with high binding affinity for TCDD and other aromatic hydrocarbons (Ah locus) (Nebert et al, 1972). Poland et al (1976) identified a stereospecific TCDD receptor in the hepatic cytosol of laboratory mice. The receptor has a maximum binding capacity of approximately 8×10^{-14} TCDD mol/mg of cytosol protein, or approximately 5×10^4 TCDD binding sites per cell. The receptor binds other aromatic hydrocarbons with enzyme induction properties, but the binding affinities in these instances are only 1/3 to 1/30 that for TCDD. Enzyme-inducing compounds other than aromatic hydrocarbons do not compete for this particular receptor.

The receptor is a high-molecular-weight protein that serves as a carrier in the nuclear uptake of TCDD (Carlstedt et al, 1979; Greenlee et al, 1979; Okey et al, 1979, 1980; Weaver, 1980). Once within the nucleus, the TCDD receptor complex binds to a specific set of genes in the Ah locus, which initiate the production of RNA responsible for the synthesis of new cytochrome P-450 enzymes.

Since the hepatic receptor affinity and the enzyme induction potency of individual TCDD isomers correspond to their LD_{50} and since the presence of the Ah locus is necessary for expression of TCDD toxicity, Poland et al (1982) have postulated that the TCDD response is mediated by its binding to the induction receptor. However, the sole presence of the Ah locus, although essential, is not sufficient for the full expression of toxicity. Many tissues respond to TCDD by enzyme induction but not all with an adverse reaction and, while animal species vary widely in their sensitivity to TCDD, they do not differ in their liver receptor concentration or affinity. The authors suggest that TCDD can induce enzymes in all tissues that contain the receptor, but activation of an additional battery of genes to control the expression of a fully developed toxic response only occurs in some tissues. The composition of these additional genes may vary in different tissues but their presence is essential for the manifestation of TCDD toxicity; thus, enzyme induction may be only a secondary sign not directly related to the toxicity of TCDD.

Analytical assays were developed for the quantification and characterization of the TCDD cytosolic receptor (Gasiewicz et al, 1982; Mason et al, 1982; Gasiewicz et al, 1984); they confirm that the hepatic cytosolic receptor in the Sprague-Dawley rat is similar to the one described in the "responsive" genetic strains of mice (Poland et al, 1976). Genetic strains that are nonresponsive to TCDD toxicity have very low or unmeasurable levels of the receptor in the liver (Poland et al, 1976; Okey et al, 1979; Gasiewicz et al, 1982). High concentrations of the receptor are localized in the liver, lung, intestine, and kidney, which produce the highest levels of induced AHH activity in all tested animal species (Nebert et al, 1969). Gasiewicz et al (1984) reported that the receptor concentrations in the liver and lung of the Sprague-Dawley rat rapidly increase after birth then decline after 21 days of age; levels in the thymus, on the other hand, remain constant between 2 and 70 days. In the adult rat, the thymus contains the highest concentrations of the receptor.

Recent laboratory data indicate that the thymic epithelium and its differentiation is the primary and most sensitive site of TCDD toxicity. Administration of TCDD produced genetically dependent thymic atrophy in mice (Poland et al, 1980) and suppressed a variety of lymphocytic functions (Voss et al, 1973; Faith et al, 1977; Faith at al, 1979). Clark et al (1981) found that thymic generation of cytotoxic lymphocytes (CTX) is particularly sensitive to inhibition, since it occurs at a dose level that is 10 to 100 times lower than the dose required for enzyme induction in the liver (weekly dose of 0.001 ug/kg BW). However, TCDD is not toxic when added directly to a culture of thymic cells, and an indirect mechanism was postulated for its suppressive effects on the immune system (Knutson et al, 1980; Clark et al, 1981). Mason et al (1982) reported the presence of TCDD cytosol receptor in the murine thymus, and Clark et al (1983) suggested that TCDD produces immunosuppression by acting on the differentiating process of lymphoid cell population within the thymus rather than on mature immunocompetent cells in the peripheral lymphoid organs. Recently, replacement of peripheral lymphocytes in the irradiated bone marrow of mice with a genetically different sensitivity to TCDD indicated that the immunosuppressive effect of TCDD on the thymic generation of allospecific cytotoxic T-lymphocytes (CTL) is predetermined by the sensitivity of the radio-resistant tissue of the host, not by the grafted lymphomyeloid cells. Although the impaired generation of CTL was accompanied by increased activity of suppressor T-cells, the authors concluded that TCDD acts indirectly through receptors in the thymic epithelium rather than on the lymphomyeloid tissue (Nagarkatti et al, 1984). Their results were confirmed by Greenlee et al (1984), who co-cultivated thymocytes with thymic epithelium in the cell culture. Subsequent treatment with TCDD altered the capacity of the thymic epithelium to support the intrathymic differentation of T-lymphocytes.

Since the symptoms of TCDD toxicity resemble manifestations of thyroid dysfunction--eg, loss of body weight, alopecia--the role of the thyroid gland in mediating the toxicity of TCDD has been suggested (Bastomsky, 1977; Neal et al, 1979). However, Gasiewicz et al (1980) observed unchanged mortality in euthyroid rats given the lethal dose of TCDD and maintained at constant or increasing body weight by parenteral nutrition. In contrast, administration of the same lethal dose resulted in no mortality in athyroid (thyroidectomized) animals in spite of the fact that their body weight losses were not different from the TCDD-treated euthyroid animals. Supplementation of thyroidectomized animals with thyroid hormone (T4) brought mortality to the level of the control group 31 days after injection (Rozman et al, 1984 a) but to only one-half of the control group at 90 days (Rozman et al, 1984 b). Triiodothyronine (T3) was equally effective (Scheufler et al, 1984).

The whole body half-life of TCDD is between 20 and 30 days in the rat (Rose et al, 1979) and guinea pig (Gasiewicz, 1979; Nolan, 1979), and 10 to 12 days in the hamster (Olson et al, 1980). Differences in the rates of elimination cannot completely explain differences in toxicity: guinea pigs and rats have identical half-lives but their susceptibilities to TCDD (LD_{50s}) are different. The discrepancy is more striking in the Syrian hamster whose elimination rate is nearly the same as that of the guinea pig and rat but its resistance to TCDD toxicity is larger by several orders of magnitude.

Similarly, the accumulated body burden of TCDD does not seem to be related to toxicity, for the Syrian hamster can tolerate up to 5,000 ug of TCDD per kg of tissue without signs of liver toxicity.

Some investigators think that the more resistant animal species, such as the beagle dog, convert TCDD to metabolites at a higher rate (Poiger et al, 1982). Weber et al (1981) found the toxicity of the crude canine biliary metabolite to be at least 100 times lower than that of TCDD. The detoxification process occurs primarily through hydroxylation and methylation of the tetrachloro- molecule to the trichloro- metabolite and finally cleavage of one or both ethereal bridges in the dioxin molecule (Poiger et al, 1982). TCDD metabolites, however, differ among individual animal species; for example, different biotransformation products were identified in the laboratory rat (Poiger et al, 1982). Olson et al (1983) found that most of the metabolites in the hepatocytes, bile, and urine of the Syrian hamster are glucuronic acid conjugates; the 1-hydroxy derivative of the tetrachloro- isomer was the most abundant metabolite in the hepatocytes. Administration of P-450 cytochrome inhibitors, such as SKF-525, slightly decreased the metabolic rate of TCDD (Neal, 1983). This is consistent with previous findings wherein pretreatment with enzyme inducers decreases the lethality of TCDD, but administration of P-450 blockers increases it (Beatty et al, 1978).

Mutagenicity

No conclusive evidence of mutagenicity has been observed in microbial assay studies of TCDD. Of more relevance to man are the results on mammalian cells--ie, HeLa; Balb-3T3, normal mouse fibroblasts; SV101, virus (SV40)-transformed 3T3 mouse fibroblasts; human foreskin fibroblasts and normal human lymphocytes (Beatty et al, 1975). There was no significant growth inhibition in the cell cultures, nor were there discernible ultrastructural changes under electron microscopy.

Rogers et al (1982) examined the mutagenicity of TCDD in L-5178Y mouse lymphoma cells. Doses of 0.1 to 0.5 ug TCDD/ml induced significant mutations in the methotrexate-, excess of thymidine-, and thioguanine-selection systems. No induced mutations were noted in the ouabain or cytosine-arabinoside systems. The authors concluded that mutagenic effects of TCDD show similarities with proflavin and other acridines, its genotoxicity being exerted by DNA intercalation (Wassom et al, 1978).

In baby hamster kidney cells (BHK cell transformation system), 2,3,7,8-TCDD was also positive, the dichloro- and trichloro- isomers were weakly positive, and the octachloro- and unsubstituted dioxins were negative; unfortunately, the authors provided no specific information on the test concentrations (Hay et al, 1983).

Tests for chromosomal aberrations in bone-marrow cells of laboratory rats were negative after a single administration of TCDD (Green et al, 1975) but positive after 13 weeks of chronic dosage (Green, 1977). Similarly, single doses of TCDD did not induce dominant lethal mutations (Khera et al, 1973).

It may be concluded that the positive data on the mutagenic activity of TCDD are isolated in the literature and refer mainly to high TCDD concentrations in closed laboratory systems. The positive findings contrast sharply with data of Poland et al (1979) who examined the in vivo covalent binding of TCDD to rat liver macromolecules using minimal levels of TCDD to interact with DNA. Although 18% to 64% of the administered dose accumulated in the liver, virtually all of the deposited radioactivity was extractable. Only 6 pmol of TCDD per mol of nucleotide residuum was associated with the DNA. This represents a binding level that is four to six orders of magnitude lower than that of most chemical carcinogens, and it contradicts the probability that covalent binding of TCDD to DNA and subsequent somatic mutation is responsible for the oncogenic potency of TCDD.

Oncogenicity

Several investigators have examined the tumor-initiating and -promoting potencies of TCDD on mouse skin. DiGiovanni et al (1977) found little or no tumor-causing or tumor-promoting properties of TCDD, and Berry et al (1978, 1979) found an inhibitory effect on skin tumors in mice.

In contrast, dermal application of TCDD was carcinogenic in Swiss-Webster mice (NTP Rept 80-32, 1980); the females had integumentary fibrosarcomas, while results from the males were inadequate. Poland et al (1982) tested the capacity of TCDD to promote tumor formation in the skin of hairless and haired HRS/J inbred mice, which have a genetically different sensitivity for TCDD. The authors found a powerful promoting effect of TCDD in the "responsive" hairless mouse but not in the resistant heterozygote (haired) strain. This effect was 100 times stronger than that of tetradecanoyl-phorbol acetate (TPA). It is important to note that TCDD produces acneiforme changes in the skin of a hairless mutant that are identical to those observed in human skin (Knutson et al, 1982).

In 1982, the International Agency for Cancer Research (IARC) reviewed all studies in which TCDD was tested orally in mice and rats and dermally in mice; the agency declared that the evidence for TCDD's carcinogenicity in animals was sufficient (IARC, 1982). The experimental evidence came primarily from two sources: 1) chronic feeding studies where only the highest administered level (0.1 ug TCDD/kg BW per day) produced an increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue in the Sprague-Dawley rat (Kociba, 1973, 1978), and 2) studies by the National Toxicology Program (NTP) in which TCDD given by gavage was carcinogenic in the B6C3F1 mouse; hepatocellular carcinomas were observed in both sexes of mouse but only female mice and male rats developed follicular cell thyroid adenomas (NTP-Rept 80-31).

The positive initiating effects of TCDD in the chemical carcinogenesis process conflict with indications that neither TCDD nor its metabolites form covalent adducts with cellular DNA. Poland et al (1979) found no significant DNA binding in vivo after administration of radioactive labeled TCDD; similar results were reported for in vitro TCDD binding (Piper et al, 1973; Vinopal et al, 1973; Ghiasuddin et al, 1975; Rose et al, 1976; Nelson et al, 1977).

Pitot et al (1980) tested TCDD's potential for promoting hepatocarcinogenesis following partial hepatectomy and a single initiating dose of diethylnitrosamine (DEN); TCDD was thereafter administered subcutaneously at two levels every two weeks for seven months. The higher dose (1.4 ug/kg BW) produced five hepatocellular carcinomas, whereas the animals treated only with DEN had no carcinomas and only a few enzyme-

altered foci. The authors concluded that TCDD is a potent promoter of hepatocarcinogenesis. Similar conclusions were reported in TCDD promotion studies on the skin of the "responsive" strain of the HRS/J hairless mouse (Poland et al, 1982).

Teratogenicity

2,4,5-T containing 30 ppm of TCDD in two strains of mice (C57BL/6 and AKR) and one strain of rats, produced increased incidences of cleft palate in mice and cystic kidney in the rat (Courtney et al, 1970); when comparably dosed with a sample containing only 1 ppm of TCDD, teratogenesis was not evident (Emerson et al, 1971). A gavage dose in the rat of 0.125 ug/kg/day from the 6th-15th days of gestation showed only a slight effect that was much pronounced at the level of 0.5 ug/kg/day (Sparschu et al, 1971).

In utero exposure of mice to TCDD during the final half of the gestation period produced fetuses with poorly developed lymphatic systems and fatty infiltrates in the liver (Neubert et al, 1973).

Prenatally, TCDD causes hydronephrosis, which according to Gibson (1976), may be only a sign of delayed maturation and not permanent damage inasmuch as the test animals were taken by cesarean section. Gibson did find altered PAH transport from kidney slices of neonate animals even where there was no evidence of a lesion; the significance of these data in the adult is unknown. TCDD also caused postnatal hydronephrosis in a suckling pup whose foster mother had been dosed beforehand with the compound (Moore et al, 1973).

A low proportion of kidney abnormalities also was found in the rabbit; Giavini (1982) considers the kidney to be the target organ of TCDD's effect in many animal species. No other severe teratological effects from TCDD have been described in the literature.

Reproductive Effects

It is still unclear whether TCDD affects spermatogenesis in lower animals (Wassom et al, 1977, 1978) and primates (Allen, 1979) by a direct action on testicular parenchyma or only secondarily as a result of cachexia produced by the high level of exposure. The maternal and embryofetal toxicity of TCDD is comparable among the various species; eg, 0.1 ug/kg BW was a "no effects" level in the mouse (Smith et al, 1976), rat (Sparschu et al, 1971), and rabbit (Giavini et al, 1982).

Pregnant female rabbits were treated orally with 0.1, 0.25, 0.5, and 1.0 ug TCDD/kg BW on the 6th to 15th days of gestation. No abortions occurred at the lowest dose of 0.1 ug/kg; two, five, and three dams aborted at the higher doses of 0.25, 0.5 and 1.0, respectively. The high abortion rate was ascribed to severe maternal toxicity and the incidence of total resorptions, which increased at the higher doses. No living fetuses were found at term in the group with the highest exposure. No external malformations occurred, and histological examination did not reveal any signs of teratogenicity (Giavini et al, 1982).

More recent data (McNulty, 1983) on pregnant Rhesus monkeys treated with 0.2, 1.0, and 5.0 ug TCDD/kg BW between 20 to 40 days of pregnancy revealed no increase in abortion rate or maternal toxicity from the lowest dose. At 1.0 ug/kg BW, however, there were three abortions out of four animals. Maternal toxicity symptoms were recorded in 50% of the animals and one out of four mothers died. Abortions and maternal deaths occurred in all animals fed 5.0 ug/kg BW. Inasmuch as the Rhesus monkey seems to be particularly sensitive to TCDD and the administered doses greatly exceeded the potential levels of

environmental exposure, the pharmacokinetics of TCDD during pregnancy--particularly the transfer of the toxin to placenta and fetus--must be investigated further. The fetuses were reported dead before the abortion, with no abnormalities except for small changes in the sebaceous glands.

