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Ambient Water Quality Criteria for 2, 3, 7, 8 - Tetrachloro-dibenzo - p - dioxin

AMBIENT WATER QUALITY CRITERIA FOR

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Prepared By
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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including groundwater. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisfaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, have been developed by EPA.

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CRITERIA DOCUMENT

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

CRITERIA

Aquatic Life

Not enough data are available concerning the effects of 2,3,7,8-TCDD on aquatic life and its uses to allow derivation of national criteria. The available information indicates that acute values for some freshwater animal species are >1.0 µg/ℓ; some chronic values are <0.01 µg/ℓ, and the chronic value for rainbow trout is <0.001 µg/ℓ. Because exposures of some species of fishes to 0.01 µg/ℓ for <6 days resulted in substantial mortality several weeks later, derivation of aquatic life criteria for 2,3,7,8-TCDD may require special consideration. Predicted bioconcentration factors (BCFs) for 2,3,7,8-TCDD range from 3000-900,000, but the available measured BCFs range from 390-13,000. If the BCF is 5000, concentrations >0.00001 µg/ℓ should result in concentrations in edible freshwater and saltwater fish and shellfish that exceed levels identified in a U.S. FDA health advisory. If the BCF is >5000 or if uptake in a field situation is greater than that in laboratory tests, the value of 0.00001 µg/ℓ will be too high.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to 2,3,7,8-TCDD exposure through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero. This criterion is based on the non-threshold assumption for 2,3,7,8-TCDD. However, zero may not be an attainable level at this time.

Therefore, the levels that may result in an increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 1.3×10^{-7}, 1.3×10^{-8} and 1.3×10^{-9} μg/ℓ, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 1.4×10^{-7}, 1.4×10^{-8} and 1.4×10^{-9} μg/ℓ, respectively. If these estimates are made for consumption of water only, the levels are 2.2×10^{-6}, 2.2×10^{-7} and 2.2×10^{-8} μg/ℓ, respectively.

INTRODUCTION

The major source of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (CAS Number 1746-01-6) appears to be as a contaminant formed during the production of 2,4,5-trichlorophenol (2,4,5-TCP) from 1,2,4,5-tetrachlorobenzene (Milnes, 1971; Kimmig and Schulz, 1957; Firestone et al., 1972). 2,4,5-TCP is the major chemical feedstock in the production of several herbicides including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-T esters and Silvex. Each of these chemicals may contain 2,3,7,8-TCDD as a contaminant (Buser and Bosshardt, 1974; Courtney et al., 1970; Edmunds et al., 1973; Zitko and Choi, 1971). It has also been reported that 2,3,7,8-TCDD may be formed during the pyrolysis of chlorinated phenols (Buu-Hoi et al., 1971a,b), chlorinated benzenes (Buser, 1979) and polychlorinated diphenyl ethers (Lindahl et al., 1980), and thus can also be emitted by municipal incinerators (Rappe et al., 1983a; Lustenhouwer et al., 1980; Olie et al., 1982, 1983). There is no clear evidence that 2,3,7,8-TCDD is a typical contaminant in the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Woolson et al., 1972; Henshaw et al., 1975; Cochrane et al., 1981).

Physical Properties

2,3,7,8-TCDD is a symmetrical, nearly planar molecule with the empirical formula $C_{12}H_4Cl_4O_2$. The four chlorine atoms are indistinguishable from one another (Poland and Glover, 1973). 2,3,7,8-TCDD is a white crystalline solid with a melting point range of 302-305°C (Sparschu et al., 1971; Elvidge, 1971) and has a molecular weight of 321.9. The vapor pressure of this compound is estimated to be 10^{-6} mm of Hg (0.1 mPa) at 1 atmosphere and 25°C (Mabey et al., 1981). The Henry's constant has been estimated to be 2.1×10^{-3} atmosphere m^3 mol^{-1} (Mabey et al., 1981). 2,3,7,8-TCDD is lipophilic, exhibiting some solubility in fats, oils and

other relatively nonpolar solvents, and is only slightly soluble in water (0.2 μg/ℓ) (Crummet and Stehl, 1973; Norris, 1981). The solubility of 2,3,7,8-TCDD in various organic solvents is given below (Crummet and Stehl, 1973):

Solvent	Solubility (ppm)
lard oil	44
benzene	570
o-dichlorobenzene	1400
chloroform	370
acetone	110
n-octanol	50
methanol	10

The partition coefficient of 2,3,7,8-TCDD in a water:hexane system has been reported to be 1000 (Matsumura and Benezet, 1973). The octanol/water partition coefficient (K_{ow}) has been calculated by the methods of Hansch and Leo (1979) and has been experimentally measured. Calculated values for log K_{ow} range from 6.84-7.28, and a measured value of 6.15 has been reported (see Section B, Bioaccumulation).

Cocontaminants of 2,3,7,8-TCDD in Chlorinated Products

2,3,7,8-TCDD is only one of many trace contaminants found in some chlorinated industrial products including a few chlorinated phenols, a few chlorinated phenoxy acids (especially the herbicides 2,4,5-T and Silvex) and hexachlorophene. Among the other trace contaminants found in these products are members of the polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated diphenylethers (PCDPEs), polychlorinated phenoxyphenols (PCPPs), polychlorinated biphenyls (PCBs) and polychlorinated benzenes (PCBz). Of these, some possess properties that make them difficult to separate analytically from the 2,3,7,8-TCDD isomer (Kimbrough, 1974; U.S. EPA, 1980; Bumb et al., 1980; Rappe et al., 1983b).

Synthesis

2,3,7,8-TCDD has been synthesized by several methods in moderate yield (e.g., reaction of dichlorocatechol salts with o-chlorobenzene by refluxing in alkaline dimethylsulfoxide; chlorination of dibenzo-p-dioxin in the presence of ferric chloride and iodine; UV irradiation of PCDDs of high chlorine content; Ullman reaction of chlorinated phenolates at 180-400°C; pyrolysis of chlorinated phenolates and chlorinated phenols; heating 1,2,4-trichloro-5-nitrobenzene and 4,5-dichlorocatechol in the presence of base). These processes have been reviewed in U.S. EPA (1980).

Chemistry

2,3,7,8-TCDD is considered to be relatively stable toward heat, acids and alkalies. It begins to decompose at 500°C, and at a temperature of 800°C, virtually complete degradation occurs within 21 seconds (Stehl et al., 1973). From a theoretical equation for thermal dissociation constant

$$K = 10^{15.5} \exp(-80{,}000/RT)\ sec^{-1}$$

K = dissociation constant, R = universal gas constant, T = temperature for tetrachlorodibenzo-p-dioxins formulated by Staub and Tsang (1983), the 99.99% gas phase dioxin dissociation at 727°C will require about 15.4 minutes. The same equation predicts a 99.99% decomposition of tetrachlorodibenzo-p-dioxins in 0.3 seconds at 977°C. Gamma radiation degrades the molecule (Fanelli et al., 1978). 2,3,7,8-TCDD can be perchlorinated (Hutzinger et al., 1972).

2,3,7,8-TCDD is transformed very slowly in aquatic systems. Of the four transformation processes (photoreaction, biotransformation, hydrolysis and radical oxidation) that control the fate of a chemical in aquatic media only the first two processes are throught to effect the transformation of 2,3,7,8-TCDD (Matsumura et al., 1983). In organic solvents, 2,3,7,8-TCDD undergoes reductive photodechlorination at wavelengths <320 nm (Crosby et

al., 1971; Liberti et al., 1978). In aqueous solution hydroxylative dechlorination probably occurs, although this has not been seen. Liberti et al. (1978) showed that 2,3,7,8-TCDD spread over silica gel, aluminum, glass, ceramic tile and marble in the absence of an organic solvent showed various decomposition rates on UV irradiation. Little decomposition occurred on glass or marble, but substantial degradation occurred on silica gel and aluminum. Also, 1:1 ethyl oleate/xylene was found to be a satisfactory H donor. Plimmer et al. (1973) reported that a 2,3,7,8-TCDD suspension in distilled water remained unchanged when irradiated with a sunlamp. Similarly, a thin dry film of 2,3,7,8-TCDD on a glass plate or 2,3,7,8-TCDD on dry and wet soils showed negligible photodegradation after irradiation with sunlamps (Crosby et al., 1971). In contrast, 2,3,7,8-TCDD in methanol solution, or a benzene solution of 2,3,7,8TCDD in water in the presence of a surfactant underwent substantial photodegradation under sunlamp or sunlight irradiation (Plimmer et al., 1973; Crosby et al., 1971). Nestrick et al. (1980) experimentally determined the photolytic half-life of 2,3,7,8-TCDD in n-hexa-decane under sunlamp irradiation to be ~57 minutes. The surfactant, 1-hexyldecylpyridinium chloride, sensitized the photodecomposition in aqueous solution (Botre et al., 1978). The evolution of 2,3,7,8-TCDD from more highly chlorinated PCDDs on UV irradiation from sunlight is unlikely since dechlorination in organic solvents and in the presence of artificial UV sources occurs preferentially at the 2,3,7,8-positions (Buser and Rappe, 1978; Nestrick et al., 1980).

Analytical Methods for TCDD

Most of the current analytical methods used for the identification and quantitation of 2,3,7,8-TCDD are based upon gas chromatography/mass spectrometry (GC/MS). This method provides both high sensitivity (detection at

low-ppt levels) and required selectivity (Crummett and Stehl, 1973; Tiernan et al., 1975; Taylor et al., 1975; Buser and Bosshardt, 1976; Buser, 1977; U.S. EPA, 1980; Tiernan, 1983). Unfortunately, the GC/MS method is expensive, time consuming and difficult. Elaborate quality control and quality assurance of analytical methods are necessary. Radioimmunoassay and electron capture-GC have also been developed; both were essentially screening techniques (Karasek and Onuska, 1982).

Sampling Method -- Two types of sampling methods can be used for collecting aqueous samples for TCDD. In the first method, no preconcentration of the samples during collection is made. Grab samples are collected in clean amber glass bottles of 1 ℓ or 1 quart capacity fitted with screw caps lined with clean Teflon or aluminum foil (U.S. EPA, 1982). The sample containers must be kept refrigerated at 4°C and protected from light during collection and shipment of grab and composite samples. All samples must be extracted within 7 days and completely analyzed within 40 days of extraction (U.S. EPA, 1982).

The second method is the preconcentrative method of sample collection (DiDomenico et al., 1980). In this method, 2-20 ℓ of water are allowed to pass through a 12 cm long x 1.5 cm internal diameter XAD-2 column. The XAD-2 columns containing the polychlorinated dioxins should be protected from light and kept at 4°C during transportation and storage.

Analysis -- An appropriate volume of water (depending on the desired detection limit) with added internal standard of either $^{13}C_{12}$ or $^{37}Cl_4$ 2,3,7,8-TCDD in the amount of 2.5-25 ng (Harless et al., 1980; U.S. EPA, 1982) can be extracted with hexane (DiDomenico et al., 1980), dichloromethane (U.S. EPA, 1982; Harless et al., 1980) or petroleum ether

(Van Ness et al., 1980). Judging from the recovery data (U.S. EPA, 1982; DiDomenico et al., 1980; Harless et al., 1980), dichloromethane appears to be a better solvent.

The extract containing TCDDs can be cleaned up by acid and base wash (Harless et al., 1980; U.S. EPA, 1982; Van Ness et al., 1980) and by subsequent liquid chromatography with an alumina column (Harless et al., 1980; Van Ness et al., 1980); however, U.S. EPA (1982) recommends another cleanup step using silica gel liquid chromatography, which may be necessary for wastewater but may be unnecessary for drinking water and clean surface water samples. The final separation and analysis is performed by low resolution GC-HRMS (Van Ness et al., 1980; Harless et al., 1980) or high resolution GC-HRMS or LRMS (U.S. EPA, 1982). The U.S. EPA (1982) method derived from the method of Buser and Rappe (1980) seems to be an appropriate one because it recommends using a 50 m Silar 10C capillary column that resolves 2,3,7,8-TCDD from its other isomers. This same column can resolve 1,2,3,7,8-penta-CDD from other penta-CDDs, and 1,2,3,6,7,8-, 1,2,3,7,8,9- and 1,2,3,4,7,8-hexa-CDDs from other hexa-CDDs (Rappe et al., 1983a). Other suitable columns include SP-2330, SP-2340 and DB-5 (Tiernan, 1983). Harless et al. (1980) reported that TCDD in water can be accurately determined down to a concentration of 0.03 ppt. However, for determination of <1 ppt, rigorous measures must be taken to avoid the possibility of sample contamination during collection, storage, transportation or analysis.

Gas Chromatography/Mass Spectrometry (GC/MS): The mass spectrometral pattern of 2,3,7,8-TCDD is very similar to the spectra of other tetrachlorodibenzo-p-dioxins. Since other compounds (e.g., certain polychlorinated biphenyls) present in the sample extract can also give rise to mass spectral

ions at the same nominal masses as TCDDs (m/e 320 and m/e 322), two approaches are being used to increase specificity (U.S. EPA, 1980).

The first approach of applying high resolution mass spectrometry ($M/\Delta M$ >9000) to increase the selectivity makes use of the small difference in the "exact" masses of TCDDs ($C_{12}H_4Cl_4O_2$ having an "exact" mass of 321.8936) compared with compounds of similar molecular weight. Application of the optimum chromatographic conditions and columns to maximize the resolution of compounds is necessary before the MS step.

The second approach to avoid the problem of interferences from closely related compounds is to make use of low-resolution mass spectrometry incorporated with a more selective separation step such as capillary column GC (Buser and Rappe, 1980; Rappe et al., 1983b) or high performance liquid chromatography followed by GC (Nestrick et al., 1979). The former method can be used for all PCCDs and PCDFs; the latter method is selective in characterizing only the TCDDs (Bumb et al., 1980).

The following criteria have been outlined by Harless et al. (1980) for confirmation of 2,3,7,8-TCDD residues:

1. Correct GC/MS retention time for 2,3,7,8-TCDD.
2. Correct isotope ratio for the molecular ions 320 and 322.
3. Correct simultaneous response for the molecular ions 320, 322 and 328.
4. Correct responses for the co-injection of sample fortified with ^{37}Cl-TCDD and 2,3,7,8-TCDD standard.
5. Response of molecular ions 320 and 322 must be >2.5 times the noise level.

Supplemental criteria that Harless et al. (1980) suggested for highly contaminated extracts are:

1. COCl loss indicative of TCDD structure
2. GC/MS peak-matching analysis of molecular ions 320 and 322 in real time to confirm the 2,3,7,8-TCDD elemental composition.

Summary of Health Effects

2,3,7,8-TCDD is one of the most toxic substances known. It exhibits a delayed biological response in many species and is highly lethal at low doses to aquatic organisms, birds and mammals. It has been shown to be acnegenic, fetotoxic, teratogenic, mutagenic (in a limited number of mutagenicity tests) and carcinogenic, and affects the immune responses in mammals.

These findings, in conjunction with the wide distribution of contaminated products and its extreme stability in the environment, lead to the conclusion that 2,3,7,8-TCDD represents a potential hazard to both aquatic and terrestrial life, and makes 2,3,7,8-TCDD one of the major concerns for public health.

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Aquatic Toxicology*

Introduction

Most of the available data related to effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) on aquatic life have been generated by Norris and co-workers, Isensee and co-workers, Matsumura and co-workers, and Helder. Much of the available information is from studies involving freshwater microcosms. Although such studies are intended to provide information on fate of a test material, some data concerning effects on aquatic life are also obtained. No tests have been conducted using saltwater organisms.

The last literature search for information that could be used in this chapter was conducted in November, 1983.

Acute Toxicity to Aquatic Animals

Although the data available concerning 2,3,7,8-TCDD do not allow calculation of an acute value for any species, some useful information does exist. Data published by Miller et al. (1973) and Norris and Miller (1974) indicate that the 96-hour LC_{50}s for a worm, *Paranais* sp., a snail, *Physa* sp., and larvae of the mosquito, *Aedes aegypti*, would be >0.2 µg/ℓ, whereas those for the coho salmon, *Oncorhynchus kisutch*, and the guppy, *Poecilia reticulata*, would be >1 and >10 µg/ℓ, respectively. Based on microcosm studies in which concentrations in water were measured at 2-day intervals, the 96-hour LC_{50} for fingerling channel catfish, *Ictalurus punctatus*, would be >0.24 µg/ℓ, whereas those for *Daphnia magna* and a

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*An understanding of the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Life and Its Uses (Stephan et al., 1983) is necessary in order to understand the following text and tables.

snail, Physa sp., would be >1.3 µg/ℓ (Isensee and Jones, 1975; Isensee, 1978). Yockim et al. (1978) did not observe acute toxicity to D. magna, a snail, Helosoma sp., or the mosquitofish, Gambusia affinis, exposed for over 96 hours to a measured concentration of 0.0024-0.0042 µg/ℓ. Helder (1980, 1981, 1982a) found that the 96-hour LC_{50}s for embryos of northern pike, Esox lucius, and embryos and yolk-sac fry of rainbow trout, Salmo gairdneri, would be >0.01 µg/ℓ; the 96-hour LC_{50} for juvenile rainbow trout would be >0.1 µg/ℓ. Although no 48- or 96-hour LC_{50}s or EC_{50}s can be calculated, the available data indicate that those for the coho salmon, guppy, D. magna, and a snail, Physa sp., are >1.0 µg/ℓ.

Chronic Toxicity to Aquatic Animals

No standard chronic toxicity tests have been conducted on 2,3,7,8-TCDD with aquatic animals, but several exposures that have been conducted for other purposes do provide some information concerning chronic toxicity. Because Miller et al. (1973) used static long-term exposures, no conclusions can be drawn concerning chronic toxicity from their exposures of A. aegypti or a snail, Physa sp., but it can be concluded that 0.2 µg/ℓ would cause chronic toxicity to a worm, Paranais sp. A 96-hour exposure to an initial concentration of 0.0056 µg/ℓ resulted in 55% mortality among coho salmon within 60 days (Miller et al., 1973, 1979); thus 0.0056 µg/ℓ would cause chronic toxicity to this species. Similarily, 0.1 µg/ℓ would cause chronic toxicity to the guppy, because exposure to 0.1 µg/ℓ for 5 days killed all individuals within 40 days (Norris and Miller, 1974). In microcosms in which the concentrations of 2,3,7,8-TCDD were measured at 2-day intervals, both D. magna and a snail, Physa sp., reproduced at 1.3 µg/ℓ (Isensee and Jones, 1975; Isensee, 1978). Exposure to a measured concentration of 0.0024-0.0042 µg/ℓ killed all exposed mosquitofish and channel

catfish within 20 days (Yockim et al., 1978). Based on effects caused by 96-hour exposures, 0.001 μg/ℓ would cause chronic toxicity to rainbow trout and 0.01 μg/ℓ would chronically affect northern pike (Helder, 1980, 1981, 1982a). Branson et al. (1983) reported that a 6-hour exposure to 0.1 μg/ℓ adversely affected rainbow trout after 64-139 days. Apparently 0.001 μg of 2,3,7,8-TCDD/ℓ would cause unacceptable chronic toxicity to rainbow trout and 0.01 μg/ℓ would be chronically toxic to coho salmon, mosquitofish, channel catfish and northern pike; 1.3 μg/ℓ may not be chronically toxic to D. magna or a snail, Physa sp.

Toxicity to Aquatic Plants

The few data available on the toxicity of 2,3,7,8-TCDD to aquatic plants are also from microcosm studies. The alga, Oedogonium cardiacum, and the duckweed, Lemna minor, were not affected by 30-day exposures to 1.3 μg/ℓ and 0.71 μg/ℓ, respectively (Isensee and Jones, 1975; Isensee, 1978). Yockim et al. (1978) did not observe any adverse effects on O. cardiacum exposed to a measured concentration of 0.0024-0.0042 μg/ℓ for 32 days.

Bioaccumulation

Several equations have been developed for predicting the steady-state bioconcentration factor (BCF) for an organic compound from its octanol-water partition coefficient (Kenaga and Goring, 1980; Veith et al., 1980; Veith and Kosian, 1983). Several estimated values (Leo, 1979; Mabey et al., 1982; Neely, 1983) and one measured value (Neely, 1979, 1983; Kenaga, 1980; Branson, 1983) have been reported for the octanol-water partition coefficient for 2,3,7,8-TCDD. Use of various equations with four available values for the partition coefficient, K_{ow}, results in the predicted BCFs shown in Table 1. The predicted BCFs range from 3000-68,000 using the measured value of the partition coefficient and from 7000-900,000 using the calculated values.

TABLE 1

Predicted Bioconcentration Factors for 2,3,7,8-TCDD Based on Estimated and Measured Values of the Octanol-Water Partition Coefficient (K_{ow})

Equation	Reference	log K_{ow}			
		Measured	Estimated		
		6.15[a]	6.84[b]	7.14[c]	7.28[d]
log BCF = 0.542 log K_{ow} + 0.124	e,f	2,870	6,780	9,860	11,700
log BCF = 0.76 log K_{ow} - 0.23	f	27,800	93,000	157,000	201,000
log BCF = 0.79 log K_{ow} - 0.40	g	28,700	101,000	174,000	224,000
log BCF = 0.635 log K_{ow} + 0.7285	e	43,000	118,000	183,000	225,000
log BCF = 0.85 log K_{ow} - 0.70	f,g	33,700	130,000	234,000	308,000
BCF = 0.048 K_{ow}	g	67,800	332,000	663,000	915,000

[a]Branson, 1983
[b]Mabey et al., 1982
[c]Neely, 1983
[d]Leo, 1979
[e]Kenaga and Goring, 1980
[f]Veith et al., 1980
[g]Veith and Kosian, 1983

Several measured BCFs have been reported for 2,3,7,8-TCDD. Using microcosm studies in which the concentrations in water were measured at 2-day intervals for 30-33 days, Isensee and Jones (1975) and Isensee (1978) obtained BCFs of 390-13,000 for the alga, O. cardiacum, a snail, Physa sp., and D. magna. In a separate 32-day microcosm study in which the measured concentrations of 2,3,7,8-TCDD ranged from 0.0024-0.0042 µg/ℓ, BCFs for O. cardiacum, Physa sp., and D. magna ranged from 660-7070 from the seventh day to the end of the test.

In a different kind of test channel catfish were held in a cage in a discharge plume in a river for 28 days. Four 24-hour composite water samples were analyzed for 2,3,7,8-TCDD. A whole-body BCF of 2,000 was obtained (U.S. EPA, 1983; Thomas, 1983). In a laboratory bioconcentration test rainbow trout were exposed to 0.107 µg/ℓ for 6 hours and followed through a 139-day depuration period. The resulting projected steady-state BCF was 5450 if growth dilution was not taken into account, and 9270 if growth dilution was taken into account. These values are for the whole body; the concentration of 2,3,7,8-TCDD in muscle was about one-half that in the whole body (Branson et al., 1983).

Corbet et al. (1983) conducted bioconcentration tests on 1,3,6,8-TCDD with the fathead minnow, Pimephales promelas, and rainbow trout, using a 4-day uptake phase and 48-day depuration phase. All results were based on radioactivity measurements, because no confirmatory analyses were performed. The projected steady-state BCFs were 1061 with the fathead minnow and 469 with the rainbow trout. The authors concluded the environmental behavior of 1,3,6,8-TCDD is quite different from that of 2,3,7,8-TCDD, based on a 10- to 15-fold difference in measured clearance rate constants and the fact that the projected BCF for 1,3,6,8-TCDD was much less than a predicted BCF for 2,3,7,8-TCDD.

Information on maximum permissible tissue concentrations is available from two sources. Hawkes and Norris (1977) found that feeding activity and growth decreased and fin erosion and liver pathology increased when a portion of the diet fed to young rainbow trout for 105 days contained 2.3 mg of 2,3,7,8-TCDD/kg of food. These effects were not observed when a portion of the diet contained 0.0023 mg/kg. A diet containing 2.3 mg of 2,3,7,8-TCDD/kg is obviously unacceptable for rainbow trout, but it is not known how low the average concentration in the diet would have to be to prevent unacceptable effects on survival, growth and reproduction.

The U.S. FDA issued a human health advisory on fish containing 0.000050 mg of 2,3,7,8-TCDD/kg; the FDA believed there was little cause for concern if the average concentration in fish was <0.000025 mg/kg (Hayes, 1981).

Other Data

Because delayed effects had been observed in tests on 2,3,7,8-TCDD with mammals, several studies were conducted to determine whether delayed effects also occurred with fishes. In tests with a number of species, exposures that lasted <6 days caused substantial mortality several weeks later (Table 2).

The bullfrog was less sensitive to injected 2,3,7,8-TCDD than many mammalian species (Beatty et al. 1976).

Unused Data

Publications, such as those by Lamparski et al. (1979), Niemann et al. (1983) and Ryan et al. (1983), that only dealt with analytical methodology for measuring 2,3,7,8-TCDD in aquatic life were not used. Publications by Baughman and Meselson (1973), Young et al. (1975), Harrison et al. (1979), Harless and Lewis (1982), O'Keefe et al. (1983), Harrison and Crews (1983),

TABLE 2

Other Data on Effects of 2,3,7,8-TCDD on Aquatic Organisms

Species	Duration	Effect	Result (µg/ℓ)	Reference
Coho salmon, *Oncorhynchus kisutch*	96 hours	50% dead in 56 more days	0.0056	Miller et al., 1973, 1979
Rainbow trout (embryo), *Salmo gairdneri*	96 hours	Some mortality in 24 weeks	0.01	Helder, 1981
Rainbow trout (yolk-sac fry), *Salmo gairdneri*	96 hours	All dead in 24 weeks	0.01	Helder, 1981, 1982a
Rainbow trout (yolk-sac fry), *Salmo gairdneri*	96 hours	Growth retarded for 23 weeks	0.001	Helder, 1981, 1982a
Rainbow trout (juvenile), *Salmo gairdneri*	16 hours/day for 4 days	All dead in 27 days	0.1	Helder, 1981, 1982a
Rainbow trout (juvenile), *Salmo gairdneri*	16 hours/day for 4 days	Growth reduced for 10 weeks	0.01	Helder, 1981, 1982a
Rainbow trout, *Salmo gairdneri*	6 hours	Fin rot, hemorrhaging and death after 64 days	0.1	Branson et al., 1983
Northern pike (embryo), *Esox lucius*	96 hours	Nearly all dead in 23 days	0.01	Helder, 1980, 1982a
Northern pike (embryo), *Esox lucius*	96 hours	Slight reduction in growth up to 21 days	0.0001	Helder, 1980

TABLE 2 (cont.)

Species	Duration	Effect	Result (µg/ℓ)	Reference
Guppy, *Poecilia reticulata*	120 hours	Killed 18%	10	Norris and Miller, 1974
Guppy, *Poecilia reticulata*	120 hours	All dead in 17 more days	0.1	Norris and Miller, 1974
Guppy, *Poecilia reticulata*	24 hours	10% dead in 41 more days	0.01	Miller et al., 1979
Bullfrog (tadpole), *Rana catesbeiana*	50 days	No deaths	1000 µg/kg (i.p.)	Beatty et al., 1976
Bullfrog (adult), *Rana catesbeiana*	35 days	No deaths	500 µg/kg (i.p.)	Beatty et al., 1976

i.p. = intraperitoneal

Harless et al. (1983) and Stalling et al. (1983) reported concentrations in aquatic organisms but did not report enough data on the concentrations in water to allow calculation of a BCF. Botre et al. (1978) and Ward and Matsumura (1978) only dealt with fate of 2,3,7,8-TCDD and presented no data on effects on aquatic life.

The bioconcentration tests of Matsumura and Benezet (1973) and Matsumura (1977) were static and usually lasted for only a few days. The concentration of 2,3,7,8-TCDD in water was not measured adequately by Tsushimoto et al. (1982). Isensee and Jones (1975) reported BCFs based on dry weight; fortunately Isensee (1978) reported results of the same tests based on wet weight. Helder et al. (1982) exposed rainbow trout to fly ash and an extract of fly ash and no conclusions can be drawn concerning effects of 2,3,7,8-TCDD on aquatic life. The data of Zullei and Benecke (1978) were not used because the test species was not identified well enough to allow a determination of whether it is resident in North America. Young et al. (1976, 1978), Esposito et al. (1980), Helder (1982b), Kenaga and Norris (1983) and a report of the National Research Council of Canada (1981) only contained data published elsewhere.

Summary

The data that are available concerning the effects of 2,3,7,8-TCDD on aquatic organisms and their uses do not allow the calculation of an acute or chronic toxicity value for any freshwater animal species. Data available from various studies do indicate, however, that the acute values for several freshwater species are >1.0 µg/ℓ. Similar data indicate that the chronic value for rainbow trout is <0.001 µg/ℓ and that chronic values for several other species are <0.01 µg/ℓ. Effects were not observed on the two plant species exposed to 1.3 µg/ℓ.

Estimates of the bioconcentration factor for 2,3,7,8-TCDD range from 3000-900,000. Measured BCFs have been reported for a variety of species and range from 390-13,000. The U.S. FDA issued a health advisory for fish containing more than 0.000050 mg of 2,3,7,8-TCDD/kg. A concentration of 2.3 mg of 2,3,7,8-TCDD/kg in a portion of the diet affected rainbow trout. Exposures of <6 days resulted in deaths among four species of fishes several weeks later.

No tests have been conducted on 2,3,7,8-TCDD with saltwater species.

National Criteria

Not enough data are available concerning the effects of 2,3,7,8-TCDD on aquatic life and its uses to allow derivation of national criteria. The available information indicates that acute values for some freshwater animal species are >1.0 μg/ℓ; some chronic values are <0.01 μg/ℓ, and the chronic value for rainbow trout is <0.001 μg/ℓ. Because exposures of some species of fishes to 0.01 μg/ℓ for <6 days resulted in substantial mortality several weeks later, derivation of aquatic life criteria for 2,3,7,8-TCDD may require special consideration. Predicted bioconcentration factors (BCFs) for 2,3,7,8-TCDD range from 3000-900,000, but the available measured BCFs range from 390-13,000. If the BCF is 5000, concentrations >0.00001 μg/ℓ should result in concentrations in edible freshwater and saltwater fish and shellfish that exceed levels identified in a U.S. FDA health advisory. If the BCF is >5000 or if uptake in a field situation is greater than that in laboratory tests, the value of 0.00001 μg/ℓ will be too high.

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Mammalian Toxicology and Human Health Effects

EXPOSURE

Water and Soil Related

The amount of human exposure of 2,3,7,8-TCDD attributable to drinking water alone cannot be readily determined. A National Academy of Sciences (NAS) document states that 2,3,7,8-TCDD has never been detected in drinking water using methods with limits of detection in the parts per trillion (ppt) range (NAS, 1977). The two most likely sources of 2,3,7,8-TCDD contamination are discharge of contaminated industrial effluents, and washouts from contaminated disposal sites. However, even after contamination 2,3,7,8-TCDD should remain strongly sorbed to sediments and biota (Isensee and Jones, 1975). In one study, >90% of 2,3,7,8-TCDD in the aquatic media was present in the sorbed state (Ward and Matsumura, 1978). The possibility of 2,3,7,8-TCDD leaching into the groundwater appears remote. Helling (1971), Kearney et al. (1972) and Helling et al. (1973) found that 2,3,7,8-TCDD tended to remain on or near the surface of the soil. The mobility of 2,3,7,8-TCDD in five different soil types was examined by Kearney et al. (1973). They found that decreasing mobility of 2,3,7,8-TCDD was associated with increasing organic content of the soil. Based on this observation, and the fact that dioxins were relatively immobile in all soils tested, they concluded that underground water supplies probably would not be contaminated with 2,3,7,8-TCDD. Similar conclusions were made by Matsumura and Benezet (1973) who hypothesized that any movement in the soil environment would most likely occur via horizontal transfer of soil and dust particles.