Studies of Human Populations

Introduction

The most significant information about the acute and long-term effects in man of Agent Orange, 2,4-D, 2,4,5-T, and their toxic contaminants is provided by the clinical, epidemiological, and pathological studies of the personnel who handled and sprayed the defoliant in Vietnam, as well as those who dedrummed the defoliant at the close of the conflict. The populations that were most heavily exposed were industrial workers exposed during routine manufacture and use, and in several instances when the reaction process went out of control. These workers provide critical information regarding the acute, chronic, and long-term effects of the parent herbicides, as well as their contaminants, especially 2,3,7,8-TCDD. Approximately 18 distinct industrial populations of record have been exposed, and several of these as a result of such "runaway" reactions. In some instances, workers have beeen exposed both to a trichlorophenol runaway reaction as well as the routine production and use of trichlorophenol. In both situations, 2,3,7,8-TCDD has been the major toxic contaminant.

Another source of data is workers who formulated or sprayed 2,4,5-T or were exposed to areas where the herbicide had been applied. This chlorophenoxy compound, later known to contain varying amounts of TCDD, was widely used from 1948 - 1970 in large scale farming, family gardens, forest management, and weed control along roadsides and railroad rights-of-way. A gauge of the extent of such human exposure in the US is provided by the production figure in 1964 for domestic use alone--9.8 million pounds.

Recently, both scientific and lay communities have become concerned about contamination of the environment by the inappropriate use and disposal of the phenoxy herbicides and of TCDD-containing materials, such as in Missouri. As a consequence, pilot studies have been carefully designed to determine the health effects of TCDD and its related compounds in at least one heavily contaminated area.

In studies of all populations, the critical components are proper design of the study, carefully defined population cohorts, and suitable controls. It should be recognized, however, that in the study of industrial populations, which are likely to be the most heavily exposed, it is often not possible to satisfy completely the requirements for randomization, for percent participation, or for knowledge of exposure levels. This is particularly true for long-term follow-up investigations.

Vietnam Veterans (Studies Complete or Partially Complete)

--Ranch Hand Program

According to Lathrop et al (1984), the personnel who filled the tanks and flew the missions in Operation Ranch Hand are currently the most relevant human population for study. The purpose of the Ranch Hand (RH) program, an ongoing, two-phase, highly sophisticated epidemiological investigation of this population, has been to determine whether there are long-term health effects from occupational exposure to Agent Orange and other herbicides during military service in Vietnam. The matched cohort design in a nonconcurrent prospective setting has incorporated mortality, morbidity, and follow-up studies on 1,174 Air Force "Ranch Hand" personnel who were involved in the actual spray

operations; these men are being compared to a matched control group of Air Force C-130 cargo personnel who had no exposure to Agent Orange. The first phase, a mortality study (USAF, 1983) published 30 June 1983, has shown no unusual findings that can be attributed to the herbicide, though the number of deaths is not substantial at present.

The second phase of the study, released in February 1984, is designed to determine morbidity incidence. The 270-page report is a myriad of detailed findings, including investigations of many of the major categories of disease and of most of the human organ systems; such as malignancies, neurological and psychological assessments, fertility dysfunction, and evaluations of hepatic, dermatologic, cardiovascular, immunologic, hematologic, pulmonary, renal, and endocrine functions. Considered a baseline, it too has shown no definitive clinical endpoints, such as soft tissue sarcoma, porphyria cutanea tarda, or chloracne. Information has been derived from two sources: 1) a specially designed questionnaire given to the 2,706 respondees, who were about equally divided between the RH group and its controls, and 2) a physical examination on a total of 2,269 individuals, again about equally divided between Ranch Hand personnel and controls.

Positive findings included significantly more minor birth defects (eg, birth marks) in the RH group compared to the controls. The fertility and reproductive results are preliminary, based largely upon subjective self-reports that await medical record and birth certificate verification. Although an excessive number of neonatal deaths and physically handicapped children occurred in the RH group, the data--from unverified subjective questionnaire reporting--must be confirmed from medical records and birth certificates. A large number of basal cell carcinomas of the skin were reported in the RH group, but these data were not adjusted for sunlight exposure, the recognized primary cause of these cancers. A more complete analysis of this data is planned for the first follow-up examination. Although biochemical differences between the RH and control groups existed, the results were still within normal limits.

Negative findings indicated that there were no significant differences between the RH and control groups with respect to the occurrence of systemic cancers; clinically significant blood abnormalities; or cardiovascular, renal, pulmonary, hepatic, and neurologic effects. Among the exposed, the absence of chloracne, soft tissue sarcomas, and porphyria cutanea tarda was noted.

Periodic follow-up examinations--at 3-, 5-, 15-, and 20-year intervals--will be compared to the baseline information. The preliminary nature of this report is repeatedly emphasized. Many of the group differences were based largely upon recall or elicited through the questionnaire and subjective data; although a subtle effect of differential reporting is suggested, the data have not been fully evaluated.

Additional work on the baseline data is still required, including data-base refinement, establishment of follow-up examination requirements, and collaboration with other dioxin research projects.

While it is premature to accept these findings as conclusive, they "should be reassuring to Ranch Handers" because "no major clinical health problems" have surfaced and the men are "overall (in) good general health for (their) age."

* * * *

On 11 February 1985, the US Air Force released a second mortality report (Wolfe et al, 1984). Like the first mortality study, which appeared in January 1983, this report did not reveal any statistically significant differences in the number of deaths between the

Ranch Hand group and its comparison members. Ranch Hand officers and flyers had slightly lower death rates and RH ground personnel had a slightly higher death rate than their respective comparisons; none of the differences was statistically significant and there was no apparent relationship between exposure and mortality. Compared to US white males, the RH officers and enlisted men, as well as their comparisons, are living significantly longer than expected; the pattern is similar to the patterns of the DOD retirees and civil service personnel. There is presently no evidence of increased mortality in persons exposed to herbicides during the Ranch Hand operations in Southeast Asia. Though the elapsed time has been sufficient for the development of clinically significant conditions, it may still be too early to see the development of conditions that might be attributable to herbicide exposure.

--CDC Birth Defects Study

Aimed at determining if there is any possible association between Vietnam service and subsequent male parentage of congenitally malformed offspring, this case-control study by the Centers for Disease Control was based on the experiences of parents of selected babies born during 1968 through 1980. From among 13,000 live- and stillbirths registered with the Metropolitan Atlanta Congenital Defects Program (MACDP), 7,133 cases with serious or major birth defects according to the International Classification of Disease, 8th revision (ICD-8), were chosen; a serious defect was defined as being associated with premature death, the cause of a substantial handicap, or the need for surgery or extensive medical care. These cases were matched with 4,246 controls according to race, year of birth, and hospital of birth.

The results (Erickson et al, 1984) provide "strong" evidence that Vietnam veterans are at no greater risk than other men for siring babies with all types of serious structural birth defects combined. The evidence for Agent Orange-associated risks is weak; if there is any risk from exposure, it is either small or it is limited to select groups of veterans or to specific types of defects. There is no explanation for the likely association between Agent Orange exposure and spina bifida, cleft lip with or without cleft palate, or congenital neoplasms; these associations may be an element of chance or the result of some unknown bias or uncontrolled confounding factor.

--Estimate of Vietnam Troop Exposure

In an effort to evaluate the possible toxic effects of the dioxin in Agent Orange on Vietnam veterans, Stevens (1981) calculated a minimum toxic dose (MTD) from animal and human data, an intake transfer factor (from human data), and from data on the spraying operation of Agent Orange in Vietnam, he calculated the fraction of the MTD to which a soldier would have been exposed. The calculation of the MTD (0.1 ug/kg) was based on primate studies of TCDD exposure and human studies of Yusho oil contaminated with tetrachlorodibenzofuran (TCDF). The average intake transfer factor (1:2,050) was based on the calculation of data from an individual exposed to TCDD in a Missouri horse arena and approximately 75 individuals exposed in zone A of Seveso, Italy. From available information on the spraying operation (Operation Ranch Hand) in Vietnam, it was calculated that a 70 kg man would have been exposed to 1/14,000 of the MTD, or 0.1 ug/kg. Thus, if a veteran had been exposed to 8 ug of TCDD/m^2/day/one year tour of duty, his cumulative intake would have been 1.4 ug, or 0.02 ug/kg. This amount, Stevens concluded, could not be responsible either for the veterans' alleged systemic illnesses or the birth defects in their offspring.

Interlaboratory analyses for 2,3,7,8-TCDD in the adipose tissue from Vietnam veterans indicated a correlation between the degree of exposure to Agent Orange and the tissue levels of TCDD. For example, the "heavily" exposed subjects had the highest levels of TCDD--greater than 20 ppt (Gross et al, 1984).

--VA Agent Orange Registry
The Registry was established by the Veterans Administration in 1978 as an index to the records of those Vietnam veterans who have come to the VA for Agent Orange-related examinations. It is essentially an extract of the veteran's complete records: his name and address, examination center, brief information about military service, estimated herbicide exposure, and elements of the findings of the physical examination. Besides serving as a means of identifying and contacting the veteran about developments and the possible need for further testing, it also provides a means of detecting health trends and other characteristics about the group. Inasmuch as the information is supplied voluntarily, any comparisons that are made are not statistically valid.

As of 31 December 1983, there were 130,220 initial examinations and 31,471 follow-ups. Thus far, there is no unusual morbidity or mortality associated with either Vietnam service or Agent Orange exposure.

There have also been attempts to compare the distribution of malignant neoplasm cases in the Registry with a reference population. Subjects in the SEER (Surveillance Epidemiology End Results) program, representing about 10% of the entire US population and being fairly representative agewise, have been selected for reference; from these, the number of malignant neoplasm cases diagnosed between 1973-1977 among US males of ages 25 to 39 is expected to include most Vietnam-era veterans. As yet, there have been no significant differences in the proportion of cancers of various sites and the proportions of soft tissue sarcomas and skin cancer, though some differences--marginal, yet statistically significant--were noted for lymphomas and cancers of the buccal cavity and pharynx (Shepard, 1983; Shepard et al, 1983).

--Agent Orange Biopsy Registry of the Armed Forces Institute of Pathology
The Agent Orange Registry (AOR), in the Department of Environmental and Drug-Induced Pathology of the Armed Forces Institute of Pathology (AFIP), was begun in 1978. Its objective is to attempt to establish whether or not there is a link between Vietnam exposure to Agent Orange and the current illnesses of these veterans. Any unusual features in the findings would suggest the need for further study. A linkage might be suggestive if there were such unusual features based on past experience with some environmental diseases that have had rather selective anatomic targets and a rather limited number of induced diseases (eg, asbestos and mesothelioma, vinyl chloride and hemangiosarcoma of the liver). To date, the AOR has been unable to show any unusual disease patterns, including cancer.

Biopsy and autopsy specimens for the Registry project are received primarily from the Veterans Administration and Armed Forces. Two phases will be carried out: Phase I is to collect and evaluate the morphologic findings of veterans or active duty personnel who served in Vietnam during 1962-1973, while Phase II will be concerned with veterans or active duty personnel who had not served in Vietnam and who will serve as a matched control set for cases in Phase I.

The Phase I group is being closely scrutinized for: 1) any clustering or peaks that represent similar organ-diagnosis combinations, 2) any clustering of pathologic findings that are unusual for a site or organ, and 3) any unusual age of occurrence for a particular diagnosis.

As of March 1983, pathologic diagnoses and demographic data have been tabulated on 1,200 veterans who had service in Vietnam. Over 300 different diagnoses, involving 84 different organs or anatomic sites, were made.

No clusters of medical significance have appeared; in other words, there have been no persistent patterns of adverse health effects. Two clusters of relatively trivial lesions--lipomas (79 cases) and epidermal inclusion cysts (96 cases)--were noted, but both were benign and of no serious prognostic significance. The relatively large incidences of these lesions may be due to their superficial locations in or on the skin; being visible or palpable, there is more concern to have them removed.

Six cases with unusual features occurred singly, not as diagnostic clusters: 1) mucinous adenocarcinoma of the colon with unusual features; 2) adenocarcinoma of the jejunum with metastases, in which both site and age were unusual for a 37-year-old black male; 3) large cell undifferentiated carcinoma of the lung, probably primary in the lung, unusual for age 31; 4) anaplastic adenocarcinoma of the lung (diagnosed in 1978) and well-differentiated prostatic carcinoma (diagnosed in 1980); metachronous malignancies--the only metachronous combination out of the total 1,200 cases--two tumors of different histologic types and different sites and types; 5) carcinoma of the prostate (age 44) at an unusually young age; and 6) gonadoblastoma of the testis, sarcoma of the epididymis, and metastatic carcinoma of the inguinal lymph node.

These 1,200 cases in the Agent Orange Registry have failed to reveal "unusual" features of apparent significance, which may be a premature conclusion because: the small number of cases may not reflect rare yet significant events, and environmental factors, which may produce unusual lesions in unusual sites, also may be associated with the more common lesions in common sites.

In order to guard against missing the more common lesions, the AOR is now entering Phase II. A matched set of control cases is being developed, having as a major criterion, "no service in Vietnam." These cases will come from the same VA hospitals and from the same time period (ie, 1978-1984) as the bulk of cases in Phase I, and will be matched by age, sex, and race. The anticipated size of the Phase II groups will be about 4,000 cases.

--Single Case Reports
Isolated cases of soft tissue sarcoma (STS) among non-Ranch Hand veterans from Vietnam who were exposed to Agent Orange have been reported (Sarma et al, 1982; Schacter et al, 1984). Whether the STS cases are related to service in Vietnam or other unrelated events is yet to be determined.

--of "Dedrumming" Personnel
Approximately 200 civilians were assigned by the military to destroy the contents of about 40,000 55-gallon drums (2.22 million gallons) of surplus Agent Orange at the close of the Vietnam war.

In addition to environmental monitoring during this operation, pre- and postexposure physical examinations, including x-rays, neurological examinations, and extensive clinical chemical tests, were performed on all workers. Military personnel revealed no apparent physical effects (Young et al, 1978).

--Australian Birth Defects Study
An extensive survey of Australian veterans was conducted to examine the impact on birth rates of those who served in Vietnam. Entitled "Case-Control Study of Congenital Anomalies and Vietnam Service (Birth Defects Study)," it is the first such study of its kind to be completed. Included in the study population were Vietnam veterans, contemporary Army personnel who had not been in Vietnam and members of the community who did not serve in the Army during this period. The records of 34 hospitals

and four cytogenetic laboratories were used to identify 8,517 subjects with birth defects and an equal number of matched controls, which consisted of healthy infants that were delivered in the same hospitals. The size of each group was reduced to 127 infants with defects and 123 normals when the service records of the fathers were taken into account (Donovan et al, 1983).

No association between exposure and adverse pregnancy outcomes was found, and the risk of siring a malformed child was no higher for either the Vietnam or the non-Vietnam veteran than for other Australian males, including the National Service and Australian Regular Army Vietnam veterans (Armstrong, 1983; Lipson, 1983; Minister of Veteran Affairs, 1983).

--Vietnamese Epidemiologic Study
A major Vietnamese epidemiological study has been described in the news section of Science magazine. Among some 40,000 families in North Vietnam, there was reportedly an increased incidence of congenital abnormalities in the offspring of fathers exposed to herbicides during the Vietnam war. In addition, children born of South Vietnamese women who were directly exposed to the spraying are said to have a suggested increase in birth defects, including neural tube defects, deformities of the sensory organs and limbs, Siamese twins, and cleft lip. No definite link between Agent Orange spraying and the anomalies has been ascertained (Norman, 1983).

Vietnam Veterans (Studies In Progress)

--VA Identical Twin Study (Vietnam Experience Twins Study, VETS)
Still in the design phase, this series of complex comparisons of identical twins may make it possible to establish whether or not Vietnam service has affected morbidity. The mental and physical health status of the subjects will be surveyed by means of a five-day battery of psychological, physiological, and biochemical tests.

The study will be conducted by the VA's St. Louis facility; examinations are to be done under contract. The VA has requested the Medical Follow-up Agency (MFUA) of the National Academy of Sciences' Commission on Life Sciences to develop the registry to be used in this project. MFUA has maintained a comparable registry since 1967 of 15,000 male pairs of twins from the WWII US Armed Forces, from which useful epidemiologic studies on schizophrenia and diabetes have been obtained. It is hoped that the new registry will also be about 15,000 strong. The VA wants about 600 pairs of twins: 100 pairs in which both twins served in Vietnam, another 100 in which neither served there, and 400 pairs in which only one served in Vietnam. A computerized list of candidates is to be furnished by the Department of Defense, state twin registries, birth records of certain states (ie, those that paid a bonus to their Vietnam veterans), VA in-patient hospital records, and VA compensation and pension records. Once compiled, the registry will be useful for future investigations into the relationships between genetic and environment and disease (CLS Lifelines :2, Spring 1984).

NRC expects to complete the entire registry early in 1987.

--Vietnam Ground Troops Study
The VA was directed by Congress in January 1979 (PL 96-151) to conduct an epidemiologic study of Vietnam ground troops who may have been exposed to Agent Orange and to determine if there have been long-term effects from such exposure. The scope of the investigation was later expanded (PL 97-27 of November 1981) to include other factors, such as medications and environmental hazards or conditions, in the Vietnam experience. In January 1983, the Congress designated the Centers for Disease

Control to conduct the work for the Veterans Administration under an interagency agreement.

CDC proposes two retrospective cohort studies and one case-control study. One cohort of male US Army veterans of Vietnam conflict is to be compared with male Army Vietnam-era veterans who did not serve in Vietnam--hereafter referred to as "Vietnam Experience" study; the other cohort will be male Vietnam veterans who differ in their probable level of exposure--to be referred to as the "Agent Orange" study. Each of the two cohort studies will have mortality assessment, health interview, and clinical and laboratory assessment.

The case-control study is to evaluate the risk of contracting STS and lymphoma among Vietnam veterans--designated as the "Selected Cancers," or "Sarcoma/Lymphoma," study--and will be limited to males of draftable age during the Vietnam conflict and to veterans from all branches of the military. This study will involve a health and exposure interview. Malaria was the most significant medical complaint in Vietnam; by 1970, neuropsychiatric disorders became the second leading disease. Diarrheal, skin, and venereal diseases were also significant (US DHHS, 1983).

Inasmuch as this will be a massive piece of research, consisting of interviews and physical examinations of several thousand veterans, its rate of progress cannot be predicted. However, CDC expects to have final reports for the Agent Orange and Vietnam Experience Studies completed by 30 September 1988 and for the Selected Cancers Study by 30 September 1989 (US CDC, 1984).

Females should be studied separately, inasmuch as they would require different sampling strategies and emphases in interviews and medical examinations. Furthermore, too few women were involved for any meaningful case-control study (US DHHS, 1983).

--VA Mortality Study
This work, begun in 1982, is analyzing death certificates for rates and causes of death of some 60,000 deceased Vietnam veterans and comparing the data with those of non-Vietnam veterans. By the end of 1984, the collection and coding of data should be completed, at which time the analytical phase can commence.

--VA Case-Control Study of Soft Tissue Sarcoma
Individuals with soft tissue sarcomas, as drawn from the files of the Armed Forces Institute of Pathology, will be compared to those without such tumors, taking into account service in Vietnam and probable exposure to Agent Orange. As of March 1984, 221 hospitals have agreed to cooperate in providing two matched controls for each case of sarcoma, one of which is to be a neoplastic disease other than STS. The study is expected to be completed in 1986.

--VA Retrospective Study of Dioxins and Furans in Adipose Tissue
Since 1970, the Environmental Protection Agency (EPA) has been collecting human adipose tissue from the general population to be analyzed for residues of certain pesticides and polychlorinated biphenyls (PCBs). The bank of approximately 8,000 samples contains specimens from approximately 524 males born between 1937 and 1952, the Vietnam veteran age group. It is highly probable that many of these men served in the military during the Vietnam era and very likely that some actually served in Vietnam. The veteran status, especially Vietnam service status, for each will be established from military and VA databases. Chemical analyses of these specimens for selected chlorinated dioxins and dibenzofurans will provide data on background levels of 2,3,7,8-TCDD in the US male population and will determine if duty in the military or

service in Vietnam has affected the levels of TCDD in adipose tissue.

--Australian Mortality Study
According to a three-part retrospective study by the Australian Commonwealth Institute of Health, which compared the death rates among 19,209 Vietnam-era veterans with 26,957 non-Vietnam veterans, the Australian Vietnam veteran is not dying at any faster rate than his counterpart who did not serve there; nor are the causes of death from diseases that have been suggested as being linked to phenoxy herbicides. Neither was there a statistically significant difference in the rates from soft-tissue sarcoma or non-Hodgkin's lymphoma (US VA, 1985). These findings are similar to those of earlier ones reported in 1983.

--State-Sponsored Programs
Nineteen states--identified in the Appendix--currently have commissions or programs designed to assist their residents who were Vietnam veterans. The activities of these commissions and programs vary considerably, from registries to university-based clinical studies.

Workplace Exposures

--Monsanto (Nitro, West Virginia)
A process accident, or "runaway reaction," in the manufacture of 2,4,5-T occurred in the Monsanto Chemical plant at Nitro, West Virginia in March 1949. Those employees involved in the clean-up and repair of equipment experienced acute symptoms that were characterized by skin, eye, and respiratory tract irritation, headache, dizziness, and nausea.

Soon after the accident and on three occasions during 1949-1953, Ashe and Suskind (1949, 1950) and Suskind (1953) examined 12 of the workers who were severely affected with chloracne; an additional 26 persons with chloracne who were not connected with the accident were examined in 1953. The most frequent clinical symptoms were acneiform lesions followed by severe muscle pains in the upper and lower extremities, shoulders, and thorax; fatigue; nervousness and irritability; decreased libido; dyspnea; vertigo; and intolerance to cold. In addition to one case of sensory loss in the foot, there were four instances of hepatomegaly and other signs of liver impairment. Upon re-examination of several of the above workers in 1953, there was marked improvement in the skin lesions and a general subsidence of the noncutaneous symptoms. Some persons had persistent but inexplicable complaints of pains in the back and lower extremities, nervousness, excess fatigue, and dyspnea.

Of the 122 persons identified as having had chloracne following the accident in 1949, 121 were selected for a cohort mortality study (Zack et al, 1980). The results of the standardized mortality analysis revealed that the 32 actual deaths (from all causes) were less than the 46.41 expected deaths; this SMR of 0.69 was the only statistically significant value among the collected data. Nor was there any apparent excess of deaths from malignant neoplasms or circulatory diseases. Of the nine deaths from malignant neoplasms, five were due to lung cancers--four of them in cigarette smokers--three were from neoplasms of the lymphatic and hematopoietic tissue; one was a rare form of skin cancer, a fibrous histiocytoma, which can be classified as a soft tissue sarcoma.

A clinical epidemiological follow-up study of 367 current or former employees of the plant was reported by Suskind et al (1984). The two cohorts in this investigation consisted of 204 subjects who were clearly exposed to the 2,4,5-T process at some time from 1948-1969, and 163 subjects with no exposure to 2,4,5-T. Clinical evidence of

chloracne persisted in 53% of the exposed subjects, while no cases of chloracne were observed in the unexposed group. An association was found between the persistence of chloracne and the presence and severity of actinic elastosis of the skin, as well as between exposure and a history of gastrointestinal ulcer. Pulmonary function values of exposed workers who currently smoked were lower than values for unexposed workers who currently smoked. There was a greater frequency of low HDL cholesterol values among those with persistent chloracne. No significant differences were detected between the exposed and nonexposed groups for abnormal laboratory values of alkaline phosphatase, SGOT, SGPT, and GGPT. There were also no differences in these same parameters between the exposed who never had chloracne and those who had chloracne currently or in the past. And finally, there was neither evidence of increased risk of cardiovascular disease, hepatic disease, renal damage, central nervous system (CNS) or peripheral nervous system disorders, reproductive disorders, or birth defects nor differences in nerve conduction velocity measurements.

A health survey in 1979 by Moses et al (1984) of 226 current or former employees at Nitro between 1948 and 1977 found a history of chloracne in 52% of the 226 workers; the mean duration for residual chloracne was 26 years. An increase of abnormal gamma-glutamyl transpeptidase (GGT) was reported, with a higher mean GGT in those with chloracne compared to those without. There was a statistically significant decrease in "sensation to pin prick" in 11 of 60 subjects with current or past chloracne but none in the 34 who never had chloracne. No mention is made of age differences or other factors that could account for this finding.

--Dow Chemical (Midland, Michigan)

Ott et al (1980) examined the mortality experiences of a group of 204 workers who had been exposed to 2,4,5-T during its production from 1950-1971. Many of the workers were also potentially exposed to a number of other chemicals during this period. Only 19 episodes of acute exposure were noted during 1954-1970, which consisted largely of irritant exposures to the eyes and skin. No cases of chloracne or porphyria cutanea tarda were evident, and mortality of this limited sample was stated to be comparable to that of US white males, as well as to the "background mortality experience" at this location.

A mortality study of 61 employees who had been engaged in the production of trichlorophenol and who had been accidentally exposed in 1964 to TCDD did not disclose an elevated mortality rate; there were four actual deaths from all causes vs 7.8 expected. Overall cancer deaths were elevated (3 vs 1.6 expected), but no particular tumor type predominated. None of the findings was statistically significant (Cook et al, 1980). Of these workers, 49 developed chloracne. The three cancer deaths (identified as an adenocarcinoma, a fibrosarcoma, and a glioma with metastases) were slightly above the expected number (3 vs 1.6), but they could not be ascribed to any predominant tumor type or particular organ/tissue site (Cook et al, 1980).

In another Dow study, information was obtained from an interview questionnaire of 715 wives of men who worked in the Michigan division. It revealed no statistically significant differences in occurrences of miscarriages, stillbirths, and fetal deaths or malformations. The two groups (ie, 370 wives of men with exposure to TCDD and 345 controls, wives of men without possible exposure) were matched closely for husbands' dates of hire. Nine variables were considered to be potential confounders in some or all of the analyses: maternal age, birth control method, conditions during pregnancy, complications in labor and delivery, medications or treatments during pregnancy, alcohol consumption, smoking during pregnancy, gravidity and mother's occupation. Most of the analyses considered the conceptus as the observational unit. If a conception occurred prior to the worker's dioxin exposure, the corresponding conceptus was classified among

the "unexposed" control group. Thus, of the 1,503 total conceptions of the 370 wives, only 737 occurred after their husbands' first exposure and constituted the "exposed" conceptuses. The remaining 766 conceptuses (ie, 1,503 - 737) were combined with the 1,274 occurring among wives of the "never exposed" employees; also, 9 induced abortions within the latter group were excluded, giving a total of 2,031 "unexposed" conceptuses (Cook et al, 1983; Townsend et al, 1982).

In a report on the findings of a cross-sectional medical and morbidity surveillance of employees considered potentially exposed to TCDD from 1976-1978, few differences between cohorts were noted. An increased frequency of radiographically documented ulcer and other digestive diseases was reported in the cohort of workers who were engaged at some time in the production of 2,4,5-T and who probably had low exposure to TCDD. These effects did not occur in the "high" exposure group, which indicates that dioxin is not likely associated with digestive effects (Bond et al, 1983).

--Diamond Alkali (New Jersey)
Porphyria cutanea tarda (PCT), of varying degrees of severity, and elevated urinary uroporphyrins were discovered in 11 of 29 workers exposed to 2,4-D and 2,4,5-T during the manufacturing process; they were examined because of chloracne (Bleiberg et al, 1964). Hyperpigmentation was more prevalent in Negroes and was said to vary in degree according to the severity of chloracne. The degree of hirsutism was related to the severity of chloracne. Of 26 workers whose urine was tested for uroporphyrins, eight had elevated uroporphyrins and 19 had chloracne. Three of those with elevated uroporphyrins had no chloracne. The porphyria cutanea tarda was assumed to be chemically induced as a result of damage or insult to the liver and not of genetic origin. The skin manifestations of PCT--ie, epidermal fragility, vesiculobullous eruptions--occurred on areas of the face, ears, and hands that were exposed to sun or pressure.

A follow-up of Bleiberg et al was made in the same plant by Poland et al (1971) on 73 male workers. Moderate to severe chloracne was prevalent in only 13 out of 73 (18%) of the cases; again, it varied in degree and was accompanied by scarring, hyperpigmentation, hirsutism, and eye irritation. There was no evidence of porphyria cutanea tarda and only one case of uroporphyrinuria. The authors have inferred that porphyria cutanea tarda and chloracne are independent syndromes, although they may have the same etiologic agent. There was either no acne or only minimal lesions in 82% of employees. Occurrence of chloracne, likewise, was not significantly greater in Negroes than whites, and was seemingly not related to job location or duration of employment (hence, level of exposure); cardiovascular findings were unremarkable except for three persons with a history of myocardial infarction. The porphyria observed by Bleiberg also was observed in a heavily exposed population in Czechoslovakia. It has never been seen in populations exposed to trichlorophenol or 2,4,5-T processing alone.

--BASF AG (Ludwigshafen, Germany)
In 1953, seventy-five workers in a trichlorophenol plant were exposed to reactor products during an accident and its subsequent cleanup. Most of them suffered chloracne; 42 suffered severely and 21 of these persons also had systemic damage to internal organs or CNS disturbances. Polyneuritis, impaired senses, and liver damage were most prominent.

In 1978, a mortality study was conducted on the cohort: Of the 17 deaths (from 11 to 25 were expected, depending on the choice of control population), six were due to cancer (three from stomach cancer), five from cardiovascular diseases, one from cirrhosis of the liver, and one from urogenital disease; all other deaths were either accidental or suicidal (Huff et al, 1980). No miscarriages or abortions were reported among the wives of exposed employees who were still at work in the plant (IARC, 1977).

A mortality study was conducted by Thiess et al (1982) 27 years after the trichlorophenol process accident at the BASF AG plant. The exposed group of workers (74), matched by age and date of entry into the factory, was compared with two internal groups and three external groups. Although there were relatively few reported deaths, malignant neoplasms were consistently above expectation but beyond chance only for stomach cancer, which has a latency of 15 years.

--NV Philips (Netherlands)
In this 1963 explosion at a 2,4,5-T plant, 106 workers were probably exposed to TCDD (or related polychlorodibenzofurans). The two operators were exposed to TCDD largely by way of inhalation. Other workers, who were either part of the regular adjoining work force or were part of the clean-up and rebuilding crews, were outfitted in protective gear; they may have had only dermal contact. Chloracne occurred in 44 persons. Liver damage was not apparent, but there were a few complaints of fatigue. Of the eight deaths, one was from a pancreatic tumor and one was due to a myocardial infarction (at age 69); five other deaths also could have been from infarctions.

--Spolana (Czechoslovakia)
Jirasek et al (1973, 1974) have reported from Czechoslovakia on what is probably the most thoroughly documented group of exposed workers. From 1965-1969, 78 persons were affected by their contact with the sodium salt of 2,4,5-T, the butyl ester of 2,4,5-T and pentachlorophenol; 76 of them developed acne (sic), hypertrichosis, and hyperpigmentation. Complaints included fatigue, weakness and pain in the extremities, sweating, and headache; many had polyneuropathy. Clinical findings revealed hepatomegaly, porphyria cutanea tarda, and increases in ALA and alkaline phosphatase (Suskind, 1980). The ten-year follow-up of 55 workers by Pazderova-Vejlupkova et al (1980, 1981) emphasized the nonuniformity of TCDD's effects on individual organs and the development of illness that affects virtually every important organ system. Complete recovery is rare, except for anxiety and depression, and persistence of impaired metabolism of fats and carbohydrates can be a major cause of premature arteriosclerosis. Children who were born to exposed individuals were not adversely affected.