Nash and Beall (1980) conducted studies on the fate of 2,3,7,8-TCDD in a microagroecosystem and found that 80% of the applied 2,3,7,8-TCDD remained

in the upper 2 cm of soil. Trace amounts of 2,3,7,8-TCDD detected at depths of 8-15 cm suggested that some movement of dioxin into the soil had occurred. Analysis of water leachate samples showed no detectable 2,3,7,8-TCDD following two applications (days 0 and 35) of Silvex containing 44 ppb 2,3,7,8-TCDD. However, similar analyses of leachable samples taken 42 days after a third application of Silvex containing 7500 ppb 2,3,7,8-TCDD indicated a maximum concentration of 0.05-0.06 ppt of 2,3,7,8-TCDD.

The downward vertical migration of 2,3,7,8-TCDD into the first 1.5 cm of soil was reported around Seveso, Italy (DiDomenico et al., 1980a,b). The monitoring of Seveso soil 1 year after the accident showed that the highest 2,3,7,8-TCDD levels were not present in the topmost soil layer (0.5 cm), but very often in the second (0.5-1.0 cm) or third (1.0-1.5 cm) layers. In view of the low water solubility of 2,3,7,8-TCDD, probable explanations of this vertical migration could be saturation of sorption sites in soil, solvation of 2,3,7,8-TCDD by organic solvents (NRCC, 1981), or biotic mixing by earthworms or other soil invertebrates. Nevertheless, both studies support the view that 2,3,7,8-TCDD does not migrate readily in soils.

The photodecomposition of 2,3,7,8-TCDD on wet or dry soil under artificial and natural sunlight was studied by Crosby et al. (1971). The photodecomposition was found to be negligible in soils. Similarly, Plimmer et al. (1973a,b) determined that photodecomposition of 2,3,7,8-TCDD on soils was too slow to be detected. In a later experiment, Plimmer (1978) found that although 2,3,7,8-TCDD decomposed significantly on a precoated silica plate (~22%) in 8 hours of sunlight irradiation, practically no decomposition of 2,3,7,8-TCDD was observed from 2,3,7,8-TCDD sorbed on soil under similar conditions.

The photodegradation of 2,3,7,8-TCDD in combination with other pesticide mixtures was studied by Crosby and Wong (1977). When Agent Orange containing 15 ppm of 2,3,7,8-TCDD was applied on the surface of glass plates (5 mg/cm²), rubber plant (Hevea brasiliensis) (6.7 mg/cm²), and on the surface of sieved Sacramento loam soil (10 mg/cm²) and exposed to sunlight, 2,3,7,8-TCDD was found to photodecompose. The loss of 2,3,7,8-TCDD in 6 hours was >50% from the glass plates, ~100% from the surface of leaves and ~10% from the surface of soil. The rapid photolysis of 2,3,7,8-TCDD from these surfaces indicates that the herbicide formulation provided a hydrogen donor which probably allowed the photolysis to occur. The authors attributed the slower photolysis of 2,3,7,8-TCDD in soil to a shading effect by the soil particles.

The overall half-life of 2,3,7,8-TCDD in soil was first reported to be 1-3 years (Kearney et al., 1972). Studies performed by the U.S. Air Force suggested that the half-life of this chemical in soils under relatively dry conditions (Utah test area) was ~330 days. In more moist soils and under warm conditions (Florida test area), the half-life was ~190 days. This is consistent with the biodegradation half-life of ~0.5 year for 2,3,7,8-TCDD determined from the soil in rural Missouri after the accidental spraying of TCDD-contaminated oil (IARC, 1977). However, physical removal may be an important factor also. More recent data (Young, 1983; Wipf and Schmid, 1983) indicate that the half-life is closer to 10 years.

The half-life of 2,3,7,8-TCDD following an accidental 2,3,7,8-TCDD release from a trichlorophenol manufacturing plant at Seveso, Italy, was studied by DiDomenico et al. (1980a). The disappearance of 2,3,7,8-TCDD from the topmost soil layer after 1 year was speculated to be due to photodegradation, volatilization, or vertical movement through the soil. These

investigators estimated the first half-life of 2,3,7,8-TCDD in soil at the time of its release to be 5 months. One month after release, the next 2,3,7,8-TCDD half-life was estimated to be 1 year, whereas 17 months later it was estimated to be >10 years. It has recently been shown that radiolabeled TCDD adsorbed to soil becomes progressively more resistant to extraction (Philippi et al., 1981; Huetter and Philippi, 1982) and, therefore, the persistence of 2,3,7,8-TCDD residues in aged soil is probably greater as well.

2,3,7,8-TCDD exhibits relatively strong resistance to microbial biodegradation. Only 5 of ~100 microbial strains that have the ability to degrade persistent pesticides show slight ability to degrade 2,3,7,8-TCDD (U.S. EPA, 1980d). Ward and Matsumura (1977) reported that the half-life of 2,3,7,8-TCDD in sediment-containing Wisconsin lake waters was 550-590 days. In lake water alone, ~70% of the 2,3,7,8-TCDD remained after 589 days. Using an outdoor pond as a model aquatic ecosystem, Tsushimoto et al. (1982) and Matsumura et al. (1983) estimated the apparent half-life of 2,3,7,8-TCDD to be ~1 year. Although biodegradation may have been responsible for part of the degradation, other investigators (Huetter and Philippi, 1982) have reported the virtually complete lack of biodegradability of 2,3,7,8-TCDD.

The biodegradation half-life of 2,3,7,8-TCDD can be estimated from the theoretical rate constant values based on relative rates of transformation reported in the literature or on structure-activity analogy values given by Mabey et al. (1981). Assuming the biotransformation rate constant of 1×10^{-10} mℓ cell^{-1} hr^{-1} (Mabey et al., 1981) and the concentration of microorganisms capable of degrading TCDD as 5×10^{5} cell mℓ$^{-1}$ (Burns et al., 1981), the half-life of biodegradation is estimated to be >1 year.

2,3,7,8-TCDD on dry and wet soils showed negligible photodegradation after irradiation with sunlamps (Crosby et al., 1971). In order to explain the longer half-life of 2,3,7,8-TCDD in a model laboratory ecosystem than in an outdoor pond, Matsumura et al. (1983) and Tsushimoto et al. (1982) speculated photolysis as the most likely cause. In the outdoor environment where the intensity of sunlight was higher compared to the laboratory experiments, algae-mediated photosensitization of 2,3,7,8-TCDD may cause some photodecomposition of this compound. From the available information, it is difficult to predict the fate of 2,3,7,8-TCDD in aquatic media under environmental photolytic conditions. In the presence of hydrogen atom donating substrate(s) in surface waters, photolysis may be a significant fate process.

Although several investigators implicated volatilization as one of the major reasons for the observed disappearance of 2,3,7,8-TCDD from aqueous solution during microbial studies, little quantitative information regarding the volatilization of 2,3,7,8-TCDD from aquatic media is available. 2,3,7,8-TCDD may undergo some water-mediated evaporation in aquatic media (Matsumura et al., 1983). A transport model to estimate TCDD volatilization from a cooling pond on an industrial site on the basis of measured concentrations in the pond bottom sediment and pond surface area led to an estimated rate of 15-16 mg/year (Thibodeaux, 1983). Using the formulas of Liss and Slater (1974), a vapor pressure value of $\sim 10^{-6}$ torr (0.1 m Pa) and a solubility value of 6.2×10^{-10} mole/ℓ, NRCC (1981) calculated the volatilization half-life for 2,3,7,8-TCDD to be 6 minutes from water of 1 cm depth and 10 hours from water of 1 m depth. Evaporation half-life is directly proportional to water depth and inversely proportional to mass transfer coefficient (Thibodeaux, 1979). The limitations of the Liss-Slater theory to predict the rate of volatilization have been discussed in the NRCC

(1981) document. The Liss-Slater model does not consider terrestrial matrices (suspended solids, sediments, biota, etc.) normally encountered in natural surface water. A computerized EXAMS model, considering sorption of TCDD on the suspended and bottom sediments and otherwise employing the Liss-Slater model, gave the result that may account for the 100% of the fraction lost due to volatilization under the most favorable conditions (NRCC, 1981). The volatilization half-life for 2,3,7,8-TCDD has been estimated to be 5.5 and 12 years from pond and lake water, respectively. However, it should be remembered that these are estimated values and no experimental confirmation of these values is yet available.

Ingestion from food

The occurrence of 2,3,7,8-TCDD in food could result from (1) contamination of plant crops with 2,3,7,8-TCDD as a result of using herbicides such as Silvex and 2,4,5-T (for weed control); (2) consumption by livestock of 2,3,7,8-TCDD-contaminated forage; or (3) magnification of residues through the food chain. Conceivably, 2,3,7,8-TCDD could also be deposited on food crops after being formed during certain combustion processes (NRCC, 1981). Galston (1979) has speculated that under certain conditions 2,3,7,8-TCDD might enter the human body from a 2,4,5-T-treated food chain and might accumulate in the fat and be secreted in the milk. Studies with either the seeds or the mature plants of soybeans or oats showed that 2,3,7,8-TCDD was neither absorbed by the seeds after spraying nor taken up from the soil into the mature plants (Isensee and Jones, 1971; Matsumura and Benezet, 1973). However, young plants accumulated up to 40 ppb of 2,3,7,8-TCDD (Isensee and Jones, 1971). From the analysis of several parts of fruit trees and kitchen-garden plants such as carrots, onions, potatoes and narcissuses collected from the contaminated (400-1000 $\mu g/m^2$ of 2,3,7,8-TCDD in soil)

Seveso area in Italy, Cocucci et al. (1979) concluded that 2,3,7,8-TCDD is translocated from soil to the aerial parts of the plants, probably through the conductive vessels. This study further suggested that the plants may eliminate 2,3,7,8-TCDD by an unknown mechanism within 4-10 months after transplantation in unpolluted soils. However, the study of Cocucci et al. (1979) contradicts the investigations of Wipf et al. (1982) in which vegetation samples analyzed from the Seveso area from 1976 through 1979 strongly suggested that the contamination in vegetation was from local dust and not from plant uptake.

Unlike the Seveso incident where release of 2,3,7,8-TCDD into the environment took place, normal use of herbicides containing 2,3,7,8-TCDD impurity may not cause detectable 2,3,7,8-TCDD contamination of the crop. Jensen et al. (1983) analyzed rice grain from fields in Arkansas, Louisiana and Texas after application of 2,4,5-T (containing 0.4 ppm 2,3,7,8-TCDD) at a maximum rate of 2.25 lbs/acre. No 2,3,7,8-TCDD residues (detection limit 2-10 ppt) were found in these rice grains nor were any found in 30 samples of rice purchased in retail stores throughout the United States. Contamination of fruits, vegetables or grains in the United States with 2,3,7,8-TCDD has never been investigated.

The presence of polychlorinated dioxins in the fat of cattle that had grazed on pasture treated with 2,4,5-T has been reported (U.S. EPA, 1980d). The levels of 2,3,7,8-TCDD ranged from 4-70 ppt. Other investigators have failed to detect 2,3,7,8-TCDD (detection limit 1 ppt) in fats of cattle grazing on pasture or rangeland treated with normal applications of 2,4,5-T (Kocher et al., 1978).

Bovine milk collected after the accident in the Seveso area was analyzed by Fanelli et al. (1980). The concentration of 2,3,7,8-TCDD was found to vary from none detected (detection limit <40 ppt) to as high as 7.9 ppb. Other investigators have failed to detect 2,3,7,8-TCDD (detection limit 1 ppt) in surveillance samples of milk (after normal application of 2,4,5-T on pasture) from the states of Oklahoma, Arkansas and Missouri, or quarantined milk in the state of Michigan (Lamparski et al., 1978; Mahle et al., 1977). Firestone et al. (1979) fed pentachlorophenol containing several dioxins (not 2,3,7,8-TCDD) to lactating cows for 70 days. The concentration factor for 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin, the dioxin of highest concentration, in milk fat was ~2.4 times relative to its concentration in the diet.

The analysis of human milk and urine for 2,3,7,8-TCDD has been reported. A study of 103 samples of breast milk from mothers living in sprayed areas in the United States revealed no 2,3,7,8-TCDD at a detection limit of 1-4 ppt (U.S. EPA, 1980d). About 6 of the 9 human milk samples analyzed by Langhorst and Shadoff (1980) may have contained 2,3,7,8-TCDD at levels slightly higher than the detection limits (0.2-0.7 ppt). Because of the lack of validation of the precision and accuracy of data, however, it was concluded that 2,3,7,8-TCDD was not present.

Bumb et al. (1980) analyzed charcoal-broiled steak in order to detect any 2,3,7,8-TCDD formed as a result of the broiling process. No 2,3,7,8-TCDD was detected (detection limit 1-10 ppt).

2,3,7,8-TCDD has been reported in several species of commercial and non-commercial fish in several rivers and lakes in the United States and Canada. The levels of 2,3,7,8-TCDD in fish and shellfish as determined by various authors are given in Table 1. In some cases the values listed are means or composites of more than one organism, species or location. Values for individual analyses as high as 695 ppt in fish tissue have been reported (Harless and Lewis, 1982). The efficiency of various extraction and cleanup procedures for 2,3,7,8-TCDD analysis in fish has been discussed by Brumley et al. (1981).

Results of analyses shown in Table 1 indicate that the 2,3,7,8-TCDD levels in fish and shellfish depend not only on the sites from which they are collected, but also on the type of species collected. For example, fish and shellfish collected from Bayon Meto/Arkansas River, Tittabawassee/Saginaw River, Saginaw Bay, Lake Ontario, Lake Huron, and Cayuga Creek showed higher levels of 2,3,7,8-TCDD than those collected from Lake Erie, Lake Michigan, Lake Superior and the Atlantic Ocean. In addition, certain types of aquatic species that are bottom feeders, have high fat content or are carnivorous, such as catfish, carp, trout and salmon, showed higher levels of 2,3,7,8-TCDD than bass, bullhead or suckers. The influence of 2,3,7,8-TCDD levels in surrounding water on the bioconcentration of fish species is not known.

Cordle (1983) estimated the potential maximum human daily intake of 2,3,7,8-TCDD for residents of the Great Lakes region who regularly consume Great Lakes fish. Within the population subset consuming these species (~17 million individuals), daily consumption of fish tissue was 15.7 g at the 90th percentile, and 36.8 g at the 99th percentile. Within the smaller subset consuming pike (number of individuals not specified), daily consumption at the 99th percentile was 83.95 g. For hypothetical mean 2,3,7,8-TCDD

TABLE 1

Levels of 2,3,7,8-TCDD in Fish and Shellfish

Type/Section of Fish	Sampling Site	Concentration[a] (ppt)	Reference
Edible flesh[b]	Bayou Meto/Arkansas River	480	Mitchum et al., 1980
Catfish	Bayou Meto/Arkansas River	ND (7)-50	Mitchum et al., 1980
Buffalo	Bayou Meto/Arkansas River	ND (7-13)	Mitchum et al., 1980
Predator[b]	Bayou Meto/Arkansas River	15-230	Mitchum et al., 1980
Bottom feeder[b]	Bayou Meto/Arkansas River	77	Mitchum et al., 1980
Rock bass/muscle	Lake Ontario/Lake Erie/ Welland Canal	ND (<2)	Josephson, 1983
Eel, Smelt and Catfish/muscle	Lake Ontario/Lake Erie/ Welland Canal	2-39	Josephson, 1983
Crayfish	Bergholtz Creek, Love Canal	3.7	Smith et al., 1983b
Catfish, Bass and Walleyed pike	2,4,5-T contaminated water-shed in Arkansas and Texas; Tittabawassee and Saginaw Rivers	ND (5-10)	Shadoff et al., 1977; U.S. EPA, 1980d; Buser and Rappe, 1980
Lake trout/whole body	Lake Ontario	51-107	O'Keefe et al., 1983
Chinook salmon/skinless fillet	Lake Ontario	26-39	O'Keefe et al., 1983
Coho salmon/skinless fillet	Lake Ontario	20-26	O'Keefe et al., 1983
Rainbow trout/skinless fillet	Lake Ontario	17-32	O'Keefe et al., 1983

TABLE 1 (cont.)