--Coalite (United Kingdom)
The Coalite episode, which occurred in Derbyshire, UK in April 1968, was first described by May (1973). This also involved an overheated reactor used to synthesize TCP; the explosive rupture of the kettle was accompanied by detonation of the released glycol and chlorobenzene vapors. Seventy-nine cases of chloracne were initially recorded; many were severe but resolved within four to six months. There was no evidence of depression, weight loss, malignancies, or liver, kidney, or cardiac impairment. The epidemiological survey of 1977-1978 covered only 41 of the 90 exposed workers and revealed some abnormalities in blood chemistry and immune function. There were no chromosomal abnormalities in the circulating lymphocytes. The levels of serum cholesterol and triglycerides were elevated, while the gamma-glutamyl transpeptidase was abnormally high. Both IgD and IgM were lower than the controls. Twenty-nine of the original 41 workers were re-examined, but immunoglobulins were not measured.

No statistically significant differences between groups were reported when chromosomal aberration and sister chromatid exchange studies were performed on blood samples of workers who were exposed, possibly exposed, or not exposed to TCDD (Blank et al, 1983).

Sell (1983) reviewed for AMA's Advisory Panel on Toxic Substances a report on the outcome of an industrial accident at the Coalite plant. In his opinion, there were no immunologic differences between the three groups of workers; ie, 1) those exposed to

TCDD and with chloracne, 2) those exposed to TCDD but without chloracne, and 3) those who were unexposed and had no chloracne.

--Vertek Chemical (Jackonville, Arkansas)
This plant was involved in the production of 2,4,5-T and 2,4,-D since 1957. The recent discovery of toxic wastes leaking from drums stored above ground at the plant led to a health survey in 1979 of 190 current or former workers. Nerve conduction velocity (NCV) measurements were done on 55 workers who were prescreened for a negative history of diabetes, neurological disease, or excess alcohol consumption; all 55 were considered to be at risk for exposure to phenoxy herbicides. There were no concurrent exposures to other possible neurotoxic agents in any of the 190 workers, although prior exposure to such agents was reported in nine workers and prior exposure to 2,4-D was reported in two. The control group was similarly prescreened and found to have had no significant exposure to neurotoxic agents. NCV measurements of the median motor, median sensory, and sural nerves were performed without knowledge of a subject's employment history. Limb temperature also was measured. Slowed NCV in one or more nerves was reported in 46% of the study group compared to 5% of the controls. The conduction velocities of the median motor and sural sensory nerves were significantly slower in the study group vs the control group. In addition, slowed sural sensory velocity correlated significantly with duration of employment (Singer et al, 1982).

It is appropriate to note that neurologists and neurotoxicologists regard NCV measurements as a useful research procedure when carried out in connection with a thorough neurological examination. The ambient conditions and technical skill requirements, however, are such that it has limited use as a field study examination.

--NIOSH Dioxin Registry
The National Institute for Occupational Safety and Health has established a dioxin registry to analyze causes of death among workers at 14 manufacturing sites for dioxin-containing products (Fingerhut, 1984a). The Registry was established in late 1979 to identify male workers exposed to substances such as TCP, 2,4,5-T, silvex, and pentachlorophenol that might contain polychlorinated dioxins and to describe the processes for manufacturing these compounds. The first use of the Registry data is a mortality analysis, which is expected to be completed in 1985. In this mortality study, a subcohort of persons who had chloracne will be analyzed separately.

One value of the Registry is its exposure matrix, which will characterize exposure conditions for the specific processes and will then delineate both high- and low-exposure populations. The factors that will be taken into account in the exposure matrix include the product, the process, the operating conditions, temperatures and solvents, job duties, and analyses of dioxin concentrations in products.

A strict definition of exposure has been utilized. The Registry includes only production workers assigned to the processes of interest, maintenance personnel assigned to those departments and clean-up workers or others identified by the companies as being involved in process accidents and who may not have their work assignments documented.

The Registry has already identified 6,000 production workers at 14 companies. The quantity and quality of the records, which date back as far as the 1930s, vary tremendously; included are personnel records, payroll records, union records, medical records, insurance records, workers' compensation records, and Social Security records.

--NIEHS-IARC International Phenoxy Herbicides Registry
In 1981, the National Institute of Environmental Health Sciences of the NIH initiated

support for a feasibility study on the development and maintainence of an international registry--exclusive of the USA--of phenoxy herbicide-exposed populations. The populations were to include production workers, formulators, and users. Workers' records and environmental data from more than 25 plants are under consideration. Some European countries are reluctant to release their health and environmental data, and there are still questions about the pooling of data from different sources and the assembling of a sufficient number of records for an estimate of excess frequency of outcomes.

--Isolated Exposures to TCDD

Oliver (1975) described three laboratory workers who were exposed to "pure" TCDD. Two of the three had typical chloracne. All had abnormal serum cholesterol levels but without porphyria. Two had gastrointestinal symptoms, one complained of blurred vision and impaired muscular coordination, and another complained of "neuralgia of the left thigh." Two years after the exposure, two of the men experienced personality changes, neurological disturbances, and hirsutism.

Exposed Herbicide Users

--Swedish Railway Workers

The first study of this group, who had worked with both phenoxy acid (2,4-D and 2,4,5-T) and amitrole herbicides (Axelson et al, 1974), indicated that amitrole was probably solely responsible for an excess of tumors. A case-control analysis of the data later revealed the possibility of a masked tumor-inducing effect of the phenoxy acids (Huff et al, 1980).

--Other Swedish Workers

Follow-up on an earlier line of investigation led Hardell and coworkers to additional cases of soft tissue sarcomas, which again seemed related to occupational exposures to phenoxy acids or chlorophenols. Malignant histiocytic lymphomas* were clustered among a group of hospital admissions in Sweden from January to September 1978; 14 of 17 patients were in occupations that utilized phenoxy acids or chlorophenols (eg, forestry, wood and paper mills, farming). Inasmuch as both 6-fold tumor increases (Hardell, 1977, 1979) were attributed to impurities, such as TCDD, a case-control (case-referrent) study by Eriksson et al (1981) was launched; their objective was to confirm the previous findings and to clarify whether the impurities were causative. The new risk ratio (5.7) for soft tissue sarcomas from exposure to phenoxy acids or chlorophenols was of the same order of magnitude as before; furthermore, a risk ratio of 4.2 was obtained for other phenoxy acids that are free of dioxins, such as MCPA, 2,4-D, mecoprop, and dichlorprop.

In a review of the occupational histories of patients with malignant lymphomas, Olsson et al (1981) reported a possible relationship between non-Hodgkin's lymphoma of the skin and exposure to phenoxy acids.

In another investigation by Hardell et al (1981), exposure to organic solvents, chlorophenols, and/or phenoxy acids resulted in a relative risk factor of 4.1 for malignant lymphoma compared to a risk factor of 0.9 in the unexposed control group. These results seem to indicate an increased risk for malignant lymphoma in workers exposed to phenoxy acids, chlorophenols, or organic solvents.

---

* Neoplasm sites included frontal sinuses, bladder, ileum, retroperitoneal, parotid and submandibular areas, femur, and groin.

Yet another investigation by Hardell et al (1982) reported a sevenfold increase in the incidence of nasal and nasopharyngeal cancers, particularly among woodworkers, due to chlorophenols. Cabinet makers, with no exposure to chlorophenols, had a doubled (but insignificant) risk of nasal cancer. Exposure to phenoxy acids resulted in a doubled (but insignificant) risk of nasal and nasopharyngeal cancer.

Analysis of geographical clusters of malformations in Sweden failed to identify any one etiological factor responsible for the clustering (Ericson et al, 1983).

--Finnish Railway and Forestry Workers
A similar group of 30 workers in Finland, who were exposed to TCDD-containing herbicides, will be compared to a matched control group to see if there are any long-term effects from their exposures. Thus far, no differences are apparent (Huff et al, 1980).

--New Zealand Birth Defects Study
Smith et al (1982b) surveyed by questionnaire the pregnancy outcomes of 548 New Zealand professional sprayers and their wives, who were also occupationally exposed to phenoxy herbicides, comparing them with a control group of 441 agricultural contractors. Inasmuch as the authors' preliminary results showed little difference in rates of congenital defects between cases and controls, this study redefined the extent of exposure by subdividing the group of sprayers; there was still no suggestive evidence that 2,4,5-T adversely affected pregnancy outcomes. Although the men had an increased risk (1.19 times) of siring children with defects, the wives' risk of miscarriage was only 0.89; the differences were not statistically significant and no other effect on the women's reproductive cycle was apparent. The several inherent sources of bias in the study were explained, but even when they were excluded the results were unchanged.

--Long Island Railroad Birth Defects Study
In 1979, NIOSH investigated a reported excess of birth defects among the children of about 1,400 maintenance workers of this New York railroad who were exposed to 2,4,5-T. Of 170 live births in the study, there were less than the expected number of all combined major defects but a significant excess of minor defects. The latter, however, is believed to be due to diagnostic bias (Honchar, 1982).

General Population

--Seveso, Italy Inhabitants
In 1976, an explosive release of TCDD from a trichlorophenol reactor at the ICMESA plant occurred in the vicinity of Seveso, Italy. This is the largest single population--over 37,000 persons--to have been potentially exposed to varying doses of this compound. Two years after the incident, the acute and mid-term health effects were assessed; mild chloracne, mainly in a small group of children, healed quickly. Subclinical peripheral nerve impairment was reported; there also was liver involvement but without apparent functional disorders. Immunoresponse was not altered nor was susceptibility to infectious diseases increased (Reggiani, 1979). This incident has been described more fully in AMA's 1981 report.

The long-term epidemiologic survey of the 220,000 residents of the Seveso area is expected to go on for many years. A progress report emphasized the preliminary nature of the findings and reiterated the above conclusions of Reggiani (Pocchiari et al, 1979; Huff et al, 1980). Except for the skin, no organs or body functions were impaired. No derangement of gestation, no fetal lethality and loss, no gross malformations, no growth

retardation at term, and no cytogenetic abnormalities have yet occurred.

Analysis of the livers of 144 sheep which died in the Seveso area following the accident disclosed that tympanism was the cause of death, not TCDD (Anon, 1980).

The primary biological effect on the inhabitants was skin irritation, largely as the result of alkali mist. However, up to the end of a year and over a six-month period, about 200 cases of chloracne were identified, primarily among children. It is interesting to note that no cases of chloracne were observed among the ICMESA workers either prior to or following the accident, including workers involved in clean-up operations.

Spontaneous abortions and birth defects were monitored by the Birth Defects Registry (established in 1978), but except for hemangiomas and benign neoplasms, the data did not show an association between malformations and TCDD exposure.

A controlled study of children with chloracne was not completed because too few of the affected children wished to participate. An Occupational Health Surveillance study was established soon after the accident and a large amount of data was collected between 1976-1980; however, the "lack of adequate study designs" and the difficulties in coping with "prevailing clinic characteristics" precluded any definitive conclusions. Nevertheless, the high incidence of "abnormals" in the liver function test declined markedly during the four-year surveillance period.

Neurologic studies by the Clinica del Lavoro Luigi Devoto of Milan revealed only three or four cases of suspected neuropathy in which either the motor or sensory nerve conduction velocity was reduced. These findings and "subclinical signs of neuropathy" in 16 other subjects could not be evaluated properly because there was no control group and no information on alcohol intake and history of diabetes.

In late 1980, a cross-sectional study was designed to evaluate hepatoxic and neurotoxic effects in 188 ICMESA workers compared to an unexposed control group of 305 workers from the metallurgical and mechanical industries outside the Seveso area. The battery of laboratory tests included lung function tests (FVC, FEV), SPE and ulnar nerve electromyography, SGOT, GGT, alkaline phosphatase, total bilirubin, cholesterol serum level, triglycerides, total serum protein and electrophoresis, prothrombin time, hematocrit, hemoglobin, WBC and differential, and total urinary porphyrin. No statistically significant differences between the two groups were found.

Thus, five years after the accident, no significant adverse health effects were detected in the ICMESA workers. Likewise, both the mortality study and Cancer Registry have yielded very little conclusive information, though eight cases of soft tissue sarcoma were found in the group of 222,000 residents living in the buffer zone outside of the more highly contaminated zones A and B.

Merlo (1983), who was associated with the Seveso data analysis group from 1979-83, said that,

> "... knowledge of the TCDD health effects comes from animal studies and human occupational exposures occurring during the manufacture of trichlorophenol. Short-term effects have been identified. Long-term effects are still under study. Unfortunately, due to the lack of proper studies in the early phase of the 'Seveso Program,' reevaluation of these data--such as has been done with Hiroshima and Nagasaki records--has to be excluded. Only well designed epidemiologic studies carried out carefully will enable us to draw conclusions about the occurrence of

chronic health effects and their relationship with TCDD exposure."

Tenchini et al (1983) performed a cytogenetic analysis of maternal and fetal tissues from induced abortions in TCDD-exposed and nonexposed women. They found a statistically significant increased incidence of aberrant cells and a greater number of aberrations per damaged cell in the dioxin-exposed group vs controls. While some effect of maternal exposure to the toxic agent cannot be ruled out, variations in cell culture growth are more likely to be the cause of the observed cell changes.

--Times Beach, Missouri

The Centers for Disease Control (CDC) was called in by the State of Missouri to find an explanation for the unusual occurrence of animal deaths and reports of family illness near a horse riding arena in Missouri (Donnel et al, 1983; Kimbrough, 1983). In 1973, the toxic agent was identified as TCDD, which was later traced to TCDD-contaminated still-bottom waste from a TCP process in Verona, Missouri. It seems that, in 1971, approximately 29 kg (64 pounds) of TCDD-contaminated waste from a chemical plant in southwest Missouri was mixed with waste oils and applied to soil for dust control at 241 sites in the eastern part of the State. At one site, the Shenandoah horse stables, as much as 35,000 ppb of TCDD was measured in the soil. In addition to several other horse arenas, there was an urban residential area with a population of 2,100, later referred to as "site-21," or "Times Beach." The situation in this town came to light when a flood struck the major portion of the town and led to a near-total evacuation of the community. As early as 1971, the unpaved streets of the town also had been sprayed with TCDD-contaminated oil. Soil samples taken in the aftermath of the flood from both the shoulder of the road and the ditches contained TCDD. While they did not have the greatest degree of TCDD contamination in Missouri, they reached a peak of 98 ppb.

Since there was no established rationale or methodology in Missouri for determining "safe" levels of TCDD in soil, the Missouri Department of Natural Resources (DNR) in collaboration with CDC and EPA reviewed environmental sampling data and the Remedial Action Marker Plan (RAMPS); they jointly recommended appropriate strategies for intervention. Hence, on 23 December 1982, CDC advised that the evacuated residents of the town should not return to their homes until additional samplings were carried out.

Contaminated Times Beach soils were given by gavage to guinea pigs and rats by McConnell et al (1984) to determine the degree of bioavailability of dioxin on soil. A characteristic clinicopathologic syndrome developed in the guinea pig, AHH was induced in the rat and both species of animals had TCDD in their livers. Hence, TCDD in soil was believed to have a "high biological availablity after ingestion." Kimbrough has cited the bioavailability of TCDD to be as much as 30% to 50% of the original amount contained on the soil matrix. CDC based its risk assessment on a "decision of prudence" and several assumptions that were presumed to be in the best interests of the public's health and safety. One such assumption was that a child would be most vulnerable to the contamination and might ingest as much as 1 to 10 g of soil. Thus, from the chronic animal toxicity data of Kociba and the National Toxicology Program, the safe human dose was judged to be in the picogram range, and "1 ppb of dioxin in soil is a reasonable level at which to begin consideration of action to limit human exposure for contaminated soil" (Kimbrough et al, 1984). (The EPA has meanwhile published a methodology for estimating human exposure and cancer risk associated with five different pathways of exposure to 2,3,7,8-TCDD contaminated soil (Schaum, 1984).)