Type/Section of Fish	Sampling Site	Concentration[a] (ppt)	Reference
Brown trout/skinless fillet	Lake Ontario	8-162	O'Keefe et al., 1983
White perch/skinless fillet	Lake Ontario	17-26	O'Keefe et al., 1983
White sucker/skinless fillet	Lake Ontario	ND (3.2)-10	O'Keefe et al., 1983
Smallmouth bass/skinless fillet	Lake Ontario	5.9	O'Keefe et al., 1983
Brown bullhead/skinless fillet	Lake Ontario	3.6	O'Keefe et al., 1983
Carp, Goldfish/skinless fillet	Cayuga Creek	87	O'Keefe et al., 1983
Northern pike/skinless fillet	Cayuga Creek	32	O'Keefe et al., 1983
Pumpkin seed/skinless fillet	Cayuga Creek	31	O'Keefe et al., 1983
Rock bass/skinless fillet	Cayuga Creek	12	O'Keefe et al., 1983
Coho salmon/skinless fillet	Lake Erie	1.4-<3.5	O'Keefe et al., 1983
Walleye pike/skinless fillet	Lake Erie	2.6	O'Keefe et al., 1983
Smallmouth bass/skinless fillet	Lake Erie	1.6-<2.4	O'Keefe et al., 1983
Carp, Goldfish/skinless fillet	Lake Erie	ND (2.6)	O'Keefe et al., 1983
Lake trout/whole body	Lake Huron	21	O'Keefe et al., 1983
Carp/skinless fillet	Lake Huron	26	O'Keefe et al., 1983
Channel Catfish/skinless fillet	Lake Huron	20	O'Keefe et al., 1983

TABLE 1 (cont.)

Type/Section of fish	Sampling Site	Concentration[a] (ppt)	Reference
Sucker/skinless fillet	Lake Huron	25	O'Keefe et al., 1983
Yellow Perch/skinless fillet	Lake Huron	ND (8.7)	O'Keefe et al., 1983
Coho salmon/skinless fillet	Lake Michigan	ND (3.8)	O'Keefe et al., 1983
Rainbow trout/skinless fillet	Lake Superior	1.0	O'Keefe et al., 1983
Perch, Sucker	Saginaw Bay	ND (3.8)-25	Niemann et al., 1983[c]
Catfish	Saginaw Bay	14-37	Niemann et al., 1983[c]
Carp	Saginaw Bay	23-47	Niemann et al., 1983[c]
Catfish	Bayou Meto/Arkansas River	ND (3.8)	Niemann et al., 1983[c]
Bottom feeders[b]	Bayou Meto/Arkansas River	ND (6.7)-12	Niemann et al., 1983[c]
Lake trout	Lake Ontario	34-54	Niemann et al., 1983[c]
Rainbow trout	Lake Ontario	43	Niemann et al., 1983[c]
Ocean haddock	Atlantic Ocean	ND (4.6)	Niemann et al., 1983[c]
Carp	Lake Huron	3-28	Stalling et al., 1983
Channel catfish	Tittabawassee, Saginaw and Grand Rivers	28-695	Harless and Lewis, 1982
Carp	Tittabawassee, Saginaw and Grand Rivers	ND (7)-153	Harless and Lewis, 1982

TABLE 1 (cont.)

Type/Section of Fish	Sampling Site	Concentration[a] (ppt)	Reference
Yellow perch	Tittabawassee and Saginaw Rivers	ND (5)-20	Harless and Lewis, 1982
Smallmouth bass	Grand River	7-8	Harless and Lewis, 1982
Sucker	Tittabawassee River and Saginaw Bay	ND (4)-21	Harless and Lewis, 1982
Lake trout	Lake Michigan	ND (5)	Harless and Lewis, 1982
Lake trout/whole body	Lake Ontario at Burlington, Canada	61.2	Ryan et al., 1983
Rainbow trout/whole body	Lake Ontario at Toronto Harbor, Canada	32.3	Ryan et al., 1983
Lake trout/whole body	Lake Huron at Burnt Island, Canada	30.4	Ryan et al., 1983
Ocean haddock/fillet	East Coast, Canada	ND (1-10)	Ryan et al., 1983

[a]When not detected, the detection limit is indicated within the parentheses.

[b]Organisms not further identified in the report.

[c]Only the GC/MS results of these authors are included in tabulation

[d]These are the mean concentrations in samples showing detectable levels of 2,3,7,8-TCDD.

ND = Not detected

residue levels of 25-100 ppt, estimates of daily intake would thus range from 0.39-8.4 ng 2,3,7,8-TCDD/day. As shown in Table 1, tissue residues for species in certain areas do fall within this range.

In order to derive an ambient water quality criterion for the protection of human health fom the harmful effects of 2,3,7,8-TCDD, it is necessary to estimate the average level of exposure of the U.S. population which would result from a particular concentration of 2,3,7,8-TCDD in ambient fresh or estuarine waters (45 FR 79348). Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980a). The results were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980).

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic species to the concentration in water. Several regression equations can be used to estimate a BCF value for 2,3,7,8-TCDD from its octanol-water partition coefficient (K_{ow}). Using three calculated values of K_{ow}, the regression equations estimate the BCF in the range of 7000-900,000. Using the only measured K_{ow} value, the regression equations predict a BCF for 2,3,7,8-TCDD in the range of 3000-68,000. The available measured BCFs, however, range from 390-13,000. The sources of the theoretical and experimental BCF values cited here can be found in detail in Section B. Until further information is available, the U.S. EPA's best current estimate for the BCF of 2,3,7,8-TCDD in aquatic organisms is 5000. Thus a BCF of 5000 will be used in the "Criterion Formulation" section to estimate the human exposure to 2,3,7,8-TCDD which would result from consumption of aquatic organisms taken from 2,3,7,8-TCDD-contaminated waters. If the BCF is actually >5000 or if uptake in a field situation is greater than in laboratory tests, human exposure will be underestimated.

Inhalation

No data pertaining to the inhalation exposure of 2,3,7,8-TCDD were found. However, the spraying of older formulations of 2,4,5-T containing 2,3,7,8-TCDD impurity may lead to a concomitant exposure to 2,3,7,8-TCDD. Exposure could be through spray drift and through the vapor phase. From microagroecosystem chamber and field studies, Nash and Beall (1980) determined the atmospheric concentration of 2,3,7,8-TCDD at various times after the application of emulsified and granular Silvex (1.3-2.0 kg/ha Silvex) containing 44 ppb and 7.5 ppm 2,3,7,8-TCDD impurity, respectively. Using tritiated 2,3,7,8-TCDD, these authors found that atmospheric concentrations of 2,3,7,8-TCDD vary not only with the number of days elapsed after application (lower concentration at longer time period), but also with formulation (granular form gave lower concentration than emulsifiable concentrate), and the 2,3,7,8-TCDD impurity present in Silvex (higher impurity levels produced higher atmospheric concentrations). Depending on these variables, the atmospheric concentration in microagroecosystem chambers, expressed in fg/m^3 (10^{-15} g/m^3), was found to vary from 0.09 fg/m^3 (granular Silvex applied at 1.3 kg/ha, concentration measured 35 days after application) to 79,800 fg/m^3 (emulsified Silvex applied at 2.0 kg/ha, concentration measured during application).

Air filter samples collected from Elizabeth, NJ, after an industrial fire on April 22, 1980, were analyzed for 2,3,7,8-TCDD by Harvan et al. (1981). Of the nine samples analyzed by collision-induced-dissociation mass-analyzed ion kinetic energy spectrometry by these authors, one contained 20 pg of 2,3,7,8-TCDD, four contained <9 pg, and four others probably contained 5-12 pg of unspecified TCDD isomer which was not the 2,3,7,8-TCDD isomer.

The atmospheric concentrations of 2,3,7,8-TCDD near two hazardous waste sites have been monitored. In one study, U.S. EPA (1982) failed to detect any 2,3,7,8-TCDD in the atmosphere (detection limit 1-20 ppt) at the Love Canal, NY, area. In another study of a waste disposal site near Jacksonville, AR, Thibodeaux (1983) reported an average concentration of 1100 ppt of 2,3,7,8-TCDD in two air particulate samples collected near the disposal site.

The levels of 2,3,7,8-TCDD in atmospheric dust was monitored in the Seveso, Italy, area between 1977 and 1979. The concentrations of 2,3,7,8-TCDD were found to be in the range of 0.06-2.1 ppb of dust using dustfall jars, and 0.17-0.50 ppb of dust by high volume sampling (DiDomenico et al., 1980c).

Another source of atmospheric emission of polychlorinated dioxins is incineration. The concentrations of TCDDs in fly ash from municipal incinerators have been studied by several authors (Eiceman et al., 1979, 1980; Nestrick et al., 1982; Karasek et al., 1982; Bumb et al., 1980; Buser and Bosshardt, 1978; Tiernan et al., 1982; Taylor et al., 1983). The TCDD isomer known to be the most toxic (e.g., 2,3,7,8-TCDD) was either not detected or detected at a low level. The quantities emitted in incinerators vary, probably because of differing efficiencies, and since few municipal incinerators have been reliably characterized for PCDD/PCDF emissions over extended time intervals, the data base is still inadequate. Whereas Bumb et al. (1980) and Buser and Rappe (1980) detected 0.4 ng/g of 2,3,7,8-TCDD in the fly ash from a United States municipal incinerator, the U.S. EPA concluded that emissions from five municipal waste combustors did not present a public health hazard for residents living in the immediate vicinity (CEQ, 1981). 2,3,7,8-TCDD has been detected in the emissions of some municipal

waste incinerators in Europe (Gizzi et al., 1982; Benfenati et al., 1983; Taylor et al., 1983; Olie et al., 1982, 1983; Lustenhouwer et al., 1980; Barnes, 1983). For an industrial boiler in the United States where pentachlorophenol (PCP) was known to have been burned, Rappe et al. (1983) reported ~5 ppm PCDDs in the baghouse and bottom ash. However, >90% of the PCDDs were lower chlorinated congeners than octa-CDD, the expected dimerization product of PCP. Among the large number of isomers found, only a small amount of 2,3,7,8-TCDD could be quantified.

Analyses of soot samples from a transformer fire in Binghamton, NY, in February 1981, revealed that 2,3,7,8-TCDD (0.6 ppm) and 1,2,3,7,8-penta-CDD were the dominating isomers of the PCDDs formed (Buser and Rappe, 1983; Rappe et al., 1983). The origin of the polychlorinated dioxins was probably the chlorobenzenes in the transformer oil (Buser, 1979). Analyses of wipe tests from a garage adjacent to this accident site did reveal the presence of polychlorinated dibenzo-p-dioxins prior to the cleaning of the garage. Following the clean-up, no contamination was found (Tiernan et al., 1982; Tiernan, 1983).

Dermal

Dermal exposure to 2,3,7,8-TCDD is likely to be most significant during the spraying of 2,4,5-T. Lavy et al. (1980) determined the exposure levels of applicators spraying 2,4,5-T (ESTERON 245) during typical applications in a forest. The average dermal exposure to 2,4,5-T was estimated to be 0.6 mg/kg bw. If the 2,3,7,8-TCDD content in 2,4,5-T is assumed to be <0.1 ppm and the absorption rate is assumed to be the same, an exposure of 0.6 mg/kg of 2,4,5-T will correspond to <60 pg/kg bw of 2,3,7,8-TCDD for dermal exposure. Lavy et al. (1980) found a slightly lower level of 2,3,7,8-TCDD concentration (~12.5% lower) than the predicted value. No 2,3,7,8-TCDD was detected in any of the urine samples (detection limit 1.7 ng/ℓ).

PHARMACOKINETICS

The pharmacokinetics of 2,3,7,8-TCDD has been investigated in a number of laboratory animals, and there are several recent reviews on this subject (Neal et al., 1982; Gasiewicz et al., 1983a; Olson et al., 1983). This section will examine our current understanding of the absorption, distribution, metabolism and excretion of 2,3,7,8-TCDD in various mammalian species.

Absorption

The dermal and gastrointestinal absorption of 2,3,7,8-TCDD have been investigated in several species. No studies are available on the pharmacokinetics of 2,3,7,8-TCDD through the inhalation route of exposure.

Absorption From the Gastrointestinal Tract

Experimentally, 2,3,7,8-TCDD is generally administered in the diet or by gavage in an oil vehicle. In Sprague-Dawley rats given a single oral dose of 1.0 μg [^{14}C]2,3,7,8-TCDD/kg of bw, absorption from the intestinal tract was estimated at ~83% (Rose et al., 1976). With repeated oral dosing at 1.0 μg/kg/day (5 days/week x 7 weeks), absorption was observed to be approximately that observed for the single oral dose. With a much larger single oral dose, 50 μg/kg bw, ~70% of the dose was absorbed by Sprague-Dawley rats (Piper et al., 1973). In these studies, the chemical was administered by gavage in acetone:corn oil (1:25 or 1:9). One study in the guinea pig reported that ~50% of a single oral dose (quantity not mentioned) of 2,3,7,8-TCDD in acetone:corn oil was absorbed (Nolan et al., 1979). The gastrointestinal absorption of 2,3,7,8-TCDD was also examined in the hamster, the species most resistant to the acute toxicity of this toxin (Olson et al., 1980a). Olson et al. (1980b) administered hamsters a single, sublethal, oral dose of [1,6-^{3}H]-2,3,7,8-TCDD in olive oil (650 μg/kg) and reported that 74% of the dose was absorbed. When 2,3,7,8-TCDD was

administered to rats in the diet at 7 or 20 ppb (0.5 or 1.4 μg/kg/day) for 42 days, 50-60% of the consumed dose was absorbed (Fries and Marrow, 1975). These findings indicate that over a wide range of doses and under these experimental conditions, 2,3,7,8-TCDD is generally well absorbed from the gastrointestinal tract of the three species that have been examined.

Contact with 2,3,7,8-TCDD in the environment would most often involve exposure to a complex mixture containing the toxin, as opposed to the above experimental situation, where 2,3,7,8-TCDD was administered in the diet or through an oil vehicle.

The influence of dose and vehicle or adsorbent on gastrointestinal absorption has been investigated in rats by Poiger and Schlatter (1980), using hepatic concentrations 24 hours after dosing as an indicator of the amount absorbed. They found a linear relationship between ng 2,3,7,8-TCDD administered in 50% ethanol (for doses of 12-280 ng, equivalent to 0.06-1.4 μg/kg) and the percentage of the dose in hepatic tissues (36.7-51.5%). At the next higher dose of 1070 ng, however, the percentage fell off to about 42%. Their results regarding the influence of vehicle or adsorbent on gastrointestinal absorption have been summarized in Table 2. Administration of 2,3,7,8-TCDD in an aqueous suspension of soil resulted in a decrease in the hepatic levels of 2,3,7,8-TCDD as compared with hepatic levels resulting from administration of 2,3,7,8-TCDD in 50% ethanol. The extent of the decrease was directly proportional to the length of time the 2,3,7,8-TCDD had been in contact with the soil. When 2,3,7,8-TCDD was mixed in an aqueous suspension of activated carbon, absorption was almost totally eliminated (<0.07% of the dose in hepatic tissues).

TABLE 2

Percentage of 2,3,7,8-TCDD in the Liver of Rats 24 Hours After Oral Administration of 0.5 mℓ of Various Formulations Containing TCDD*

Formulation	TCDD Dose (ng)	No. of Animals	Percentage of Dose in the Liver
50% Ethanol	14.7	7	36.7 ± 1.2
Aqueous suspension of soil (37%, w/w) that had been in contact with TCDD for:			
10-15 hours	12.7, 22.9	17	24.1 ± 4.8
8 days	21.2, 22.7	10	16.0 ± 2.2
Aqueous suspension of activated carbon (25%, w/w)	14.7	6	≤0.07

*Source: Poiger and Schlatter, 1980

Philippi et al. (1981) and Hutter and Philippi (1982) have shown that radiolabeled 2,3,7,8-TCDD becomes progressively more resistant with time to extraction from soil. Similarly, the feeding of fly ash, which contains PCDDs, to rats in the diet for 19 days resulted in considerably lower hepatic levels of PCDDs than did the feeding of an extract of the fly ash at comparable dietary concentrations of PCDDs (van den Berg et al., 1983). The PCDDs were tentatively identified as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD and the difference in hepatic levels noted between fly ash-treated and extract-treated rats was greater for the more highly chlorinated isomers than it was for 2,3,7,8-TCDD. These results indicate the importance of the formulation or vehicle containing the toxin(s) on the relative bioavailability of 2,3,7,8-TCDD, PeCDD and HxCDDs following oral exposure.