The Missouri Department of Health and CDC decided, after a review of animal data and consultation with other health professionals, that there would be a risk of developing

"adverse health effects" if there were continued residential exposure to soil having more than 1.0 ppb of TCDD. The two agencies then went on to initiate four public health actions in January 1983 that would provide:

--health education of the medical and public health communities, as well as the general public, regarding current understanding of health effects of dioxin exposures;
--dermatologic screening clinics for the general public;
--a registry for potentially exposed individuals; approximately 2,000 interviews were conducted; and
--the design and implementation of a "highest risk" cohort.

--Missouri Pilot Study
An investigation by questionnaire of two groups of residents was conducted; one group of 68 persons was at risk, having been exposed agriculturally to dioxin, while the control group of 36 had little or no history of exposure. Both were matched by age; sex; socioeconomic status; history of alcohol, tobacco, and pesticide usage; and according to their relative proportion of work in a hazardous occupation. The medical examination included separate neurologic and dermatologic examinations and a broad range of laboratory tests, one series of which was intended to assess cell-mediated immunity.

An initial review of data did not indicate any statistically significant differences between the high-risk and low-risk study groups. No consistent indications of increased disease prevalence was found in the dioxin-exposed group, although there was an apparent trend of urinary tract abnormalities and some immune function changes (T_4:T_8 cell ratios were less than 1.0). Functional tests of the immune system did not indicate any overall abnormalities. No cases of chloracne or porphyria cutanea tarda were reported. Follow-up studies were recommended (Donnel et al, 1983; Webb et al, 1984).

And finally, the authors of the report entitled "Missouri Dioxin Health Studies," (published by the Missouri Division of Health and CDC in collaboration with St. Joseph's Hospital of Kirkwood, Missouri and St. Louis University Hospital) stated that,

> "... it is conceivable that uptake of dioxin from contaminated soils was generally less than estimated for this study group or that chronic exposures to environmental TCDD have actually induced little or no adverse health effects as measured in this study."

The Missouri Department of Natural Resources has meanwhile added to its registry of uncontrolled hazardous waste sites three new locations that are contaminated with dioxin: Minker-Stout and Lacy Manor sites in Jefferson County and Erxleben site in St. Louis County.

--St. Louis, Missouri (Trucking Employees)
A recent survey of present and former workers employed at trucking terminals that had been sprayed for dust abatement with TCDD-contaminated oil disclosed a 59-year-old man with a sarcoma and porphyria cutanea tarda (PCT). A truck driver for 21 years, he had worked at one of the contaminated terminals for several years; he also was exposed to TCDD while unhooking trailers in the terminal. The PCT improved with treatment; however, in February 1983, a sarcoma in the proximal right femur and pelvis involving the adjacent soft tissue was diagnosed. It was not possible to determine whether the tumor originated in the bone or in the soft tissue (Hope et al, 1984).

--Alsea, Oregon

In the Alsea basin of Oregon, 2,4,5-T and silvex were used on a seasonal basis to eradicate unwanted vegetation from forest lands. In 1978, there was some concern that such spraying was responsible for a number of spontaneous abortions in the area. All told, 13 miscarriages were reported from nine women. As a result of much publicity and public concern, the EPA conducted a study--Alsea I--to assess the facts and to determine whether the spraying was indeed responsible. EPA concluded that there was no relationship between the spraying and the incidence of miscarriage; the rate was within the expected limits. The study was harshly criticized because the exposures were not documented and the statistical treatment was improper. Thus, Alsea II was begun in October 1978, to study a much larger area and population than Alsea I and to compare miscarriages within the first 20 weeks of pregnancy with the spraying of 2,4,5-T. A high probability of an elevated miscarriage rate shortly after spraying was reported to the press, but this claim was proved to be without merit.

review of Alsea II by Oregon State University--A critical analysis of Alsea II was conducted by an interdisciplinary team of Oregon State University scientists (Wagner et al, 1979). It did not support any of EPA's conclusions in the Alsea II study. The team contended that:

there was no control population in the study;

data on miscarriages were unreliable and inaccurate;

medical practices were profoundly different in the three areas under consideration;

information on use of 2,4,5-T was inaccurate and incomplete;

variations in hospitalized miscarriages in all the areas were well within expectations;

differences existed in topography, climate, and demography;

data collection was incomplete and failed to account for patterns of and differences in medical practice among areas.

The panel termed the EPA's hospitalized spontaneous abortion indices (HSAI) values to be unreliable because:

there was substantial variation in the magnitude of the HSAIs for any one month among the six years, and

the peak HSAI in June was due to a single large excursion only in 1976, while the seasonal cyclic peaks were consistent with random variations.

Furthermore,

alleged increase in the use of 2,4,5-T was actually due to more complete collection of spray data;

there was no statistically significant cross-correlation except for the apparent increase in the June HSAI--deletion of the single June 1976 HSAI value removed the significant correlation; and

the HSAI increase in June 1976 was identified only with a location where human exposure was least likely to have occurred.

Hence, according to the OSU panel, there was:

no significant correlation between 2,4,5-T use and the HSAI, and

EPA's study was seriously flawed because of incomplete and inaccurate data.

review of Alsea by FIFRA's Scientific Advisory Panel--This panel of the Federal Insecticide, Fungicide and Rodenticide Act reviewed the evidence and found neither immediate nor substantial threat to human health or the environment when silvex or 2,4,5-T was applied to rice, rangeland, orchards, or sugarcane and when used for certain non-crop purposes.

review of Alsea II by others--Lamm (1979) also declared the Alsea II study to be epidemiologically unsound and the report useless. Both statistically and epidemiologically, the report does not support any relationship between herbicide spraying and miscarriages. Lamm (1980) has since claimed that only four of the ten women cited by EPA in the Alsea II study underwent spontaneous abortions; the remainder were hospitalized for other reasons.

New Zealand's Allan H. Smith (1979) termed the Oregon study of highly doubtful validity.

Robinson (1979) of Australia emphasized that the EPA information in no way confirmed a hazard to human health from 2,4,5-T when it was used in a careful manner and according to label instructions on the container. A comprehensive review by the Australian Department of Health (1977) concluded that there was no evidence that 2,4,5-T caused human birth defects.

Canadian Review of TCDD's Health Hazards

Canada's Expert Advisory Committee on Dioxins has evaluated the available published information and concluded that:

> Epidemiological data on the effects of dioxins on Canadian populations, when taken in isolation, are insufficient to prove any significant association with disease. Considering that it is impossible to prove the absolute safety of a substance--not to mention the multiplicity of dioxin sources and its mixtures with other chemicals, and the small size of the Canadian population--additional epidemiological studies solely on a Canadian population would be unlikely to provide a definitive identification of dioxin-associated diseases. Therefore, risk assessments associated with dioxin exposure must rely on world-wide epidemiological data in conjunction with toxicological studies on laboratory animals.
>
> Similarities between the signs and symptoms in humans and laboratory animals justify the concern about human exposure to dioxins; hence, a "virtually safe dose" for 2,3,7,8-TCDD was calculated from animal data. The corresponding dose for a one-in-a-million lifetime risk of cancer in humans ranged from 0.02 to 0.07 pg/kg (mean = 0.03) of body wt/day. Another estimate of cancer risk assumed a dose threshold, for humans was estimated to be between 0.2 and 1 pg/kg of body wt/day, based on dividing the "no observed effect level" (or NOEL) from animal studies by an arbitrary safety factor, usually 5,000 or 1,000.

The potential risks of adverse effects on human reproduction were also evaluated from animal data, inasmuch as epidemiological data on reproductive toxicity and teratogenesis were of such poor quality that no association could be determined. Fetotoxicity and teratogenicity in laboratory animals occurs at doses near those which cause maternal toxicity. A 1000-fold safety factor was arbitrarily applied to the NOEL for reproductive effects in animals, thereby resulting in an "acceptable daily intake" of 1 pg/kg of body wt/day. This estimate is not much higher than the level to which some high-risk groups in Canada are exposed (Willis, 1983).

Cancer Studies

CDC Selected Cancers Study

See under "Vietnam Veterans (Studies in Progress), --Vietnam Ground Troops Study."

NIOSH Review of Reports of Soft Tissue Sarcoma and Dioxin Exposure in Seven Chemical Workers

According to Fingerhut (1983), the first suggestion of an association between these tumors and TCDD began with clinical observations in Sweden in 1977 and two published studies of Hardell and others. Four separate studies of industrial populations were then reported in the United States in 1980. The mortality analyses, which were conducted by Monsanto and Dow epidemiologists, included workers exposed during process accidents as well as during the uneventful processing of TCP to 2,4,5-T. The authors indicated that there was no excess of mortality due to dioxin exposure. Honchar and Halperin (1981) reviewed the studies and noted that of the total 105 deaths, three were reported to be due to soft tissue sarcomas--a frequency of 2.9%, which contrasts with 0.07% for the general population. Subsequently, a fourth person from the Dow cohort was reported to have STS (Cook, 1981), and Johnson et al (1981) and Moses et al (1981) provided case reports of three additional cases of soft tissue sarcoma in chemical workers.

Fingerhut and others (1984b) undertook an effort to evaluate the accuracy of reports of dioxin exposure and soft tissue sarcoma pathology in these seven chemical workers. Tissue specimens of the persons with alleged soft tissue sarcomas were examined by two STS pathologists, Dr. Franz Enzinger at the Armed Forces Institute of Pathology and Dr. William Russell. Of the four cases in the Dow and Monsanto cohorts, whose causes of death were derived from death certificates, only two were found to have soft tissue sarcomas, and these two also had chloracne. The three other individuals were confirmed as cases of soft tissue sarcoma, but one was a truck driver who had been a clerk and maintenance worker but with no recorded assignment to dioxin-contaminated processes. The other two cases were a father and son. The son was never assigned to a dioxin-contaminated process during his 2.5 years of employment before he died; the father, a utility worker who worked for 11 days in the pentachlorophenol process area, had a generalized liposarcoma.

This review of seven cases neither affirmed nor rejected the association alleged by the Swedish workers because there was no adequate comparison group that could be used. In this country, we have no mortality rates for soft tissue sarcomas that are based on pathologic review of tissue specimens; the only available rates are derived from death certificates. According to a review by Percy et al (1981), the death certificate does not accurately estimate the diagnosis of STS. The authors, from NCI's Biometry Branch, have shown that only around 50% of STS cases list STS on the death certificates, while only around 50% of the death certificates listing STS also have a corresponding diagnosis

from hospitals during 1970-1971, 252 reported soft tissue sarcomas, but only 54.8% of these were confirmed by pathologists of soft tissue sarcomas. On the other hand, only 56.3% of the pathology diagnoses of soft tissue sarcoma were recorded as such on death certificates.

There are several other dilemmas in the study of these tumors: Not only is the death certificate inadequate as a source of diagnostic information, but the tumors are often misclassified because of the anatomic-based rules for the International Classification of Disease (ICD) System. For example, if a sarcoma occurs in either the stomach or liver, it will not be classified under ICD-171 for soft tissue sarcoma but as a tumor of the stomach or liver. Only 60% of the histological diagnoses of STS in the Swedish studies are listed under ICD-171, the remaining 40% are in other ICD categories.

Fingerhut and others (1984b) suggest that the diagnosis of the original pathologist may not be accurate; thus, experts in soft tissue pathology may be needed necessary to confirm original diagnoses. Additionally, the histological subtypes of STS are difficult to diagnose. Among the above seven cases reviewed by expert pathologists, there was agreement by the experts that five were soft tissue sarcoma. However, the experts themselves disagreed on the subtypes subtypes for two persons.

A causal relationship between soft tissue sarcoma and dioxin exposure has not yet been confirmed or excluded. Since the incidence of soft tissue sarcoma in the general population is very low, most of the study designs require a very high relative risk for the sarcoma to be detected (Coggen et al, 1982).

NCI Case-Control Study of Lymphoma and Soft Tissue Sarcoma

According to Hoar (1983), the first disclosure of herbicide-related cancers was that of Swedish railroad workers exposed to amitrole, 2,4-D and 2,4,5-T, which showed elevated rates of cancer incidence (Axelson et al, 1974). Next in line were the case-control studies by Hardell and his collaborators wherein associations were described between phenoxyacetic acid or chlorophenol exposure and soft tissue sarcomas (STS), non-Hodgkin's lymphomas (NHL), and Hodgkin's disease (HD). Risks for all three cancers were increased five- to sixfold, irrespective of the presence of polychlorinated dibenzodioxins or dibenzofurans as contaminants.

A five- to sixfold increase in risk is a remarkable finding for epidemiologists; furthermore, such an increase is of especially great concern because there is widespread potential for exposure to phenoxyacetic acids and chlorophenols. Apart from their use as herbicides, these compounds act as blue-stain retardants in lumber, as slime control agents in cutting oil fluids and in the paper and pulp industry, as wood preservatives, and as water-proofing agents for leather and textiles. Thus, if the epidemiologic associations suggested by the Swedish research are proven to be causally related, these agents could be responsible for a large share of STS, NHL, and HD cases in the United States.

The National Cancer Institute (NCI) consequently is comparing herbicide exposure among reported cases of soft tissue sarcoma and lymphoma in Kansas; subjects have been matched with a control group of similar age, sex, and county of residence. The work is expected to be completed in late 1984 or early 1985.

NCI has considered various approaches for further investigations. Cohort studies of pesticide applicators are underway. A duration-related association between lung cancer and employment as an applicator has already been reported by Blair et al (1983). Unfortunately, these cohorts are far too small for conclusive evidence in a disease as

rare as STS. A case-control study of STS, NHL, and HD with particular attention to herbicide exposure was planned, with concentrated effort on agricultural uses; for example, on wheat, corn, sorghum, soybeans, alfalfa, and pasture land. Inasmuch as herbicides are often accompanied by extremely heavy applications of insecticides, which could confound the results of the study, the choice of crop was narrowed down to wheat. Wheat requires a large amount of herbicide but comparatively little insecticide and fungicide application.

Kansas, a major wheat-producing state, was chosen as the site for the study. The herbicide most commonly used there has been 2,4-D; nevertheless, substantial amounts of 2,4,5-T also have been used in addition to a number of other chemicals. According to a 1981 report of the Ohio Agricultural Research and Development Center entitled "Pesticide Use on Major Crops in the North Central Region," about 5.2 million pounds of 2,4-D and 1.3 million pounds of 2,4,5-T were applied in 1978--amounts far greater than in any of the other North Central states. Kansas also has a population-based cancer registry, from which figures for cancer incidence can be obtained without having to rely on death certificates; as noted above, the reporting of sarcomas and lymphomas on death certificates is known to be extremely inaccurate.

The study population consists of white males, aged 21 and older, who have been diagnosed with STS, NHL, or HD during residence in Kansas. From 1976-1982, there were 200 with STS and 173 with HD; a sample of 200 was selected from those diagnosed with NHL during 1979-1981.

The control group consists of white males from the general population of Kansas. Three controls were selected for each case, matched on age ($\pm$ 2 years) and vital status. Controls for live cases, aged 65 years or older, were selected from the Medicare file; controls aged 64 years or younger were selected by random-digit telephone dialing. For deceased members, the controls were selected from Kansas state mortality files, with an additional match on the year of death; excluded were those persons whose cause of death was STS, NHL, HD, a malignancy of an ill-defined site (ICDA code 195), homicide, or suicide. In the case of deceased subjects, the next-of-kin were interviewed. One-half of the STS and NHL and one-third of the HD cases died before the study could be initiated.

The same controls are intended to be used for comparing the three different series--ie, sarcomas, NHL, and HD cases--thus, only 1,008 controls are needed instead of approximately 1,700. Seventy men who were diagnosed with colon cancer in 1981 were chosen as a "cancer control series" in order to evaluate the possibility of recall-bias among cancer patients.

Pathology specimens are being reviewed to confirm the diagnosis of STS, NHL, or HD, which is important because these conditions are difficult to diagnose accurately; also, the original pathologists used a variety of schemes for categorizing the tumors into subgroups. Not only are consistent diagnoses a requisite, but there must also be agreement on the subgroup terminology. Subgroups may be important with respect to etiology; some subgroups may be associated with herbicide use, while others may not. After about 45% of the specimens had been reviewed, it was noted that some cases belonged to other series in the study--a so-called NHL is actually an HD or an HD should be an STS. Then too, a few instances of malignancies have been found that are not eligible; for example, one multiple myeloma was considered to be benign and an STS was rediagnosed as nodular fasciitis. If the number of such misclassifications is large enough, it may be possible to evaluate observation- and recall-bias in these situations with conditions that may be unrelated to herbicide exposure.

Each telephone interview has taken from about 25 minutes to one hour. Corroborative evidence of the self-reported herbicide exposure will be obtained from farm suppliers by asking them about the kinds of crops grown and the purchases of herbicides and insecticides. Such verification is an important step, giving a crude evaluation of potential observation-bias--a criticism of the Swedish studies. All interviews and verification were to have been completed in the early part of December 1983. The data were to be analyzed by computer and reported by summer of 1984.

New Zealand Soft Tissue Sarcoma Case-Control Study

In addition to the Swedish studies, a case-control study of STS has been reported by researchers from New Zealand, where phenoxy herbicides were used extensively for many years. A preliminary report by Smith et al (1982a) found no association between soft tissue sarcoma and the use of phenoxyacetic acid pesticides. None of the persons with soft tissue sarcoma in the study had worked as a licensed herbicide applicator. In this study 102 males with STS, as indicated from the New Zealand Cancer Registry between 1976-1980, and 306 controls (with cancers other than STS) were matched by age and year of registration.

The 102 STS cases, or their next-of-kin, and "other cancer" controls were interviewed by telephone for information concerning their occupations. No excess of STS was seen in the category for agriculture and forestry (odds ratio = 1.03, 90% confidence limits = 0.9, 1.8); farmers showed a slight excess of 1.45 (0.8, 2.7) despite extensive use of phenoxy herbicides, but this excess may be due to misclassification. Neither complete work histories nor histories of actual herbicide use are available. Although misclassification of exposure status may dilute the risk estimates, the amount of misclassification cannot explain the differences between the Swedish and New Zealand studies.

Lifetime work histories for 82 of the New Zealand STS cases and their 92 controls with other types of cancer were taken by telephone. The results of the interviews showed a 60% increase in risk of STS if the probable or definite exposure to phenoxy herbicides for more than one day occurred at least five years prior to the cancer registration; there was a 30% increase in risk if exposure was at least a total of 5 days and occurred more than 10 years prior to registration. Increases in risk for chlorophenol exposure were 50% and 60%, respectively. Little control for potential confounding factors was done (Smith et al, 1984).

Finland

No deaths from STS or lymphomas were revealed from a survey of mortality data on 1,926 herbicide sprayers employed from 1955-1971, although exposures were admittedly low and of short duration (Anonymous, 1983; Riihimaki et al, 1982).

US, Pacific Northwest

In the Pacific Northwest, there is a case-control study of approximately 280 STS cases, 540 NHL cases, and 125 liver cancer cases, all diagnosed between 1980-1983. The cases originated in 13 counties in western Washington state, where phenoxy herbicides and chlorophenols are used extensively in agriculture, forestry, and the wood products industry. Cancer incidence data are available from a population-based cancer registry. Approximately 1,100 subjects were selected by random-digit dialing to serve as controls for each of the three types of cancer. In-person interviews of cases and controls, or their next-of-kin, were used to collect detailed residential and occupational histories and other risk factor information. Information on geographical area and extent of herbicide

application will be combined with the residential histories to construct exposure indices. Occupational exposures will be ranked and given an exposure index value. The estimated time of completion is 1985.

An update was provided by its principal investigator (Milham, 1982), who concluded that the mortality patterns of 200 white males, ages 20 and above, from 1950-1979 do not support a link between soft tissue sarcomas, Hodgkin's disease, and non-Hodgkin's lymphomas and exposure to phenoxy herbicides or chlorophenols.

New York State

Several herbicide-related studies have been conducted in New York State. One of these was of 281 males diagnosed with STS during 1962-1980 and who were between 18 and 29 years of age during 1962-1971. A living control was selected for each case from drivers' license registration files matched on age, sex and place of residence. For each deceased case, a second deceased control was obtained from the New York State mortality files, matching year of death, age, years of education, sex, race and health systems area. Cancer deaths were excluded. The telephone interviews with the subjects or their next-of-kin focused on exposure to herbicides while in military service; other factors, such as occupation, histology and anatomic site of the tumors, were also examined. No statistically significant association was found between STS and Vietnam service or military service in general, or any other variable that could relate to herbicide exposure (Greenwald et al, 1984).

Another investigation (Lawrence et al, 1985), using the mortality odds ratio (MOR), compared the causes of death among New York State veterans who had served in Vietnam with those of that era who had not served there. The study group excluded New York City and consisted of 1,496 Vietnam-era veterans, 555 of which had been in Vietnam. The highest MORs involved "non-motor vehicular injuries of transport" (2.18), other accidents and burns (1.37) and homicide (1.59); no association was shown between herbicide exposure and cause of death.

Michigan State Department of Public Health

A high and increasing death rate due to connective tissue cancers among white women was observed in 9 widely dispersed Michigan counties. For Midland County, the location of a Dow Chemical facility, a total of 13 deaths due to these cancers occurred over a period of 20 years, but there were none among white women during 1980 and 1981. In some instances, the onset of the disease had begun before the subjects had moved to Midland; then again, it is unclear whether the apparent increase was real or an artifact of death certification and coding. A case-control study will include all patients with a biopsy-proven diagnosis of STS from 1970-1983 -with the exception of mesotheliomas--in the specified eight counties. Three hospital patients without a history of cancer will be selected as controls for each case, matching on sex, race, and five-year age period. Interviews will cover occupational and residential history, smoking habits, hobbies, medical history, family history of malignancy, and diet. The report is estimated to be completed by October 1985.

A preliminary finding by the Michigan Department of Public Health in 1983 did not establish a link with any factor in the environment, including TCDD. Though there were increases in mortality due to connective and soft tissue cancers in some of the 28 other US counties where TCDD might occur as a result of chemical manufacturing, as compared to other counties that were suspected of being devoid of the contaminant, there was no predominant pattern of excesses. One might expect a predominant excess

if TCDD were responsible.

Italy

A case-control study of 100 cases of STS identified from hospital pathology department logs from 1981-1983 and 300 controls is being conducted in three counties where phenoxy herbicides have been used in the growing of rice. Both sexes will be included due to the large number of women who have worked in the rice fields. Two controls per case will also be identified from the pathology logs and will be matched on sex, age, and province of residence; other cancer patients have been excluded. A second control group for cases from larger towns will be drawn randomly from the general population and from death records. A reasonable completion date is 1985.

Denmark

First, there will be a cohort study of workers at a plant where phenoxy acids have been produced since 1947. Workers from newer plants may also be included. After its completion, a case-control study of STS similar to the Swedish studies is planned.

Immunological Response to TCDD

In Animals

Sell (1983) reviewed for AMA's Advisory Panel on Toxic Substances the available literature that might pertain to TCDD's effect on the immune system. It is known that rats and mice develop atrophy of the thymus gland when fed even "safe" doses of TCDD; their T-cells become cytotoxic, and eventually the animals die. T-cells, or T-lymphocytes, are the "policemen of the immune system" and are involved in the regulation of the immune system. Antibodies are identified according to the function of the T-cells and B-cells or their subsets. It is important to learn why these cells are activated. The most sensitive immune system test is based on the production of cytotoxic T-lymphocytes. Immunosuppresive drugs, such as cyclosporine, interfere with interleukin-2 and inhibit the growth of T-helper cells.

Published data from animal studies, however, are meager and conflicting, which makes their interpretation difficult. For example, the administered dose, usually dissolved in acetone, often may be given with no indication of what the animal actually received. Nevertheless, an NIH study on mice given high doses of TCDD (5 ug/kg of body wt, or 0.1 ug/mouse) for 270 days revealed no defects; moreover, an examination of the thymus, spleen, and lymphocytes revealed no abnormalities, and their antibody response was satisfactory.

In Humans

Information about TCDD's action on the human immune system is even more scarce, although immunologic research has been carried out by the National Institutes of Health since 1976. According to Sell, there is presently no conclusive evidence that TCDD adversely affects the human immune system. Reggiani reported earlier (Arch Toxicol (Suppl 2):291-302 (1979)) that immunoresponse was not altered nor was susceptibilty to infectious diseases increased among the inhabitants of Seveso, Italy.

Sell also reviewed a report on the outcome of an industrial accident at the Coalite plant in Bolshover, England. In his opinion, there were no immunologic differences between three groups of workers; ie, 1) those exposed to TCDD with chloracne, 2) those exposed

to TCDD without chloracne, and 3) those who were unexposed and had no chloracne.

Conclusions

The studies to date on the human health effects of Vietnam exposures to Agent Orange do not reveal a clear relationship between serious illness and exposure. A number of important studies are still in progress; until they or others that may be deemed necessary are completed, no final conclusions can be drawn.

Workplace exposures, on the other hand, have involved components of Agent Orange, especially 2,4,5-T and its contaminant TCDD, and represent a different level of biologic experience. Definable and measurable effects on specific organ systems can be described for acute, subchronic, and long-term exposures. Chloracne is the marker for biologically effective exposure in humans, and it may persist for as long as 30 years.

A wide range of adverse reactions in animals has been observed, which are species-dependent. For example, the guinea pig is most sensitive to TCDD, while the hamster is one of the least sensitive. Adverse reactions in animals include thymic atrophy in all species, induction of hepatic enzymes in the mouse and rat, and teratogenicity and reproductive effects in rodents and non-human primates. Except for chloracne, however, TCDD has not demonstrated comparable levels of biologic activity in man; that is to say, no long-term effects on the cardiovascular and central nervous systems, the liver, the kidney, the thymus and immunlogic defenses, and the reproductive function -in the male, female or offspring--have been demonstrated.

The final answers concerning military exposures to Agent Orange, as well as exposures during the production and application of phenoxy herbicides in general, will require additional, carefully designed morbidity and laboratory studies using all of the sensitive technical strategies of modern science. The answers will come only through the objective, well designed and scholarly approaches of the community of scientists.

References

Albro PW, Corbett BJ: Extraction and clean-up of animal tissues and subsequent determination of mixtures of chlorinated dibenzo-p-dioxins and dibenzofurans. Chemosphere 1977;7:381.

Allen JR, Barsotti DA, Van Miller JP, et al: Morphological change in monkeys consuming a diet containing 500 ppt of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Cosmet Toxicol 1977;15:401-10.

Allen JR, Barsotti DA, Lambrecht LK, et al: Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann NY Acad Sci 1979;320:419-25.

Anonymous: More Seveso tests. Chem Week 1980(Dec) :21.

Anonymous: Agent Orange research update; soft-tissue sarcomas. AO Rev 1983(Apr);2(1).

Anonymous: Toxic Materials News 1984(Aug 11):222.

Arensault RD: Pentachlorophenol and contained chlorinated dibenzodioxins in the environment. American Wood Preservers Association, 1976.

Armstrong B: Australians report no link between service in Vietnam and birth defects among offspring. Epidemiol Monit 1983(Mar);3(4):1.

Ashe WF, Suskind RR: Reports on chloracne cases, Monsanto Chemical Co., Nitro, West Virginia. Reports of the Kettering Laboratory, Dec 1949 and Apr 1950.

Australian Dept of Health: 2,4,5-T and human birth defects. Report of the Division of Public Health, Department of Health, Australia, 1977.

Axelson O, Sundell L: Herbicide exposure, mortality and tumor incidence; an epidemiological investigation on Swedish railroad workers. Work-Environ Health 1974;11:21.

Balarajan R, McDowall M: Congenital malformations and agricultural workers. Lancet 1983;1:1112-1113.

Bastomsky CH: Enhanced thyroxine metaboism and high uptake goiters in rats after a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 1977;101:292-296.

Baughman RW, Meselson M: An analytical method for detecting TCDD (dioxin); levels of TCDD in samples from Vietnam. Envl Hlth Persp 1973a;5:27-35.

Baughman RW, Meselson M: An improved analysis for tetrachlorodibenzo-p-dioxin. In: Chlorodioxins — Origin and Fate. EH Blair (ed), American Chemical Society, Washington, DC, 1973b.

Beatty PW: Studies on the metabolism and possible mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Doctoral thesis, Vanderbilt University, 1978.

Beatty PW, Lembach KJ, Holscher MA, et al: Effects of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) on mammalian cells in tissue cultures. Toxicol Applied Pharmacol 1975;31:309-312.

Beatty PW, Vaughn WR, Neal RA: Effects of alteration of rat hepatic MFO activity on the toxicity of 2,3,7,8-TCDD. Toxicol Appl Pharmacol 1978;45:513-519.

Berry DL, Slaga TJ, DiGiovanni J, et al: Studies with chlorinated dibenzo-p-dioxins, polybrominated biphenyls and polychlorinated biphenyls in a two-stage system of mouse skin tumorigenesis; potent anticracinogenic effects. Annals NY Academy of Sciences 1979;320:405-414.

Berry DL, Slaga TJ, Wilson, NM, et al: Transplacental induction of mixed-function oxygenases in extraOhepatic tissues by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem Pharmacol 1977;26:1383-1388.

Blair A, Grauman DJ, Lubin JH, Fraumeni Jr JF: Lung cancer and other causes of death among licensed pesticide applicators. JNCI 1983;71(1):31-38.

Blank CE, Cooke P, Potter AM: Investigations for genotoxic effects after exposure to crude 2,4,5-trichlorophenol. Br J Indus Med 1983;40:87-91.

Bolton L: Seveso dioxin; no solution in sight. Chem Eng 1978;**85**(22):78.

Bond GG, Ott MG, Brenner FE, et al: Medical and morbidity surveillance findings among employees potentially exposed to TCDD. Br J Indus Med 1983;**40**:318-324.

Buser HR: Polychlorinated dibenzo-p-dioxin; separation and identification of isomers by gas chromatography-mass spectrometry. J Chromatography 1975;**114**:95-108.

Buser HR: Polychlorinated dibenzo-p-dioxins and dibenzofurans; formation, occurrence and analysis of environmentally hazardous compounds. Department of Organic Chemistry, University of Umea, Sweden and Swiss Federal Research Station, Waedenswill, Switzerland, pp 9-21, 1978.

Buser HR, Bosshardt HP: Polychlorinated dibenzo-p-dioxins, dibenzofurans and benzenes in fly ash of municipal and industrial incinerators. Mitt Geb Lebensmittelsunters Hyg 1978;**69**(2):191-199.

Buser HR, Bosshardt HP, Rappe C: Identification of polychlorinated dibenzo-p-dioxin isomers formed in fly ash. Chemosphere 1978;**7**(2):165-172.

Buser HR: Formation of polychlorinated dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs) from the pyrolysis of chlorobenzenes. Chemosphere 1979;**6**:415-424.

Buu-Hoi NP, Chanh PH, Sesque G, et al: Organs as targets of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) intoxication. Naturwissenschaften 1972;**59**:174-175.

Carlstedt-Duke JMB: Tissue distribution of the receptor for 2,3,7,8-tetrachloro-dibenzo-p-dioxin in the rat. Cancer Res 1979;**39**:3172-3176.

Clark DA, Gauldie J, Szewczuk MR, et al: Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc Soc Exp Biol Med 1981;**168**:290-299.

Clark DA, Sweeney G, Safe S, et al: Cellular and genetic basis for suppression of cytotoxic T cell generation by haloaromatic hydrocarbons. Immunopharmacol 1983;**6**:143-153.

Coggen D, Acheson ED: Do phenoxy herbicides cause cancer in man? Lancet 1982;**1**:1057-1059.

Cook RR: Dioxin, chloracne and soft tissue sarcoma. Lancet 1981; **I**:618-619.