Information on the absorption of 2,3,7,8-TCDD through the skin is found only in a study by Poiger and Schlatter (1980). The authors administered 26 ng 2,3,7,8-TCDD in 50 μℓ methanol to the skin of six rats. After 24 hours, the liver contained 14.8±2.6% of the dose. By comparing to the hepatic levels obtained after oral administration in 50% ethanol (in the same study), the amount absorbed from a dermal application can be estimated at ~40% of the amount absorbed from an equivalent oral dose. This comparison assumes that hepatic levels are valid estimates of the amount absorbed from both oral and dermal routes and that absorption from methanol is equivalent to absorption from 50% ethanol. As compared with dermal application in methanol, dermal application of 2,3,7,8-TCDD to rats in vaseline or polyethylene glycol reduced the percentage of the dose in hepatic tissue to 1.4 and 9.3%, respectively, but had no observable effect on the dose of 2,3,7,8-TCDD required to induce skin lesions (~1 μg/ear) in the rabbit ear assay.

Application of 2,3,7,8-TCDD in a soil/water paste decreased hepatic 2,3,7,8-TCDD to ~2% of the administered dose and increased the amount required to produce skin lesions to 2-3 μg in rats and rabbits, respectively. Application in an activated carbon/water paste essentially eliminated absorption, as measured by percent of dose in the liver, and increased the amount of 2,3,7,8-TCDD required to produce skin lesions to ~160 μg. These results suggest that the dermal absorption and acnegenic potency of 2,3,7,8-TCDD are dependent on the formulation (vehicle or adsorbent) containing the toxin.

Distribution

The tissue distribution of 2,3,7,8-TCDD in a number of species is summarized in Table 3. From these data it is apparent that 2,3,7,8-TCDD distributes preferentially to the liver and adipose tissue of most species that have been examined. Piper et al. (1973) used a single oral dose of [^{14}C]2,3,7,8-TCDD to study distribution and excretion in male Sprague-Dawley rats. Most of the radioactivity (53.2%) was excreted via the feces, but the urine and expired air accounted for 13.2 and 3.2%, respectively. Analysis of the tissues after 3 days showed liver and adipose tissue to contain the highest percent of the dose per gram of tissue, with 3.18 and 2.60%, respectively.

Rose et al. (1976) also examined the distribution of [^{14}C]2,3,7,8-TCDD in the rat. Twenty-two days after a single oral dose of 1.0 μg/kg, liver and adipose tissue had retained most of the ^{14}C activity, with 1.26 and 1.25% of the label retained per gram of tissue, respectively. With repeated oral doses, the activity was again localized mainly in the liver and adipose tissue, but the liver had five times as much radioactivity as did the fat.

TABLE 3

Tissue Distribution of 2,3,7,8-TCDD

Species	Route of Administration	Tissues with the Highest Concentration of 2,3,7,8-TCDD	References
Rat	oral	liver	Fries and Marrow, 1975
Rat	oral	liver > fat	Rose et al., 1976
Rat	oral	liver > fat	Piper et al., 1973
Rat	oral	liver > fat	Kociba et al., 1978
Rat	oral	liver > fat	Allen et al., 1975
Rat	i.p.	liver > fat	Van Miller et al., 1976
Mouse	oral	liver > fat > kidney > lung	Manara et al., 1982
Mouse	i.p.	liver > fat > kidney > lung > spleen	Manara et al., 1982
Rhesus monkey	i.p.	fat > skin > liver > adrenal = thymus	Van Miller et al., 1976
Golden Syrian hamster	i.p. or oral	liver > fat	Olson et al., 1980a
Guinea pig	oral	fat > liver > adrenals > thymus > skin	Nolan et al., 1979
Guinea pig	i.p.	fat > liver > skin > adrenals	Gasiewicz and Neal, 1979

NA = Not applicable

With the single oral dose, no radioactivity was detected in either the urine or expired air, indicating that most if not all of the elimination of 2,3,7,8-TCDD and/or its metabolites was through the feces. With repeated oral doses, the ^{14}C activity was also excreted primarily through the feces, but significant amounts were found in the urine, especially of the female rats. Male rats given 1.0 μg/kg/day of 2,3,7,8-TCDD for 7 weeks excreted an average of 3.1% of the cumulative dose in the urine while the female rats excreted an average of 12.5% in the urine (Rose et al., 1976). Fries and Marrow (1975) have also reported evidence of sex differences in tissue distribution in rats. During 42 days of administration of 2,3,7,8-TCDD, ~85% of the total body residue of male rats was located in the liver, while 70% of the total body residue of female rats was located in this organ.

Studies performed by Van Miller et al. (1976) on rhesus monkeys and rats using single i.p. doses of tritiated 2,3,7,8-TCDD (400 μg/kg bw) showed that while rats had over 40% of the 2,3,7,8-TCDD in the liver 7 days after dosing, the monkeys had only about 10% in the same organ at that time. In two strains of mice, the liver contained ~35% of an administered dose of 2,3,7,8-TCDD 1 day after oral or i.p. administration (Manara et al., 1982). The liver was also found to be the major site of accumulation of 2,3,7,8-TCDD in the hamster, with 20% of the dose localized in the liver (5.3% of dose/g liver) at 3 days following a sublethal dose of 650 μg ^{3}H-2,3,7,8-TCDD/kg (Olson et al., 1980a). In all three species, 1-22 days after single-dose oral or i.p. administration, levels of 2,3,7,8-TCDD in adipose tissue were generally slightly lower than levels in the liver, and were considerably higher than concentrations in other tissues (Piper et al., 1973; Rose et al., 1976; Van Miller et al., 1976; Olson et al., 1980a; Manara et al., 1982), including the thymus (Rose et al., 1976; Van Miller et al., 1976; Olson et al., 1980a).

Kociba et al. (1978) found that female rats maintained on a daily dietary 2,3,7,8-TCDD intake of 0.1 μg/kg/day for 2 years had an average 2,3,7,8-TCDD content of 8100 ppt in fat and 24,000 ppt in the liver. Rats given 0.01 μg/kg/day had an average of 1700 ppt of 2,3,7,8-TCDD in the fat and 5100 ppt in the liver. For both of these daily dosages the liver:body fat ratio of 2,3,7,8-TCDD was 3:1. At the lowest dose level of 0.001 μg/kg/day, both fat and liver contained an average of 540 ppt 2,3,7,8-TCDD. Kociba et al. (1976) presented evidence that steady-state had been reached after <13 weeks of feeding of 2,3,7,8-TCDD.

McNulty et al. (1982) reported that 2 years after administration of a single oral dose of 1 μg/kg of 2,3,7,8-TCDD to an adult rhesus macaque monkey, tissue levels of the compound were 100 ppt in adipose tissue and 15 ppt in liver. These results indicate that prolonged retention of 2,3,7,8-TCDD may occur in this species. The tissue distribution of 2,3,7,8-TCDD in the guinea pig appears to be similar to the monkey, with the highest concentration of the toxin being found in adipose tissue (Gasiewicz and Neal, 1979; Nolan et al., 1979). The interspecies difference in the tissue distribution of 2,3,7,8-TCDD may be related to the relative adipose tissue content of a given species and/or the affinity of 2,3,7,8-TCDD for the hepatic microsomal fraction; however, the significance of these differences remains in doubt. For example, the hepatotoxicity of 2,3,7,8-TCDD in a given species does not appear to be related to the hepatic concentration of the toxin (Neal et al., 1982).

2,3,7,8-TCDD has been demonstrated to be teratogenic and fetotoxic in the rat (see Teratogenicity section); the ability of 2,3,7,8-TCDD to gain access to the developing fetus of Fischer 344 rats following a single oral dose of [^{14}C]2,3,7,8-TCDD was investigated by Moore et al. (1976). They

found low concentrations of 2,3,7,8-TCDD in the fetus at gestation days 14, 18 and 21. The radioactivity appeared to be evenly distributed throughout the fetus on days 14 and 18; however, increased levels of radioactivity were detected in fetal liver on day 21. Nau and Bass (1981) (more recently reported by Nau et al., 1982) investigated the fetal uptake of 2,3,7,8-TCDD in NMRI mice following oral, i.p. or s.c. administration of the compound at dose levels of 5, 12.5 or 25 μg/kg in dimethylsulfoxide (DMSO):corn oil or acetone:corn oil. The chemical was usually administered as a single dose 2 days prior to sacrifice. All three modes of administration produced similar maternal and embryonic or fetal levels of 2,3,7,8-TCDD at 5 and 12.5 μg/kg. At 25 μg/kg, higher maternal and fetal tissue levels were obtained with s.c. administration, and much higher levels were obtained with i.p. administration, than were obtained with oral administration. Embryonic 2,3,7,8-TCDD concentrations were maximal on gestational days 9 and 10; however, low levels were found in the embryo and fetus between gestational days 11 and 18. This sharp decrease in 2,3,7,8-TCDD concentration coincides with placentation. 2,3,7,8-TCDD concentrations in the placenta were an order of magnitude greater than in the fetus itself. The affinity of fetal liver for 2,3,7,8-TCDD was relatively low, as compared to maternal liver; however, 2,3,7,8-TCDD levels in fetal livers were 2-4 times higher than the levels in other fetal organs. An attempt was made to correlate 2,3,7,8-TCDD levels in the fetuses with the observed incidence of cleft palate, but no clear relationship was observed.

Autoradiographic studies of tissue localization following i.v. administration of [^{14}C]2,3,7,8-TCDD in DMSO to three strains of mice indicated that the liver had the highest concentration and longest retention of radioactivity in the body, followed by the nasal mucosa (Appelgren et al., 1983).

In pregnant mice, the concentration of radioactivity in the fetuses was lower than in the dams, but a similar, selective labelling of the liver and the nasal mucosa was seen in the fetuses at day 17 of gestation. In the adult animals, labelling of the adrenal cortex was about equal to that of the liver at 1 hour after dosing, but thereafter was much lower than in the liver. Labelling of the thymus, lymph nodes, bone marrow and prostate were low at all observation times (i.e., 5 minutes to 61 days after injection).

Very few data are available on the tissue distribution of 2,3,7,8-TCDD in humans. Facchetti et al. (1980) reported tissue concentration of 2,3,7,8-TCDD at levels of 1-2 ng/g in liver and $\leq$0.1 ng/g in thyroid, brain, lung, kidney and blood in a woman who died 7 months after potential exposure to 2,3,7,8-TCDD from the Seveso accident. This pattern of 2,3,7,8-TCDD distribution, however, may not be representative for humans since the woman at the time of death had an adenocarcinoma (which was not considered related to the accident) involving the pancreas, liver and lung.

In addition Young et al. (1983) reported preliminary results of the analysis of adipose tissue from soldiers exposed to Agent Orange. Two analyses were performed, one using the exact mass of 321.8936 and the other the signal profile at masses 321.8936 and 319.8965. Three groups were studied consisting of 20 veterans claiming health problems related to Agent Orange exposure, 3 Air Force officers with known heavy exposure to Agent Orange during disposal operations, and 10 control veterans with no known herbicide exposure. In the first group, 10 of the 20 had measurable levels of 2,3,7,8-TCDD (5 with 5-7 ppt, 3 with 9-13 ppt and 1 with 23 and 35 ppt and another with 63 and 99 ppt). In the second group only two officers had measurable 2,3,7,8-TCDD levels and these did not exceed 3 ppt. In the 10 control veterans, 4 had 2,3,7,8-TCDD levels between 7 and 14 ppt. Levels of

2,3,7,8-TCDD in adipose tissue did not appear to be associated in this study with ill health or any particular symptom. However, it was considered that information on background levels of 2,3,7,8-TCDD in adipose tissue was too limited to draw any firm conclusions.

Metabolism

Vinopal and Casida (1973) found no evidence of water soluble metabolites of 2,3,7,8-TCDD following incubation with mammalian liver microsomes or i.p. injection into mice. In the same experiment, only unmetabolized 2,3,7,8-TCDD was extractable from mouse liver 11-20 days after treatment. Van Miller et al. (1976) claimed that the slow elimination of 2,3,7,8-TCDD they observed in both rats and monkeys after i.p. injections suggested that 2,3,7,8-TCDD was not readily metabolized. Metabolites of 2,3,7,8-TCDD have been detected in the bile and urine of Syrian Golden hamsters after single oral or i.p. doses (Olson et al., 1980a) and in the bile of dogs following repeated direct introduction of the chemical into the duodenal lumen (Poiger et al., 1982a).

Poiger and Schlatter (1979), Ramsey et al. (1979) and Ramsey et al. (1982) demonstrated biliary excretion of several metabolites of [^{14}C]2,3,7,8-TCDD by rats after repeated oral dosing. The metabolites were tentatively identified as glucuronides of hydroxylated 2,3,7,8-TCDD. The amounts of metabolites found were small, indicating that 2,3,7,8-TCDD is only slowly metabolized in the liver. Previous work by Piper et al. (1973) using single oral doses of 2,3,7,8-TCDD concluded that, since small amounts of radioactivity were found in the urine and expired air of male rats during the first 10 days, metabolic alteration or breakdown must occur. The study by Rose et al. (1976) using oral doses stated that while the ^{14}C activity in the rat livers appeared to be present as unchanged 2,3,7,8-TCDD, a

significant amount of radioactivity found in the feces appeared to come from substances other than 2,3,7,8-TCDD; the excretion of ^{14}C in the urine also indicated that metabolism had occurred.

Poiger et al. (1982b) investigated the toxicity of 2,3,7,8-TCDD metabolites by administering extracts of bile from 2,3,7,8-TCDD-treated dogs to male guinea pigs in single oral doses equivalent to 0.6, 6.0 and 60 μg/kg of parent compound. Other groups of guinea pigs received bile extract from untreated dogs or 2,3,7,8-TCDD itself. A comparison of the mortality data at 5 weeks after dosing indicated that the acute toxicity of 2,3,7,8-TCDD to guinea pigs was at least 100 times higher than was the acute toxicity of its metabolites.

More recently, Olson et al. (1983) reported that all of the radioactivity in urine and bile from ^{14}C-2,3,7,8-TCDD-treated rats, hamsters and guinea pigs corresponded to metabolites of 2,3,7,8-TCDD. The enzymatic hydrolysis of the 2,3,7,8-TCDD metabolites from the rat and hamster altered the chromatographic profile of the metabolites, indicating the presence of glucuronide conjugates in bile and sulfate conjugates in urine (Olson and Bittner, 1983). The apparent absence of these metabolites in extracts of hamster and rat liver suggest that once formed, the metabolites of 2,3,7,8-TCDD are readily excreted (Olson et al., 1980a; Rose et al., 1976). These results also indicate that urinary and biliary elimination of 2,3,7,8-TCDD is dependent upon metabolism of the toxin. Although urine and bile appear to be free of unmetabolized TCDD, data from the hamster and rat indicate that from 10 to 40% of the 2,3,7,8-TCDD-derived radioactivity in feces represents unchanged 2,3,7,8-TCDD (Olson et al., 1983; Olson and Bittner, 1983). The daily presence of unchanged 2,3,7,8-TCDD in feces and its absence in bile suggests that direct intestinal elimination may be the

source for the fecal excretion of 2,3,7,8-TCDD. This finding demonstrates that the half-life for elimination of 2,3,7,8-TCDD may not directly reflect the in vivo rate of 2,3,7,8-TCDD metabolism in a given animal. Nevertheless, the metabolism of 2,3,7,8-TCDD does in part regulate its elimination or relative persistence in a given animal.

Several metabolites of 2,3,7,8-TCDD have recently been identified. Sawahata et al. (1982) investigated the in vitro metabolism of 2,3,7,8-TCDD in isolated rat hepatocytes. The major product was deconjugated with β-glucuronidase, derivatized with diazomethane, and separated into two compounds by high performance liquid chromatography (HPLC). These metabolites were subsequently identified as 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin. Poiger et al. (1982b) identified six metabolites in the bile of dogs that were given a lethal dose of [3H]2,3,7,8-TCDD. The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin; 2,3,7,8-trichlor-3-hydroxydibenzo-p-dioxin and 1,2-dichloro-4,5-hydroxybenzene were also identified as minor metabolites. The structures of the three remaining metabolites were not determined; however, two appeared to be trichlorohydroxydibenzo-p-dioxins and the third was apparently a chlorinated 2-hydroxydiphenyl ether.

Data on the metabolism of 2,3,7,8-TCDD suggests that reactive epoxide intermediates may be formed. Poland and Glover (1979) have investigated the in vivo binding of [1,6-^{3}H]-2,3,7,8-TCDD derived radioactivity to rat hepatic macromolecules. They found maximum levels equivalent to 60 pmol 2,3,7,8-TCDD/mole of amino acids in protein, 12 pmol 2,3,7,8-TCDD/mole of nucleotide in rRNA, and 6 pmol of 2,3,7,8-TCDD/mole of nucleotide in DNA. This corresponds to one 2,3,7,8-TCDD-DNA adduct/35 cells. Poland and Glover (1979) suggest that it is unlikely that 2,3,7,8-TCDD-induced oncogenesis is through a mechanism of covalent binding to DNA and somatic mutation.

Further studies in other species, possibly with [^{14}C]-2,3,7,8-TCDD, are needed to confirm these results and assess the relationship between covalent binding and the short and long-term toxicity of 2,3,7,8-TCDD.

Isolated rat hepatocytes in suspension have been used as an in vitro system for assessing 2,3,7,8-TCDD metabolism under various conditions (Olson et al., 1981). Data indicate that the rate of 2,3,7,8-TCDD metabolism in rat hepatocytes correlates directly with drug induced changes in hepatic cytochrome P-450 monooxygenase activity, suggesting that 2,3,7,8-TCDD is metabolized by this enzyme (Neal et al., 1982). Pretreatment of rats with 2,3,7,8-TCDD has been shown to enhance the rate of 2,3,7,8-TCDD metabolism in isolated hepatocytes, demonstrating that 2,3,7,8-TCDD can induce its own rate of metabolism. Beatty et al (1978) also found a correlation between hepatic mixed-function oxidase (MFO) activity and the toxicity of 2,3,7,8-TCDD in rats. In both naturally occurring age and sex-related differences in MFO activity, and following administration of inducers and inhibitors of MFO enzyme systems, hepatic MFO activity was directly correlated with the 20-day LD_{50}.

Olson and Bittner (1983) reported that the rate of 2,3,7,8-TCDD metabolite formation in vitro was higher in hepatocytes from the hamster than in hepatocytes from the rat. Qualitative evaluation of in vivo and in vitro metabolites by HPLC also suggested significant interspecies variability. The authors suggested that such differences in metabolism may partially explain the differences in toxicity among species.

Excretion

The following discussion assumes that elimination is a first order process. With the exception of the guinea pig, which may follow zero order kinetics (Gasiewicz and Neal, 1979), elimination data yield a straight line

on a semilogarithmic plot, indicating that elimination is a single, first order process. Hiles and Bruce (1976) have pointed out that the studies of Allen et al. (1975) and Piper et al. (1973) can be interpreted equally well by either zero or first order kinetics. The majority of the data, however, seem to support the assumption of a first order elimination process.

The excretion of 2,3,7,8-TCDD and its metabolites has been investigated in a number of species. Table 4 summarizes results on the elimination of 2,3,7,8-TCDD-derived radioactivity, following a single exposure to ^{3}H- or [^{14}C]-2,3,7,8-TCDD. These studies show that 2,3,7,8-TCDD was slowly excreted from the bodies of all species tested, with a half-life in the body of 10-43 days. In the Syrian Golden hamster, the least sensitive mammalian species to the acute toxicity of 2,3,7,8-TCDD, excretion occurred readily through both the urine (35% of administered dose, 41% of total excreted radioactivity) and feces (50% of the administered dose, 59% of total excreted radioactivity) (Olson et al., 1980b; Gasiewicz et al., 1983a). The high levels found in the urine of infant monkeys were probably due to the incomplete separation of urine and feces (Van Miller et al., 1976). In all the other species tested so far, excretion occurred mainly through the feces (80-100% of total urinary and fecal radioactivity) with only minor amounts of 2,3,7,8-TCDD metabolites found in the urine (Piper et al., 1973; Allen et al., 1975; Rose et al., 1976; Gasiewicz and Neal, 1979). Only Piper et al. (1973) reported the excretion of metabolites in the expired air. During 21 days following administration of a single oral dose of [^{14}C]2,3,7,8-TCDD to rats, 3.2% of the administered radioactivity (4.6% of the excreted radioactivity) was recovered in the expired air.

TABLE 4

Elimination of 2,3,7,8-TCDD

Species	Single Treatment µg/kg (route)	Half-Life for Elimination (days)	Relative % of TCDD-Derived Radioactivity		Reference
			Feces	Urine	
Guinea pig	2 (i.p.)	30.2 ± 5.8	94.0	6.0	Gasiewicz and Neal, 1979
Guinea pig	1.45 (oral)	22 - 43	NT	NT	Nolan et al., 1979
Rat	1.0 (oral)	31 ± 6	>99	<1	Rose et al., 1976
Rat	50 (oral)	17.4 ± 5.6	80.0	20.0	Piper et al., 1973
Rat	50 (oral)	21.3 ± 2.9	95.5	4.5	Allen et al., 1975
Rat	400 (i.p.)	NT	91.0	9.0	Van Miller et al., 1976
Monkey (adult)	400 (i.p.)	NT	78.0	22.0	Van Miller et al., 1976
Monkey (infant)	400 (i.p.)	NT	39.0	61.0	Van Miller et al., 1976
Mouse					
C57BL/65	10 (i.p.)	11.0 ± 1.2	72.0	28.0	Gasiewicz et al., 1983a,b
DBA/2J	10 (i.p.)	24.4 ± 1.0	54.0	46.0	Gasiewicz et al., 1983a,b
B6D2F$_1$/J*	10 (i.p.)	12.6 ± 0.8	72.0	28.0	Gasiewicz et al., 1983a,b
Hamster	650 (i.p.)	10.8 ± 2.4	59.0	41.0	Olson et al., 1980a
Hamster	650 (oral)	15.0 ± 2.5	NT	NT	Olson et al., 1980a

*Offspring of C57BL/6J and DBA/2J which are heterozygous at the Ah locus

NT = Not tested

Rose et al. (1976) investigated the elimination of [^{14}C]2,3,7,8-TCDD in rats given repeated oral doses of 0.01, 0.1 or 1.0 μg/kg/day Monday through Friday for 7 weeks, or a single dose of 1.0 μg/kg. In the single-dose study, no ^{14}C was excreted in the urine or expired air; in the repeated-dose study, however, 3-18% of the cumulative dose was excreted in the urine by 7 weeks. This study indicated that steady-state concentrations will be reached in the bodies of rats in ~13 weeks. The rate constant defining the approach to steady-state concentrations was independent of the dosage of 2,3,7,8-TCDD over the range studied. This is consistent with the observations of Fries and Marrow (1975) who found that the total retention in the bodies of rats was proportional to total intake. When rats were maintained on a diet containing either 7 or 20 ppb 2,3,7,8-TCDD, the amount of 2,3,7,8-TCDD retained in the body was 5.5 times the daily intake of 2,3,7,8-TCDD at 14 days, 7.5 times the daily intake at 28 days, and 10.0 times the daily intake at 42 days.

The data in Table 4 suggest some interspecies differences in the half-life for elimination (t 1/2) of 2,3,7,8-TCDD. In the hamster, the least sensitive species to the acute toxicity of 2,3,7,8-TCDD, a mean t 1/2 of 10.8 days was observed (Olson et al., 1980a,b), and in the guinea pig, the most sensitive species to the acute toxicity of 2,3,7,8-TCDD, the mean t 1/2 was 30.2 days (Gasiewicz and Neal, 1979). The observed interspecies differences in the t 1/2 of 2,3,7,8-TCDD may in part be related to the relative sensitivity of a given species to the acute toxicity of 2,3,7,8-TCDD.

The intraspecies differences in the t 1/2 of 2,3,7,8-TCDD in three mouse strains may be due to the finding that the DBA/2J strain possesses ~2-fold greater adipose tissue stores than the C57BL/6J and B6D2F$_1$/J strains (Gasiewicz et al., 1983b). The sequestering of the lipophilic toxin in

adipose tissue stores of the DBA/2J mouse may contribute to the greater persistence of 2,3,7,8-TCDD in this strain.

In all of the rat studies shown in Table 4, urinary and fecal elimination were monitored for a period of only 20-22 days, and from these data it was assumed that elimination followed a single component, first order kinetic model. Recently, Olson and Bittner (1983) examined the elimination of 2,3,7,8-TCDD-derived radioactivity in rats over a 35-day period following a single i.p. exposure at 1 μg ^{3}H-2,3,7,8-TCDD/kg. They observed first order kinetics for elimination, with a fast component having a t 1/2 of 7 days (representing 13% of total elimination) and a slow component having a t 1/2 of 75 days (87% of total). The second, slow component for elimination was evident only when urinary and fecal elimination were monitored for >30 days. This study suggests that 2,3,7,8-TCDD may be more persistent than earlier studies suggested. A preliminary study in the rhesus monkey indicates that 2,3,7,8-TCDD may be exceptionally persistent in adipose tissue. McNulty et al. (1982) estimated the apparent half-life of 2,3,7,8-TCDD in the fat of a monkey to be ~1 year.

Studies in the rat, guinea pig, hamster and mouse have found that all of the 2,3,7,8-TCDD derived radioactivity excreted in the urine and bile corresponds to metabolites of 2,3,7,8-TCDD (Olson et al., 1983). The apparent absence of 2,3,7,8-TCDD metabolites in liver and fat suggests that, once formed, the metabolites of 2,3,7,8-TCDD are readily excreted. Thus, urinary and biliary elimination of 2,3,7,8-TCDD is dependent upon metabolism of the toxin. Although urine and bile appear to be free of unmetabolized 2,3,7,8-TCDD, data from the hamster and rat indicate that a significant amount (10-40%) of unchanged 2,3,7,8-TCDD may be excreted into the feces (Olson et al., 1983). Unmetabolized 2,3,7,8-TCDD thus appears to enter the intestinal

lumen by some route other than bile (direct intestinal elimination) for a number of days following treatment. Studies in lactating rats have also found that unchanged 2,3,7,8-TCDD may be excreted in the milk of lactating animals (Moore et al., 1976; Lucier et al., 1975). Lactation, direct intestinal elimination, and perhaps sebum may serve as routes for excretion of 2,3,7,8-TCDD, which are not dependent upon metabolism of the toxin. These data suggest that the in vivo half-life for elimination of 2,3,7,8-TCDD may not directly reflect the rate of 2,3,7,8-TCDD metabolism in a given animal (Neal et al., 1982).

EFFECTS

Acute, Subacute and Chronic Toxicity

The acute LD_{50} for 2,3,7,8-TCDD in several species is shown in Table 5. The oral LD_{50} values range from 0.6 μg/kg bw for guinea pigs to 5051 μg/kg bw for hamsters (Schwetz et al., 1973; Vos et al., 1974; McConnell et al., 1978a,b; Henck et al., 1981; Olson et al., 1980b). The dermal LD_{50} for rabbits was 275 μg/kg of body weight (Schwetz et al., 1973); death was sometimes delayed as long as 40 days following acute exposure. Of the laboratory animals studied, the guinea pig was the most susceptible to the toxic effects of 2,3,7,8-TCDD (Schwetz et al., 1973; Gupta et al., 1973; Greig et al., 1973).

The acute toxicity has also been found to vary with the sex, age and strain of the test animal. Schwetz et al. (1973) found male Sherman rats more sensitive to 2,3,7,8-TCDD than females, while Beatty et al. (1978) found female Sprague-Dawley rats more sensitive than adult male rats. Thus, no general sex difference is apparent in the rat, perhaps due to strain differences in sensitivity to 2,3,7,8-TCDD. A significant sex difference was observed in the C57BL/10 mouse, with the oral LD_{50} in females being 3-fold

TABLE 5

Lethality of 2,3,7,8-TCDD Following Acute Exposure

Species/Strain	Sex/No./ Group	Route/Vehicle	Dose Tested (μg/kg)	Duration of Observation	LD_{50} (μg/kg)	Comments	Reference
Guinea pigs/ Hartley	M/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	0.6 (0.4-0.9)*	time to death was 5-34 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Guinea pigs/ Hartley	M/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	2.1 (1.5-3)*	time to death was 9-42 days, the 2,3,7,8-TCDD was 99% pure	Schwetz et al., 1973
Guinea pigs/ Hartley	M/9	gavage/corn oil	NR	30 days	2	median time to death was 17-20 days, marked weight loss, thymus atrophy, intestinal hemorrhage, no porphyria and only mild liver injury	McConnell et al., 1978a
Rats/Sherman	M/5-10	gavage/corn oil-acetone (9:1)	8 16 32 63	2-8 weeks	22	time to death was 9-27 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Rats/Sherman	F/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	45 (30-66)*	time to death was 13-43 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Rats/Sprague-Dawley	M/6	i.p./olive oil	NR	20 days	60	LD_{50} (μg/kg, mean ± SE) adult male, 60.2 ± 7.8; weanling male, 25.2 ± 1.4	Beatty et al., 1978
Rats/Sprague-Dawley	F/6	i.p./olive oil	NR	20 days	25	LD_{50} (μg/kg, mean ± SE) adult female, 24.6 ± 2.0	Beatty et al., 1978
Monkey/rhesus	F/3	gavage/corn oil	0 70 350	>35 days	<70	weight loss, edema, severe thymus atrophy, loss of hair, mild liver damage	McConnell et al., 1978b
Mice/C57Bl	M/14	gavage/corn oil-acetone (9:1)	0 100 150 200	60 days	114	time to death in the high dose group was 15-20 days, body weight loss, edema in 25% of treated animals, severe thymic and spleen atrophy, hemorrhage in the region of the eye and small intestine, liver necrosis in the centrilobular region	Vos et al., 1974

TABLE 5 (cont.)

Species/Strain	Sex/No./ Group	Route/Vehicle	Dose Tested (µg/kg)	Duration of Observation	LD_{50} (µg/kg)	Comments	Reference
Mice/C57Bl	M/9	gavage/corn oil	NR	30 days	283.7	median time to death was 22-25 days, dose-related body weight loss, thymic atrophy, increased liver weight and porphyria, gross and historic liver alterations, subcutaneous edema, intestinal hemorrhage	McConnell et al., 1978a
Mice/C57BL/10	M/5	gavage/arachis oil	85 107 135 170 213	45 days	146	95% confidence limits of 111-211 µg/kg. Most deaths occurred from 22-26 days after dosing. Signs of porphyria, edema, hemorrhage.	Smith et al., 1981
Mice/C57BL/10	F/5	gavage/arachis oil	85 107 135 170 213 269 338 426 536	45 days	>450	1 of 4 animals died at dose of 426 µg/kg	Smith et al., 1981
Mice/C57BL/6J	M/NR	i.p./olive oil	NR	30 days	132		Gasiewicz et al., 1983a,b
Mice/DBA/2J	M/NR	i.p./olive oil	NR	30 days	620		Gasiewicz et al., 1983a,b
Mice/B6D2F_1/J	M/NR	i.p./olive oil	NR	30 days	300	B6D2F_1/J mice are the offspring of C57BL/6J and DBA/2J and are heterozygous at the Ah locus.	Gasiewicz et al., 1983a,b
Rabbits/ New Zealand	M&F/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	115 (38-345)*	time to death was 6-39 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Rabbits/ New Zealand	M&F/5	i.p./corn oil	32 63 126 252 500	4 weeks	NR	time to death was 6-23 days, 2-3 animals/group died in all but the low exposure group	Schwetz et al., 1973

TABLE 5 (cont.)