Cook RR, Bodner KM: Dioxin and reproductive events. In: Tucker RE, Young AL and Gray AP (eds): Human and Environmental Risks of Chlorinated Dioxins and Related Compounds. Plenum Press, New York, 1983, pp 593-604.

Cook RR, May V: Dow Chemical worker and environmental studies. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 24 October 1983.

Courtney KD, Gaylor DW, Hogan MD, Falk HL: Teratogenic evaluation of 2,4,5-T. Science 1970;**168**:864-866.

Crosby DG: Photodecomposition of chlorinated dibenzo-p-dioxins. Science 1971;**73**:748-749.

Crosby DG, Wong AS: Environmental degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Science 1977;**195**:1337-1338.

Crosby DG: Methods of photochemical degradation of halogenated dioxins in view of environmental reclamation. In: Accidental Exposure to Dioxins; Human Health Aspects. F. Coulston and F. Pocchiari (eds), Academic Press, New York, 1982.

Crummett WB, Stehl RH: Determination of chlorinated dibenzo-p-dioxins and dibenzofurans in various materials. Envl Hlth Persp 1973;**5**:15-25.

DiGiovanni JA, Viaje A, Berry DL, et al: Tumor initiating ability of 2,3,7,8-tetrachlorodiobenxo-p-dioxin (TCDD) and Aroclor 1254 in a two-stage system of mouse skin carcinogenesis. Bull Environ Contam Toxicol 1977;**18**:522-557.

Donovan JW, Adena MA, Rose G et al: Case-control study of congenital anomalies and Vietnam service (birth defects study). Report to the Minister for Veterans' Affairs, January 1983, Australian Government Publishing Service, Canberra.

Dow Chemical Company: The trace chemistries of fire -- A source and route for the entry of chlorinated dioxins into the environment. The Chlorinated Dioxin Task Force, Michigan Division, Dow Chemical, USA, 1978.

Elson M: Lawyers map strategy here for vast Agent Orange suite. Chicago Trib, 5 October 1980.

Emerson JL, Thompson DJ, Strebing RJ, et al: Teratogenic studies of 2,4,5-trichlorophenoxyacetic acid in the rat and rabbit. Food Cosmet Toxic 19719:395-404.

Ericson A, Kallen B, Westholm P: Clusters of malformations in Sweden; a study with central registers. Envl Resch 1983;**30**:466-479.

Eriksson M, Hardell L, Berg NO, et al: Soft-tissue sarcomas and exposure to chemical substances; a case-referrent study. Br J Indus Med 1981;**38**:27-33.

Faith RE, Luster MI: Investigations on the effects of 2,3,7,8-TCDD on parameters of various immune functions. Ann NY Acad Sci 1979;**320**:564-571.

Faith RE, Moore JA: Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J Toxicol Environ Health 1977;**3**:451-464.

Fingerhut MA: NIOSH dioxin registry. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 17 November 1983.

Fingerhut MA, Marlow DA, Honchar PA, Halperin WE: The NIOSH Occupational Dioxin Registry. In: Public Health Risks of the Dioxins. Proceedings of symposium, 19-20 October 1983 at The Rockefeller University. Lowrance W (ed), William Kaufman (Los Altos, Calif.) 1984a.

Fingerhut MA, Halperin WE, Honchar PA, Smith AB, Groth DH, Russell WO: An evaluation of reports of dioxin exposure and soft tissue sarcoma pathology in US chemical workers. Scand J Work Environ Health (in press) 1984b.

Firestone D: The 2,3,7,8-tetrachlorodibenzo-p-dioxin problem; a review. In: Chlorinated Phenoxy Acids and their Dioxins; Mode of Action, Health Risks and Environmental Effects. C. Ramel (ed), Ecol Bull (Stockholm) 1978.

Gaffey WR: Monsanto worker studies. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 17 November 1983.

Gasiewicz TA, Holscher MA, Neal RA: The effect of total parenteral nutrition on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol Appl Pharmacol 1980;**54**:469-488.

Gasiewicz TA, Neal RA: 2,3,7,8-Tetrachlorodibenzo-p-dioxin tissue distribution, excretion and effects of clinical chemical parameters in guinea pigs. Toxicol Applied Pharmacol 1979;**51**(2):329-339.

Gasiewicz TA, Neal RA: The examination and quantitation of tissue cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-p-dioxin using hydroxylapatite. Anal Biochem 1982;**124**:1-11.

Gasiewicz TA, Ness WC, Rucci G: Ontogeny of the cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver, lung and thymus. Bioch Biophys Res Commun 1984;**118**:183-190.

Ghiasuddin SM, Nelson JO, Mehzer RE, et al: Comparative metabolism of environmentally significant chlorinated aromatic compounds in hepatic microsomal systems. Toxicol Appl Pharmacol 1975;**33**:152-153.

Giavini E, Prati M, Vismara C: Rabbit teratology study with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Research 1982;**27**:74-78.

Gibson JE: Perinatal nephropathies. Environ Health Perspect 1976;**15**:121-130.

Green S, Moreland F, Sheu C: Cytogenetic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on rat bone marrow cells. FDA By-lines 1977;**6**:292-294.

Greenlee WF, Dold KM, Irons RD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) inhibits the induction by thymic epithelial cells of T-lymphocyte nitrogen responsiveness. The Toxicologist 1984;**4**(4):188.

Greenlee WI, Poland A: Nuclear uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6J and DBA/2J mice. J Biol Chem 1979;**254**:9814-9821.

Greenwald P, Kovasznay B, Collins DN, Therriault G: Sarcomas of soft tissue after

Vietnam service. JNCI 1984;73:1107-1109.
Greig JB, Jones G, Butler WH, et al: Toxic effects of 2,3,7,8-tetrachloridibenzo-p-dioxin. Food Cosmet Toxicol 1973;11:585-595.
Gupta BN, Vos JG, Moore JA, et al: Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ Health Perspect 1973;5:125-40.
Gross ML, Lay JO, Lyon PO, et al: 2,3,7,8-Tetrachlorodibenzo-p-dioxin levels in adipose tissue of Vietnam veterans. Envl Resch 1984;33:261-268.
Hardell L: Malignant mesenchymal tumors and exposure to phenoxy acids; a clinical observation. Lakartidningen 1977;74:542-546.
Hardell L: Soft-tissue sarcomas and exposure to phenoxyacetic acids and cancer. Lakartidningen 1977;74:2735.
Hardell L: Malignant lymphoma of histiocytic type and exposure to phenoxyacetic or chlorophenols. Lancet 1979;I:55-56.
Hardell L, Eriksson M, Lenner P, et al: Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids; a case control study. Brit J Cancer 1981;43:169-176.
Hardell L, Johansson B, Axelson O: Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid or chlorophenol exposure. Am J Indus Med 1982;3:247-257.
Hay A: Dioxin hazards; secrecy at Coalite. Nature 1981;290:729.
Hay A, Ashby J, Styles JA, et al: The mutagenic properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Abstract, American Chemical Society meeting, Washington, DC, August 1983.
Hoar S: Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 24 October 1983.
Hoffman RE, Donnel HD: Pilot study Times Beach, Missouri. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 13 December 1983.
Homberger E, Reggiani G, Sambeth J, et al: The Seveso accident; its nature, extent and consequences. Ann Occup Hyg 1979;22:327-370.
Honchar PA, Halperin WE: 2,4,5-T, trichlorophenol and soft-tissue sarcoma. Lancet 1981;I(8214):268-269.
Honchar P: Health Hazard Evaluation Report HETA-80-039-1179. National Institute for Safety and Health, Cincinnati, 1982.
Hope W, Lischwe D, Russell W, et al: Epidemiologic notes and reports; porphyria cutanea tarda and sarcoma in a worker exposed to 2,3,7,8-tetrachlorodibenzodioxin -- Missouri. MMWR 1984;33(8):113-114.
Huff JE, Moore JA, Saracci R, Tomatis L: Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ Health Perspect 1980;36:221-240.
Inagami K, Koga T, Kikuchi M, et al: Experimental study of hairless mice following administration of rice oil used by a "Yusho" patient. Fukuoka Acta Medica 1969;60:548-553.
International Agency for Research on Cancer (IARC): Some fumigants, the herbicides 2,4-D and 2,4 5-T, chlorinated dibenzodioxins and miscellaneous industrial chemicals. IARC Monograph 15: Evaluation of the Carcinogenic Risk of Chemicals to Man, 1977.
International Agency for Research on Cancer (IARC): Chemicals, industrial processes and industries associated with cancer in humans. Evaluation of the Carcinogenic Risk of Chemicals to Humans. Monograph Suppl 4 1982; pp 238-243.
Jensen S, Renberg L: Contaminants in pentachlorophenol; chlorinated dioxins and predioxins. Ambio 1972;1(2):62-65.
Jensen S, Renberg L: Chlorinated dimers present in several technical chlorophenols used as fungicides. Envl Hlth Persp 1973;5:37-41.
Jirasek L, Kalensky J, Kubec K: Acne chlorina and porphyria cutanea tarda during the

manufacture of herbicides. Cesk Dermatol 1973;**48**:306-317.

Jirasek L, Kalensky J, Kubec K, et al: Acne chlorina and porphyria cutanea tarda, other manifestations of general poisoning, during the manufacture of herbicides. II. Cesk Dermatol 1974;**49**:145-157.

Johnson FE, Kugler MA, Brown SM: Soft-tissue sarcomas and chlorinated phenols. Lancet 1981;**II**:40.

JRB Associates: Review of Literature on Herbicides, Including Phenoxy Herbicides and Associated Dioxins, vols 1 and 2. Prepared for the Veterans Administration, Dept of Medicine and Surgery, Washington, DC, 1981.

Khera KS, Ruddick JA: Polychlorodibenzo-p-dioxins; perinatal effects and the dominant lethal test in the Wistar rats. In: Chlorodioxins--Origin and Fate. Advances Chem Series **120**, Blair EH (ed), American Chemical Soc, Washington, DC, pp 70-84.

Kimbrough R: Soft tissue sarcomas. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 17 November 1983.

Kimbrough R, Falk H, Stehr P: Health implications of 2,3,7,8-tetrachloridibenzo-p-dioxin (TCDD) contamination of residential soil. J Toxicol Environ Health 1984;**14**:47-93.

Kimmig J, Schultz KH: Berufliche Akne durch chlorierte aromatische zyklische Äther Dermatologica 1957;**115**:540-546.

Kimmig J, Schultz KH: Chlorinated aromatic cyclic esters as the cause of chloracne. Naturwissenschaften 1957;**44**:337-338.

Knutson JC, Poland A: Response of murine epidermis to 2,3,7,8-tetrachloridibenzo-p-dioxin; interaction of the Ah and hr loci. Cell 1982;**30**:225-234.

Knutson JC, Poland A: 2,3,7,8-Tetrachlorodibenzo-p-dioxin; failure to demonstrate toxicity in twenty-three cultured cell types. Toxicol Appl Pharmacol 1980;**54**:377-383.

Lamm SH: An epidemiologic assessment of the Alsea II report, 6 August 1979.

Lamm SH: (Cited in "Toxic Materials in Brief") Toxic Materials News :339, 10 Dec 1980.

Lathrop GD, Wolfe WH, Albanese RA, Moynahan PM: An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides; baseline morbidity study results. USAF School of Aerospace Medicine, Brooks AFB, 24 February 1984.

Lawrence CE, Reilly AA, Quickenton P, Greenwald P, Page WF, Kuntz AJ: Mortality patterns of New York State Vietnam veterans. Am J Public Health 1985;**75**:277-279.

Lipson A, Gaffey WR, LaVecchio F: Agent Orange and birth defects. N Engl J Med (Letters to the Editor) 1983;**309**:491-492.

Mason ME, Okey AB: Cytosolic and nuclear binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the Ah receptor in extra-hepatic tissues of rats and mice. Eur J Biochem 1982;**123**:209-215.

May G: Chloracne from the accidental production of tetrachlorodibenzodioxin. Br J Ind Med 1973;**30**:276-283.

McConnell EE, Moore JA, Haseman JK, et al: The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol Applied Pharmacol 1978;**44**:335-336.

McConnell EE, Lucier GW, Rumbaugh RC, Albro PW, Harvan DJ, Hass JR, Harris MW: Dioxin in soil; bioavailablity after ingestion by (female Sprague-Dawley) rats and (male Hartley) guinea pigs. Science 1984;**223**;1077-1079.

McNulty WP: Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin for Rhesus monkey. Bull Environ Contam Toxicol 1977;**18**:108-189.

McNulty WP: TCDD Teratogenicity and Fetotoxicity. Rockefeller University Symposium on Public Health Risk of the Dioxins, New York, New York, 19-20 October 1983.

McNulty WP, Becker GM, Cory HT: Chronic toxicity of 3,4,3,4 - and 2,5,2,5-

tetrachlorobiphenyls in Rhesus Macques. Toxicol Appl Pharmacol 1980;56:182-190.
McNulty WP, Pomerantz I, Farell T: Chronic toxicity of 2,3,7,8-tetrachlorodibenzofuran for Rhesus Macaques. Food Cosmet Toxicol 1981;19:57-65.
Merlo F: Adverse health effects in human populations exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso; an update. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 13 December 1983.
Michigan Department of Public Health: Evaluation of Soft and Connective Tissue Cancer Mortality Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewide, 4 May 1983.
Milham S Jr: Herbicides, occupation and cancer. Lancet 1982;I:1464-1465.
Minister of Veterans' Affairs: Case-control study of congenital anomalies and Vietnam service (birth defects study). Australian Government Publishing Service, January 1983.
Moore JA, Gupta BN, Zinkl JG, et al: Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ Health Perspect 1973;5:81-85.
Moore JA: A pesticide. Science 1979;203(4382):741-742.
Moses M, Lilis R, Crow KD, et al: Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichlorophenoxy-acetic acid; comparison of findings with and without chloracne. Am J Indus Med 1984;5:161-182.
Moses M, Selikoff IJ: Soft tissue sarcomas, phenoxy herbicides, and chlorinated phenols. Lancet 1981;I:1370.
Nagarkatti PS, Sweeney GD, Gauldie J, et al: Sensitivity to suppression of cytotoxic T-cell generation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is dependent on the Ah genotype of the murine host. Toxicol Appl Pharm 1984;72:169-176.
National Toxicology Program (NTP): (Rep 80-31) -- Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Osborne-Mendel rats and B6C3F1 mice (gavage study). Technical report series No. 209, NIH publication No. 82-1765, Research Triangle Park, NC, 1982.
National Toxicology Program (NTP): (Rep 80-32) -- Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Swiss-Webster mice (dermal study). Technical report series No. 201, NIH publication No. 82-1757, Research Triangle Park, NC, 1982.
Neal RA: Spectrum of metabolic effects of TCDD in animal experiments. Rockefeller University Symposium on Public Health Risk of the Dioxins, New York, New York, 19-20 October 1983.
Neal RA, Beatty PW, Gasiewicz TA: Studies on the mechanism of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Ann NY Acad Sci 1979;320:204-213.
Nebert DW, Gielen JE: Genetic regulation of aryl hydrocarbon hydroxylase induction in the mouse. Fed Proc 1972;31:1315-1327.
Nebert DW, Jensen NM: The Ah locus; genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450 mediated monooxygenases. CRC Crit Rev Biochem 1979;6:401-437.
Nelson JO, Menzer R, Kearney PC: 2,3,7,8-Tetrachlorodibenzo-p-dioxin; in vitro binding to rat liver microsomes. Bull Environ Comtam Toxicol 1977;18:9-13.
Nestrick TJ, Lamparski LL: Isomer-specific determination of chlorinated dioxins for assessment of formation and potential environmental emission from wood combustion. Anal Chem 1982;54:2292-2299.
Neubert D, Dillmann I: Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Naunyn-Schmiedberg's Arch Pharmakol Exp Pathol 1972;272(3):243-264.
Neubert D, Zens P, Rothenwallner A, Merker HJ: A survey of the embryotoxic effects of TCDD in mammalian species. Environ Health Perspect 1973 (Exptl issue 5):233-40.
Nilsson CA, et al: Chromatographic evidence for the formation of chlorodioxins from

chloro-2-phenoxyphenols. J Chromatography 1974;**96**:137-147.