Species/Strain	Sex/No./ Group	Route/Vehicle	Dose Tested (μg/kg)	Duration of Observation	LD_{50} (μg/kg)	Comments	Reference
Rabbits/ New Zealand	M&F/NR	dermal/acetone	31.6 63 126 252 500	3 weeks	275 (142-531)	time to death was 12-22 days	Schwetz et al., 1973
Hamster/ golden Syrian	M/6	gavage/corn oil-acetone (9:1)	0 300 600 1000 3000 6000	55 days	5051 (3876-18,487; 95% confidence)	time to death was 26-43 days, the liver and thymus appeared to be the primary target organs, only 1 death occurred in the 300 and 3000 μg/kg group	Henck et al., 1981
Hamster/ golden Syrian	M&F/5-6	i.p./olive oil	0 500 1000 2000 3000	50 days	>3000	significant dose-related decrease in thymus weight starting at 500 μg/kg, only 2 deaths occurred out of 11 hamsters in the 3000 μg/kg group.	Olson et al., 1980b
Hamster/ golden Syrian	M/5	gavage/olive oil	500 1000 2000 3000	50 days	1157	death generally occurred between 24 and 45 days, decrease in body weight above 2000 μg/kg, proliferative ileitis with mild to severe inflammation	Olson et al., 1980b
Dogs/beagle	M/2	gavage/corn oil-acetone (9:1)	3000	2-8 weeks	NA	all animals died	Schwetz et al., 1973
Dogs/beagle	F/2	gavage/corn oil-acetone (9:1)	30 100	2-8 weeks	NA	all animals survived	Schwetz et al., 1973

*The number in parentheses appears to indicate the range of lethal doses; however, the studies did not specify what these numbers represented.

NA = Not applicable; NR = not reported

greater than that in males (Smith et al., 1981). In a study of age-related differences, Beatty et al. (1978) reported weanling male rats to have an acute LD_{50} of 25 μg/kg in contrast to the value of 60 μg/kg in adult males. Vos et al. (1974) found 0, 17 and 44% mortality in mice of 4, 2 and 1 months of age, respectively, following 4 weekly doses of 25 μg 2,3,7,8-TCDD/kg. These limited studies suggest that young animals may be more susceptible to the acute toxicity of 2,3,7,8-TCDD. Various strains of mice have been used to study the mechanism of action of 2,3,7,8-TCDD, based on the ability of the toxin to induce enzymes that have been shown to segregate with a single genetic locus, the Ah locus (Poland et al., 1974, 1976a,b). The "non-responsive" strains (e.g., DBA/2J) appear to be less responsive to enzyme induction due to an altered receptor with lower affinity for 2,3,7,8-TCDD, in comparison to the "responsive" strains (e.g., C57BL/6J). Gasiewicz et al. (1983b) reported that the "responsive" C57BL/6J mice have an acute LD_{50} for 2,3,7,8-TCDD of 132 μg/kg, compared with an LD_{50} of 620 μg/kg in the "non-responsive" DBA/2J mice. An intermediate LD_{50} of 300 μg/kg was also reported for $B6D2F_1$/J mice, which are offspring of C57BL/6J and DBA/2J ($B6D2F_1$/J mice are heterozygous at the Ah locus). These results suggest that the acute LD_{50} for 2,3,7,8-TCDD varies with the strain of mouse and the relative activity "responsiveness" at the Ah locus.

The hepatotoxicity of 2,3,7,8-TCDD is well established, especially in rats, mice and rabbits where the hepatic lesions are particularly severe (Milnes, 1971). Sublethal doses of 2,3,7,8-TCDD in rats produced significant liver damage, characterized by fatty changes, centrilobular necrosis (Cunningham and Williams, 1972), megalocytosis, and unusual numbers of multinucleated giant hepatocytes (Gupta et al., 1973). A single dose of 0.1 μg/kg in rats produced increased liver weights (Harris et al., 1973).

In rats given single doses of 5 and 25 μg/kg 2,3,7,8-TCDD, Fowler et al. (1973) reported extensive proliferation of the smooth and rough endoplasmic reticulum, especially near the bile ducts. Twenty-eight days after dosing, the electron micrographs of the livers were indistinguishable from controls. Similar results were observed by Jones and Butler (1974) and Jones and Greig (1975). In mice, exposure to 1-10 μg/kg 2,3,7,8-TCDD/day produced liver damage as indicated by elevated SGOT, SGPT, serum LDH, alkaline phosphatase and bilirubin levels (Zinkl et al., 1973).

A number of toxic responses have been observed following exposure to 2,3,7,8-TCDD and these have been summarized for a number of species in Table 6. 2,3,7,8-TCDD toxicity exhibits marked interspecies variability, with some responses being highly species specific and confined to one or a few species. Loss of body weight or reduced weight gain and thymic atrophy are the most consistent toxic responses of 2,3,7,8-TCDD exposure in various species, with the latter being one of the most sensitive indicators of toxicity. In general, the toxicologic pattern observed with 2,3,7,8-TCDD is not unique; it also occurs with certain halogenated dibenzofurans, chlorinated biphenyls, naphthalenes, and brominated dioxins (McConnell, 1980).

An extended period was observed between treatment and death. During this period the animals had poor weight gain or loss of weight and appeared to be "wasting away". At death, loss in body weight was reported to be as great as 50% for some species (McConnell, 1980). In female Wistar rats intubated with 2,3,7,8-TCDD at a dose of 100 μg/kg, the weight loss was biphasic (Courtney et al., 1978). The initial weight loss occurred rapidly during the first 7-10 days after treatment and was associated with decreased food and water consumption. This initial phase of weight loss was reversed with the resumption of normal food intake for 4 or 5 days, only to be

TABLE 6

Toxic Responses Following Exposure to 2,3,7,8-TCDD: Species Differences[a]

	Monkey	Guinea Pig	Cow[b]	Rat	Mouse	Rabbit[b]	Chicken[b]	Hamster
Hyperplasia and/or metaplasia								
Gastric mucus	++[c]	0	+	0	0			0
Intestinal mucosa	+							++
Urinary tract	++	++	++	0	0			
Bile duct and/or gall bladder	++	0	+		++			0
Lung: focal alveolar				++				
Skin	++	0	*[d]	0	0	++		0
Hypoplasia, Atrophy, or Necrosis								
Thymus	+	+	+	+	+		+	+
Bone marrow	+	+			±		+	
Testicle	+	+		+	+		+	
Other								
Liver lesions	+	±		++	+	++	+	±
Porphyria	0	0		+	++		+	0
Edema	+	0		0	+		++	+

[a]References: Monkey (McConnell et al., 1978a; Norback and Allen, 1973; Allen et al., 1977); Guinea pig (McConnell et al., 1978a; McConnell, 1980; Moore et al., 1979; Turner and Collins, 1983); Cow (McConnell, 1980); Rat (McConnell, 1980; Kociba et al., 1978, 1979); Mouse (Schwetz et al., 1973; McConnell et al., 1978a; Vos et al., 1973); Rabbit (Kimmig and Schultz, 1957; Schwetz et al., 1973; Vos and Beems, 1971); Chicken (Schwetz et al., 1973; Norback and Allen, 1973; Allen and Lalich, 1962; Vos and Koeman, 1970); Hamster (Olson et al., 1980b; Henck et al., 1981)

[b]Responses followed exposure to 2,3,7,8-TCDD or structurally related chlorinated aromatic hydrocarbons.

[c]Symbols: 0, lesion not observed; +, lesion observed (number of "+" denote severity); ±, lesion observed to a very limited extent; blank, no evidence reported in literature.

[d]Skin lesions in cattle are observed, but they differ from the skin lesions observed in other species.

Source: Adapted from Poland and Knutson, 1982

followed by a second, more gradual, decline in food and water intake and weight until death. Providing animals with an adequately nutritious liquid diet by intubation did not appreciably alter the pattern of weight loss nor affect survival. In contrast, Gasiewicz et al. (1980) observed that providing rats with total parenteral nutrition would prevent some of the weight loss induced by 2,3,7,8-TCDD; however, there was no protection from the lethal effects of 2,3,7,8-TCDD. Also, severe thymic atrophy has been universally observed in all species given lethal doses of 2,3,7,8-TCDD, and since weight loss and thymic atrophy are both associated with malnutrition, van Logten et al. (1981) investigated the effects of dietary protein on the toxicity of 2,3,7,8-TCDD. Groups of female Fischer 344 rats administered 2,3,7,8-TCDD (20 μg/kg) and maintained on low (3.5%), normal (26%) or high (55%) protein diets maintained approximately the same body weight (gains were -0.2±3, 7±6 and 7±3 g for each dietary group, respectively) during the subsequent 10-day period. The weight gain in treated animals was 10-18 g less than that in the respective control rats. Dietary protein also had no effect on preventing or enhancing the 2,3,7,8-TCDD induced thymic atrophy.

In yet another study, Seefeld and Peterson (1983) suggest that a reduction in food intake caused by 2,3,7,8-TCDD is primarily responsible for the loss of body weight or depressed growth rate of rats. Pair-fed control rats lost weight at the same rate and to the same extent as their weight matched 2,3,7,8-TCDD-treated partners (25 or 50 μg/kg) until day 10 after treatment. At 20-35 days after treatment, the body weight of the two groups began to diverge, with the pair-fed control group having body weights that were 20-30 g higher than the corresponding 2,3,7,8-TCDD groups. They propose a hypothesis that 2,3,7,8-TCDD lowers a regulated level of "set-point" for body weight control in the rat. The ensuing change in food intake is

thought to occur secondarily to the change in "set-point". Thus, the precise mechanism for the 2,3,7,8-TCDD induced weight loss remains uncertain; however, it is evident that weight loss is a contributing factor to 2,3,7,8-TCDD induced mortality and morbidity.

Feeding a diet containing 7 ppb of 2,3,7,8-TCDD to rats caused an increase of liver weight while unexpectedly, 20 ppb caused less of a liver weight gain. After the feeding of 2,3,7,8-TCDD was discontinued, recovery was greater in the 7 ppb groups (Fries and Marow, 1975). The hepatotoxicity of 2,3,7,8-TCDD was most severe in rats, mice and rabbits (Vos and Beems, 1971; Gupta et al., 1973; Schwetz et al., 1973; Vos et al., 1974). 2,3,7,8-TCDD-induced liver alterations in the guinea pig and hamster were generally limited to the responses accompanying liver hypertrophy (Turner and Collins, 1983; Olson et al., 1980b). Limited steatosis, focal necrosis, and cytoplasmic hyalin-like bodies were also observed in the guinea pig (Turner and Collins, 1983). Comparative studies indicate that the guinea pig and hamster were the least sensitive to 2,3,7,8-TCDD-induced hepatotoxicity, which is in contrast to the 5000-fold difference in the acute LD_{50} for 2,3,7,8-TCDD in these species.

2,3,7,8-TCDD affects porphyrin metabolism and causes significantly elevated excretion of porphyrins and δ-aminolevulinic acid (see Metabolism section). Goldstein et al. (1978) showed that α-aminolevulinic acid synthetase, a rate-limiting enzyme in porphyrin synthesis, was slightly increased (2-fold) in male C57Bl mice given 4 weekly doses of 2,3,7,8-TCDD at 25 μg/kg. This dose of 2,3,7,8-TCDD increased liver prophyrin levels 2000-fold. Catabolism of porphyrin by uroporphyrinogen decarboxylase (UD) also appeared to be decreased in 2,3,7,8-TCDD-treated mice. Smith et al. (1981) reported a decrease in UD activity from ~25 to 7 nmoles/hr/g liver in

male and female C57Bl mice 3 weeks after a single oral exposure to 2,3,7,8-TCDD at a dose of 75 μg/kg. No effect of 2,3,7,8-TCDD on UD activity was observed in DBA/2 mice, which were insensitive to the induction of prophyria. A time course of changes in UD activity with length of time after exposure to 2,3,7,8-TCDD indicated a steady decline in activity starting 3 days after exposure to 2,3,7,8-TCDD, which continued until day 21 when the study was terminated. Sweeney and Jones (1978) reported similar results after 5 weekly doses of 2,3,7,8-TCDD at 25 μg/kg. In this study, the UD activity declined ~48% in C58Bl mice and only 4% in DBA/2 mice. Other factors besides the increase in α-aminolevulinic acid synthetase and the decrease in UD activity may also participate in the dramatic increase in liver porphyrin in mice, associated with exposure to near lethal doses of 2,3,7,8-TCDD.

A number of biochemical studies have resulted from the observation that 2,3,7,8-TCDD produces fatty livers and a resulting increase in total hepatic lipid content in several species. A sublethal dose of 2,3,7,8-TCDD in the rat produced an increase in triglycerides and free fatty acids and a decrease in sterol esters, while a lethal dose increased cholesterol esters and free fatty acids (Albro et al., 1978). Poli et al. (1980) treated rats with a single i.p. injection of 2,3,7,8-TCDD at doses of 2.5, 5, 10 and 20 μg/kg. At day 21 after treatment there was a dose-related increase in total plasma cholesterol and high density lipoprotein cholesterol, while no change was observed in triglycerides or very low and low density lipoproteins (VLDL and LDL, respectively). At a dose of 20 μg/kg the maximum increase in HDL cholesterol and total cholesterol occurred 30 days after treatment, and a significant elevation was still present at 60 days after treatment when the study was terminated. Slight changes in the apoproteins

of HDL from 2,3,7,8-TCDD rats and control rats were indicative of new apoprotein synthesis. Although the increase in HDL cholesterol may be in response to eliminating excess lipids, the exact function has not been clearly shown.

In contrast to rats, male Hartley strain guinea pigs given a single i.p. injection of 2,3,7,8-TCDD at a dose of 2 μg/kg had increased hyperlipidema characterized by increases in VLDL and LDL (Swift et al., 1981). In animals sacrificed 7 days after exposure to 2,3,7,8-TCDD, there was an increase in total serum lipid, cholesterol esters, triglycerides and phospholipids when comparison was made to pair-fed, weight-paired or ad libitum fed control groups. Serum-free fatty acids were not changed quantitatively; however, some qualitative changes occurred, reflecting an increase in the types of fatty acids which were abundant in the adipose tissue of guinea pigs. Analysis of lipoproteins revealed a 19-fold increase in VLDL and a 4-fold increase in LDL, with no change observed in the levels of HDL. The VLDL was also qualitatively different in the 2,3,7,8-TCDD treated animals, containing less cholesterol ester and an altered C apoprotein. The importance of these qualitative changes is unclear. The hyperlipidemia may result from the 2,3,7,8-TCDD induced mobilization of free fatty acids, which are then used in the synthesis of VLDL and are subsequently formed into LDL. The relationship of the changes in serum lipid levels to the mechanism of 2,3,7,8-TCDD toxicity needs further study.

Gupta et al. (1973) reported slight to moderate thymic atrophy in guinea pigs after 8 weekly oral doses of 0.2 μg/kg. The thymic atrophy was characterized by a decrease in the number of cortical thymocytes, reduction in size of the thymic lobules, and the absence of a demarcation between cortex and medulla. There was a relative depletion of lymphoid cells in the

spleen and the lymph nodes. In addition, moderate thymic atrophy was observed in rats after 31 daily oral doses of 1 μg/kg 2,3,7,8-TCDD. Thymic atrophy has also been noted in monkeys (Norback and Allen, 1973). In later studies 2,3,7,8-TCDD was found to suppress cell-mediated immune function in young rats without affecting humoral immune function. Suppression of T-cell function was selective in that "helper" cell function was not suppressed (Faith and Moore, 1977). Recently, the effects of 2,3,7,8-TCDD on thymus involution in rats were found not to involve the adrenal or pituitary glands and were not prevented by treatment with growth hormone (van Logten et al., 1980).