Nilsson CA, Norstrom A, Andersson K, Rappe C: Pentachlorophenol; Chemistry, Pharmacology and Environmental Toxicology. K. Ranga Rao (ed), Plenum, New York, 1978.

Nolan RJ, Smith FA, Hefner JG: Elimination and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female guinea pigs following a single oral dose. Toxicol Appl Ppharmacol 1979;**48**:A162.

Norman C: Vietnam's herbicide legacy; recent studies in Vietnam have found long-term environmental damage and a possible rise in birth defects from US spraying. Science 1983;**219**:1196-1197.

Nunn DM: Trial between Palmer et al vs Nova Scotia Forest Industries. Transcript of judicial decision, 1983.

O'Keefe PW, Meselson MS, Baughman RW: Neutral clean-up procedures for 2,3,7,8-tetrachlorodibenzo-p-dioxin residues in bovine fat and milk. JAOAC 1978;**61**:621-626.

Okey AB, Bondy GP, Mason ME, et al: Regulatory gene product of the Ah locus characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. J Biol Chem 1979;**254**:11636-11648.

Okey AB, Bondy GP, Mason ME, et al: Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in continuous cell culture lines. J Biol Chem 1980;**255**:11418-11422.

Olie L, Vermeulen PL, Hutzinger O: Chlorodibenzo-p-dioxins and chlorodibenzofurans are trace components of fly ash and flue gas of some municipal incinerators in the Netherlands. Chemosphere 1977;**8**:455-459.

Oliver RM: Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory workers. Br J Ind Med 1975;**32**:49-53.

Olson JR, Gasiewicz TA, Geiger LE, et al: The metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin. In: Mammalian Systems in Accidental Exposure to Dioxins; Human Health Aspects, Coulston F and Pocchiari F (eds), Academic Press, New York, 1983.

Olson JR, Gasiewicz TA, Neal RA: Tissue distribution, excretion and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the Golden Syrian hamster. Toxicol Applied Pharmacol 1980;**56**:78-85.

Olsson H, Brandt L: Non-Hodgkin's lymphoma of the skin and occupational exposure to herbicides. Lancet 1981;**2**:579.

Osborne R, Greenlee WF: Altered regulation of human epidermal cell proliferation and differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The Toxicologist 1984;**4**(1):188.

Pazderova-Vejlupkova J, Lukas E, et al: Chronic poisoning by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Pracov Lek 1981;**32**:204-209.

Pazderova-Vejlupkova J, Nemcova M, Pickova J, et al: The development and prognosis of chronic intoxication by tetrachlorodibenzo-p-dioxin in men. Arch Environ Health 1981;**36**(1):5-11.

Percy C, Stanek E, Gloeckler L: Accuracy of cancer death certificates and its effect on cancer mortality statistics. Am J Public Health 1981;**71**(3):242-250.

Piper WN, Rose JQ, Gehring PJ: Excretion and distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Environ Health Perspect (Exptl issue 5) 1973;241-244.

Pitot HC, Goldsworthy T, Campbell HA, et al: Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res 1980;**40**:3616-3620.

Plimmer JR, Klingebiel UI, Crosby DH, Wong AS: Photochemistry of dibenzo-p-dioxins. In: Chlorodioxins — Origin and Fate. E. Blair (ed), American Chemical Society, Washington, DC, pp 44-54, 1973.

Plimmer JR: Approaches to decontamination or disposal of pesticides; photodecomposition. In: Disposal and Decomposition of Pesticides. MV Kennedy (ed), ACS Symposium Series 73, American Chemical Society, Washington, DC, pp 12-23, 1978.

Pocchiari F, Vittorio S, Zampieri A: Human health effects from accidental release of tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. In: Health Effects of Halogenated Aromatic Hydrocarbons, Nicholson WJ and Moore JA (eds), Ann NY Acad Sci 1979(May);**320:**311-320.

Poiger H, Buser HR, Weber H, et al: Structure elucidation of mammalian TCDD-metabolites. Experientia 1982;**38:**484-86.

Poiger H, Weber H, Schlatter C: Special aspects of metabolism and kinetics of TCDD in dogs and rats. In: Chlorinated Dioxins and Related Compounds, Hutzinger O et al (eds), Pergamon series. Env Sci 1982;**5:**317-323.

Poland A, Glover E: 2,3,7,8-tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Mol Pharmacol 1980;**17:**86-94.

Poland A, Glover E: An estimate of the maximum in vitro covalent binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to rat liver protein, ribosomal RNA and DNA. Cancer Res 1979;**39:**3341-3344.

Poland A, Glover E, Kende AS: Stereospecific high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. J Biol Chem 1976;**251:**4936-4946.

Poland A, Knutson JC: 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons; examination of the mechanism of toxicity. Ann Rev Pharmacol 1982;**22:**517-554.

Poland A, Palen D, Glover E: Tumour promotion by TCDD in skin of HRS/J hairless mice. Nature 1982;**300:**271-273.

Poland A, Yang GC: Preparation and characterization of chlorinated dibenzo-p-dioxin. J Agric Food Chem 1972;**20:**1093-1099.

Rappe C: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) introduction. In: Dioxin -- Toxicological and Chemical Aspects. F. Cattabeni, A Cavallaro and G Galli (eds), SP Medical and Scientific Books, NY, pp 9-11, 1978.

Rappe C, Buser HR, Bosshardt HP: Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs); occurrence, formation and analysis of environmental hazardous compounds. CIPC Symposium, Baltimore, Maryland, 5-6 June 1979.

Rappe C, Buser HR, Kuroki H, Masuda Y: Chemosphere 1979.

Reggiani G: The estimation of the TCDD toxic potential in the light of the Seveso accident. Arch Toxicol (Suppl 2) 1979;291-302.

Riihimaki V, Asp S, Hernberg S: Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland. Scand J Work Environ Health 1982;**8:**37-42.

Robinson BD: Safety aspects of 2,4,5-T herbicides. Technote No. 5/79. Dept of Agriculture and Fisheries, Adelaide, Australia, 1979.

Rogers AM, Andersen ME, Back KC: Mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in L5178Y mouse lymphoma cells. Mutation Res 1982;**105:**445-449.

Rose JQ, Ramsey JC, Wentzler TH, et al: The fate of TCDD following single and repeated oral doses to the rat. Toxicol Applied Pharmacol 1976;**36:**209-226.

Rozman K, Rozman T, Greim H: Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced toxicity. Toxicol Appl Pharmacol 1984;**72:**372-376.

Rozman T, Rozman K, Greim H: Role of thyroid function in TCDD induced toxicity. The Toxicologist 1984;**4**(1):189.

Sarma PR, Jacobs J: Thoracic soft tissue sarcoma in Vietnam veterans exposed to Agent Orange. N Engl J Med 1982;**306**(18):1109.

Schacter L, Crum E, Abbound S, et al: Adenocarcinoma of the lung in Vietnam veterans younger than 35 years. JAMA 1984;**251**(5):604.

Schaum J: Risk Analysis of TCDD Contaminated Soil. US Environmental Protection

Agency, EPA/600/8-84/031.

Scheupler E, Rozman K, Pazdernik T, et al: Effect of thyroxine (T4) and triiodootyrosine (T) on TCDD toxicity in thyroidectonized rats. The Toxicologist 1984;**4**(1):189.

Schwetz BA, Norris JM, Sparschu GL, et al: Toxicology of chlorinated dibenzo-p-dioxins. Environ Health Perspect 1973;**5**:87-99.

Sell KW: Immunology. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 13 December 1983.

Shepard BM: A summary of current information on the VA's Agent Orange Registry. Presented before the Division of Environmental Chemistry, American Chemical Society, Washington, DC, 30 August 1983.

Shepard BM, Young AL: Twins study, soft tissue sarcomas, porphyria cutanea tarda. Presentation at meeting of AMA's CSA Panel on Toxic Substances, 23 September 1983.

Singer R, Moses M, Valciukas J, et al: Nerve conduction velocity studies of workers employed in the manufacture of phenoxy herbicides. Envl Rsch 1982;**29**:297-311.

Smith AH: Seasonal analysis of Oregon data on spontaneous abortions and 2,4,5-T spraying. Presented at ANZSERCH, Annual Conference; Dunedin, New Zealand, August 1979.

Smith AH, Fisher DO, Pearce N, Teague CA: Do agricultural chemicals cause soft tissue sarcoma? Initial findings of a case-control study in New Zealand. Community Health Stud 1982a;**6**:114-119.

Smith AH, Fisher DO, Pearce N, Chapman CJ: Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Arch Environ Health 1982b;**37**:197-200.

Smith AH,Pearce NE, Fisher DO, Giles HJ, Teague CA, Howard JK: Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. JNCI 1984;**73**:1111-1117.

Smith FA, Schwetz BA, Nitschke KD: Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-mice. Toxicol Appl Pharmacol 1976;**38**:517-523.

Sparschu GL, Dunn FL, Rowe VK: Study of teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Cosmet Toxic 1971;**9**:405-412.

Stevens KM: Agent Orange toxicity; a quantitative perspective. Human Toxicology 1981;**1**:31-39.

Suskind RR: Clinical manifestations of 2,4,5-T exposures. Paper presented at the International Workshop on Chemical and Epidemiological Follow-up After Areawide Chemical Contamination, National Academy of Sciences, Washington, DC, 1980.

Suskind RR, Hertzberg VS: Human health effects of 2,4,5-T and its toxic contaminants. JAMA 1984;**251**(18):2372-2380.

Tenchini ML, Crimaudo C, Pacchetti G, et al: A comparative cytogenetic study of cases of induced abortions in TCDD-exposed and nonexposed women. Envl Mutagenesis 1983;**5**:73-85.

Thiess AM, Frentzel-Beyme R, Link R: Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF AG on 17 November 1953. Am J Indus Med 1982;**3**:179-189.

Townsend JC, Bodner KM, Van Peenen PFD, et al: Survey of reproductive events of wives of employees exposed to chlorinated dioxins. Amer J Epid 1982;**115**(5):695-713.

Tung TT, An TT, Tam NE, et al: Le cancer primaire du foie au Vietnam. Chirurgie 1973;**99**:427-436.

Tung TT, Tugen BQTKA, Tra DX, et al: Clinical effects of massive and continuous utilization of defoliants on civilians. Vietnamese Studies 1971;**29**:53-81.

US Air Force (USAF) School of Aerospace Medicine: Project Ranch Hand II. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides; baseline mortality study results. USAF School of Aerospace Medicine, Brooks AFB, 30 June 1983.

US Centers for Disease Control (CDC): Agent Orange projects (press release). 11 December 1984.

US Department of Health and Human Services (US DHHS): Protocol for epidemiological studies of the health of Vietnam veterans. CDC Public Health Service, November 1983.

US Veterans Administration (US VA): Australia issues mortality report on Vietnam veterans. Agent Orange Rev 1985;4(1):4.

US Environmental Protection Agency (US EPA): Dioxins. Cincinnati, Ohio, 1980.

Van Miller JP, Marlar RJ, Allen JR: Tissue distribution and excretion of tritiated tetrachlorodibenzo-p-dioxin in non-human primates and rats. Food Cosmet Toxicol 1976;14:31.

Vinopal JH, Casida JE: Metabolic stability of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian liver microsomal systems and in living mice. Arch Environ Contamn Toxicol 1973;1:122-132.

Voss JG, Beems RB: Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. Toxicol Appl Pharmacol 1971;19:617-633.

Voss JG, Moore JA, Zinkl JG: Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ Health Prespec 1973;5:149-162.

Wagner SL, Witt JM, Norris LA, et al: A Scientific Critique of the EPA Alsea II Study and Report. Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon, 1979.

Wassom JS, Huff JE, Loprieno N: A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutation Res 1977/78;47:141-160.

Weaver D, Amos A, Tierney B, et al: Interaction of DNA with cytosolic 3-methylcholanthrene binding proteins from either rat or mouse liver. Carcinogenesis 1980;1:481-486.

Webb K, Ayres S, Slavin R, et al: Results of a pilot study of health effects due to 2,3,7,8-tetrachlorodibenzodioxin contamination - Missouri. MMWR 1984;33(5):54-61.

Weber H, Poiger H, Schlatter C: Acute oral toxicity of TCDD metabolites in male guinea pigs. Toxicol Lett 1982;14:117-122.

Willis RF: Canadian review of human and animal studies and dioxin sources as a basis for recommendations on government regulations. Presentation at meeting of AMA's CSA Advisory Panel on Toxic Substances, 13 December 1983.

Wolfe WH, Michalek JE, Albanese RA, Lathrop GD, Moynahan PM: Project Ranch Hand II. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides; mortality update - 1984. USAF School of Aerospace Medicine, Brooks AFB, 10 December 1984.

Young AL, Calcagani JA, Thalken CE, et al: The Toxicology, Environmental Fate and Human Risk of Herbicide Orange and its Associated Dioxin. USAF Occupational and Environmental Health Laboratory, Technical Report No. TR-78-92, Final Report, Brooks Air Force Base, Texas, 1978.

List of States with Vietnam Veteran Commissions or Programs

California	California Division of Veterans Affairs P.O. Box 1559 Sacramento CA 95814 (916) 445-9518
Connecticut	Vietnam Veterans Herbicide Information Commission Veterans' Office Southern Connecticut University 501 Cresent Street New Haven CT 06515 (203 297-4329
Georgia	Agent Orange Program Georgia Department of Human Resources 47 Trinity Avenue, S.W. Atlanta, GA 30334 (404) 656-4764
Hawaii	Agent Orange Program Hawaii Department of Health P.O. Box 3378 Honolulu, HI 96801 (808) 548-8705
Indiana	Agent Orange Program Department of Veterans' Affairs 707 State Office Building Indianapolis, IN 46204 (317) 232-3910
Iowa	Agent Orange Program Iowa Department of Health Lucas State Office Building Des Moines IA 50319 (515) 281-8220
Kansas	Agent Orange Program Kansas Department of Health/Environment Forbes Field Topeka, KS 66620 (913) 862-9360
Maine	Bureau of Health State House, Station 11 Augusta, ME 04333 (207) 298-3201
Massachusetts	Agent Orange Program 100 Cambridge Street 10th Floor, #1001 Boston, MA 02202 (617) 722-1107
Minnesota	Agent Orange Program Department of Veterans' Affairs Veterans Service Building St. Paul, MN 55155 (612) 297-4217

New Jersey	Agent Orange Commission Broad Street Bank Building 143 East State Street Trenton, NJ 08608	(609) 984-7397
Ohio	Agent Orange Program Ohio Board of Regents 30 East Broad Street Columbus, OH 43215	(614) 466-6000
Oklahoma	Agent Orange Assistance Program Oklahoma Department of Health P.O. Box 53551 Oklahoma City, OK 73152	(405) 271-4200
Oregon	Agent Orange Program Department of Veterans' Affairs Veterans Service Division 700 Summer Street, N.E., Suite 150 Salem, OR 97310-1270	(503) 378-6839
Pennsylvania	Vietnam Herbicides Information Commission Pennsylvania Department of Health P.O. Box 8380 Health and Welfare Building, Room 912A Harrisburg, PA 17105	(717) 787-1708
Rhode Island	Agent Orange Commission 242 Prairie Avenue Providence, RI 02907	(401) 521-6710
Texas	Agent Orange Program Texas Department of Health 1100 West 49th Street Austin, TX 78756	(817) 458-7251
West Virginia	Agent Orange Program West Virginia Department of Health Office of Community Health Services 1800 Washington Street East Charleston, WV 25303	(304) 348-3210
Wisconsin	Agent Orange Program Wisconsin Department of Health & Social Services Division of Health P.O. Box 309 Madison, WI 53701	(608) 266-1253

*U.S. GOVERNMENT PRINTING OFFICE : 1985 0-461-890/35651