Increased susceptibility to Salmonella infection was found in mice treated intragastrically with 2,3,7,8-TCDD at doses between 1 and 20 μg/kg bw once weekly for 4 weeks. Such increased susceptibility after 2,3,7,8-TCDD administration was not seen with Herpes virus infection (Thigpen et al., 1975). Thymus atrophy with consequent suppression in cell-mediated immunity as measured by several parameters was found by Vos et al. (1978) in mice after various doses of 2,3,7,8-TCDD up to 50 μg/kg bw. The effects were dose related. Juvenile and adult mice treated with 2,3,7,8-TCDD in their feed at 10 and 100 ppm displayed several dose related changes, including depression in total serum protein, gamma globulin and albumin. Primary and secondary antibody responses to both tetanus toxoid and sheep erythrocytes were also reduced, as well as resistance to challenge with either Salmonella typhimurium or Listeria monocytogenes (Hinsdill et al., 1980). Neonatal $B6C3F_1$ mice, exposed to prenatal (maternal dosing on day 14 of gestation) and postnatal (days 1, 7 and 14 after birth) doses of 0, 1.0, 5.0 or 15.0 μg/kg 2,3,7,8-TCDD were studied for immunotoxic effects and host susceptibility (Luster et al., 1980). In the bone marrow, hypocellularity

and depressed macrophages-granulocyte progenitor cells and pluripotent stem cells were associated with 2,3,7,8-TCDD exposure at the 5.0 and 15.0 μg/kg dose levels. Host susceptibility to L. monocytogenes and PYB6-tumor cells was tested in the 2,3,7,8-TCDD-exposed neonates. Death occurred in 73% and 40% of the L. monocytogenes inoculated (1.2×10^6 viable organisms) mice in the 5.0 and 1.0 μg/kg dose groups, respectively, compared to 28% of controls. Tumor development occurred in 44, 60 and 22% of the neonates inoculated with 5×10^4 tumor cells from the 5.0 μg 2,3,7,8-TCDD/kg, 1.0 μg 2,3,7,8-TCDD/kg and control groups, respectively. While thymic atrophy may be one of the most sensitive indicators of experimental exposure to 2,3,7,8-TCDD, animals given a lethal dose of 2,3,7,8-TCDD do not appear to die from infections, nor does a germ-free environment protect them from death (Greig et al., 1973).

In the Gupta et al. (1973) study, rats also showed degenerative changes in the renal collecting tubules, degenerative changes of the thyroid follicles, necrosis and ulceration of the glandular stomach and hemorrhage into the adrenals. This latter set of findings generally occurred at higher dose levels than the minimum dose needed to provide thymic atrophy or liver enlargement. More recent studies on the effects of 2,3,7,8-TCDD on renal functions have been carried out. Anaizi and Cohen (1978) reported an increase in the renal secretion of phenolsulfonphthalein (PCP) and a significant decrease in glomerular filtration rate (GFR) compared with controls in rats treated with 10 μg/kg (i.p.) of 2,3,7,8-TCDD. These authors attributed these effects to the toxicity of 2,3,7,8-TCDD on glomerular structures. However, other reports concluded that 2,3,7,8-TCDD causes no specific functional lesions in the kidney, rather that the effects on renal functions reflect a general toxicosis (Pegg et al., 1976).

Pronounced dermal effects with 2,3,7,8-TCDD treatment have been reported for rabbits by Schwetz et al. (1973). Milnes (1971) observed chloracne in rabbits after a single oral dose of 1 μg/kg. Dogs, though apparently less sensitive to 2,3,7,8-TCDD than rabbits (lethal effects) following oral administration, have exhibited hair loss (Schwetz et al., 1973). McConnell et al. (1978b) found facial alopecia with acne-like eruptions, blepharitis, weight loss and anemia in rhesus monkeys after single oral doses of 70 or 350 μg/kg 2,3,7,8-TCDD. Allen et al. (1977) observed loss of facial hair and eyelashes, accentuated hair follicles, dry scaly skin and gastric mucosal dysplasia in eight female rhesus monkeys fed a diet containing 500 ppt 2,3,7,8-TCDD for up to 9 months. Eventually 5 of the 8 monkeys died from severe pancytopenia. In humans, the most characteristic and frequently observed lesion produced by 2,3,7,8-TCDD and other chlorinated aromatic hydrocarbons is chloracne (Crow, 1981; Taylor, 1979). This lesion consists of hyperplasia and hyperkeratosis of the interfollicular epidermis, hyperkeratosis of the hair follicle, especially at the infundibulum, and squamous metaplasia of the sebaceous glands which form keratinaceous comedones and cysts (Kimbrough, 1974).

A number of studies have been directed toward evaluating the mechanism(s) for the toxicity of 2,3,7,8-TCDD. Such studies will ultimately provide a better estimate of man's relative sensitivity to 2,3,7,8-TCDD and other compounds having a similar mode of action. Specifically, these studies may be able to explain the marked interspecies differences in relative sensitivity to 2,3,7,8-TCDD, and thus help establish man's relative sensitivity. These studies may also some day provide for the better treatment of human exposure to these toxins.

Pharmacogenetic studies have played an important role in understanding the biologic and toxic effects of drugs and xenobiotics. Nebert and co-workers have shown that carcinogenic polycyclic aromatic hydrocarbons (PAHs) induce the cytochrome P-450-dependent monooxygenase, aryl hydrocarbon hydroxylase (AHH), in certain responsive strains of mice (e.g., C57BL/6J, BALBc, C3HF/He), whereas this PAH induction activity is minimal or non-existent in non-responsive strains (DBA/2J) (Nebert, 1979, 1982; Nebert and Jensen, 1979; Nebert et al., 1981, 1983). The gene complex responsible for the induction of AHH and several other enzymes has been designated the Ah locus, which comprises regulatory, structural and possible temporal genes. Extensive studies on genetically inbred responsive and non-responsive mice (and their backcrosses) indicate that these differences are related to the Ah regulatory gene and its gene product, the Ah cytosolic receptor protein. This receptor protein interacts with PAH ligands and the resultant PAH:Ah receptor complex translocates into the nucleus and presumably initiates the induction of AHH via a process comparable to that proposed for the steroid hormones.

Since the carcinogenic and toxic effects of PAHs are dependent on their oxidative metabolism to reactive electrophilic forms, it is not surprising that the Ah receptor plays an important role in mediating their toxicity and carcinogenicity (Kouri, 1976; Kouri et al., 1974; Benedict et al., 1973; Shum et al., 1979; Thomas et al., 1973; Legraverend et al., 1980; Robinson et al., 1975; Mattison and Thorgeirsson, 1979). Responsive mice are more susceptible to the toxic (inflammation, fetotoxicity, primordial oocyte depletion) and carcinogenic effects of PAH at organs/tissues in direct contact with the applied chemical; in contrast, non-responsive mice are more susceptible to the tumorigenic effects of PAHs at tissue/organ sites remote

from the initial site of exposure to the PAHs. These differences in susceptibility are due to several factors including AHH-mediated toxication and detoxication.

Genetic studies also support the role of the Ah receptor in mediating the toxic and biologic effects of 2,3,7,8-TCDD. Initial studies by Poland and coworkers (Poland et al., 1974, 1983; Poland and Glover, 1975; Nebert et al., 1975; Poland and Knutson, 1982) demonstrated that the microsomal AHH-inducing activity of 2,3,7,8-TCDD and 3-methylcholanthrene (MC) in several genetically inbred mice strains were similar. Like MC and related PAHs, 2,3,7,8-TCDD induced AHH in several responsive mouse strains (e.g., C57BL/6J); in contrast to MC, 2,3,7,8-TCDD induced microsomal AHH in the DBA/2J non-responsive mice; however, the ED_{50} for this biologic response was significantly higher than values reported for the responsive mice. In genetic crosses between responsive C57BL/6 and non-responsive DBA/2 mice it was also shown for both MC and 2,3,7,8-TCDD that the trait of responsiveness is inherited in a simple autosomal dominant mode (Poland and Knutson, 1982). It has been suggested that the observed differences in the activities of MC and 2,3,7,8-TCDD are related to their relative Ah receptor affinities (Poland and Knutson, 1982) and pharamcokinetic and metabolic factors, which would more rapidly diminish the "available" concentrations of MC due to metabolism and excretion.

Several studies with 2,3,7,8-TCDD in genetically inbred mice support the receptor mediated hypothesis. The induction of UDP-glucuranosyl transferase, DT diaphorase, α-aminolevulinic acid, glutathione-S-transferase B, T-aldehyde dehydrogenase and choline kinase by 2,3,7,8-TCDD or MC in genetically inbred mice has also been shown to segregate with the Ah locus (Beatty and Neal, 1976a; Owens, 1977; Kirsch et al., 1975; Dietrich et al.,

1978; Ishidate et al., 1980; Poland and Glover, 1973). Toxicology studies with genetically inbred mice confirm the role of the Ah locus in mediating several toxic effects including porphyria, immunotoxicity, a wasting syndrome, thymic atrophy and cleft palate formation (Jones and Sweeney, 1980; Poland and Glover, 1980; Courtney and Moore, 1971; Vecchi et al., 1983). Poland et al. (1982) have also linked the tumor-promoting activity of 2,3,7,8-TCDD in hairless mice to the cytosolic receptor. In vitro studies with X8 cells in culture also support the role of receptor in mediating a dose-related cell keratinization by 2,3,7,8-TCDD which resembles some of the characteristics of chloracne (Knutson and Poland, 1980). This cell line is also responsive to AHH induction and contains a cytosolic receptor binding protein.

Although the murine Ah receptor has not been characterized, several studies confirm that a protein with high affinity for MC and 2,3,7,8-TCDD is present in low concentrations in the hepatic (~30-50 fmolar) and extrahepatic tissues of responsive C57BL/6J mice (Greenlee and Poland, 1979; Okey et al., 1979, 1980; Poland et al., 1976b; Mason and Okey, 1982; Gasiewicz and Neal, 1982; Okey and Vella, 1982; Okey, 1983; Nebert et al., 1983). Although the Ah receptor has not been detected in the cytosol of DBA/2J mice, after the administration of radiolabeled 2,3,7,8-TCDD to these mice, some of the radiolabel is detected in the nuclei of the non-responsive mice. Moreover, the sedimentation characteristics of the [^{3}H]-2,3,7,8-TCDD: nuclear protein complex in DBA/2J mice are similar to those observed with the bound Ah cytosolic receptor protein in C57BL/6J mice using a sucrose density gradient centrifugation separation technique (Okey, 1983). Several reports have also demonstrated that the cytosolic Ah receptor protein migrates into the nucleus of the cell only after binding with 2,3,7,8-TCDD

(Greenlee and Poland, 1979; Okey et al., 1979, 1980) and this parallels the observations noted for the interactions between steroids and their receptor proteins.

It should be noted, however, that the binding affinity and concentration of the cytosol receptor for 2,3,7,8-TCDD in liver from guinea pig, rat, C57BL/6 mouse, rabbit and hamster are very similar despite a 5000-fold difference in LD_{50} for 2,3,7,8-TCDD between the guinea pig and hamster (Poland and Knutson, 1982; Gasiewicz et al., 1983a). Thus the affinity and concentration of hepatic cytosol receptors does not alone explain the profound interspecies variability in sensitivity to TCDD.

In a subchronic study, Kociba et al. (1976) fed rats 0, 0.001, 0.01, 0.1 or 1.0 μg 2,3,7,8-TCDD/kg of body weight by gavage for 5 days/week for 13 weeks. The dosing at 1.0 μg/kg/day caused some mortality, lethargy, decreased body weights, liver pathology, biochemical evidence of liver damage, thymic atrophy, decreased lymphatic tissues, disturbances of porphyrin metabolism and slight alterations in the hematopoietic system. There was also evidence of mild adverse effects on the male and female reproductive systems. The effects on the reproductive system included decreased size of the testis and secondary sex organs in 2 of 5 males and uteri in 4 of 5 females. The 0.01 μg/kg/day level was considered by the authors to be the no-observed-adverse-effect level (NOAEL) and the 0.001 μg/kg/day level was the no-observed-effect level (NOEL) for this treatment regimen.

The dietary administration of 2,3,7,8-TCDD to rats at dose levels equivalent to 0, 0.001, 0.01 or 0.1 μg/kg/day for three generations (Murray et al., 1979) produced effects on liver, thymus and reproduction (discussed in

the Teratogenicity section) at 0.01 and 0.1 μg/kg/day. According to the authors, the 0.001 μg/kg/day exposure was a NOAEL (however, equivocal effects were noted in some generations at this dose).

Liver toxicity was the only effect of treatment observed by histologic examination of Osborne-Mendel rats and $B6C3F_1$ mice administered 2,3,7,8-TCDD for 13 weeks in a preliminary subchronic toxicity study designed to define an acceptable dose for a chronic toxicity study (U.S. DHHS, 1980b). The animals in groups of 10 males and 10 females were administered the compound in corn oil:acetone (9:1) twice a week at doses for rats of 0.0, 0.5, 1, 2, 4 and 8 μg/kg/week, and for mice at doses of 0.0, 1, 2, 5, 10 and 20 μg/kg/week. Deaths occurred at the two high dose levels in rats, with 4 females in the 8 μg/kg/week and 1 in the 4 μg/kg/week group dying, while only 2 male rats in the 4 μg/kg/week group died. Deaths were accompanied by severe toxic hepatitis. Hepatic lesions were observed in all other rats examined in groups administered 1-8 μg/kg/week; however, not all animals in each group were submitted to necropsy. Normal liver histology was observed in the two male rats examined from the low dose groups and only threshold toxic effects occurred in the low dose female rats.

Similar effects of treatment were observed in mice, with a single death occurring in each sex at the high exposure level along with reports of hepatic lesions on histologic examination. In contrast to rats, female mice were less sensitive to the hepatotoxic effect of 2,3,7,8-TCDD than were the male mice. Hepatic lesions were observed in all dose groups of male mice, while the 1 and 2 μg/kg/week dose groups of female mice had normal livers. Although the group sizes were small, making conclusions tenuous, it appeared that sex differences in the sensitivity to the toxic effects of 2,3,7,8-TCDD occurred, and that the more sensitive sex may vary with species tested.

In a more extensive subchronic study in rats, King and Roesler (1974) followed the development of toxicity by a series of interim sacrifices during 28 weeks of exposure to 2,3,7,8-TCDD and a 12-week, post-treatment recovery period. Groups of 35 male and 35 female Sprague-Dawley rats were intubated twice weekly with 2,3,7,8-TCDD in corn oil:acetone (9:1) at doses of 0.0, 0.1 and 1.0 μg/kg/week. No treatment-related deaths occurred; however, 3 animals from each group of each sex were killed after 2, 4, 8 and 16 weeks, and 10 animals of each sex were killed after 28 weeks of treatment. In addition, 3 rats of each sex were killed 4 and 12 weeks after termination of exposure. Animals were monitored for gross changes during the study and were examined for gross and histologic changes at necropsy.

Besides a dose-related decrease in body weight gain in male rats and a decrease in body weight gain in the high dose female rats, the only effect of exposure to 2,3,7,8-TCDD was histologic changes in the liver. Liver pathology was normal in all treated groups up through the interim kill at 10 weeks. Fatty changes in the liver were considered the most important observation and the data is summarized in Table 7. The fatty changes ranged from single large lipid droplets in a few centrilobular heptocytes to lipid droplets in all centrilobular hepatocytes with extension into the midzonal hepatocytes. No clear dose-response pattern was observed in this study; however, it did appear that the severity of fatty changes was greater in female rats. During the recovery period fatty changes progressively decreased in severity, but were still present in some treated animals 12 weeks after cessation of exposure. Other histologic changes observed in the liver of a small number of animals, predominantly in the animals killed at 28 weeks, included single cell or very small areas of necrosis, increased nuclear size, subtle distortion of liver architecture, and hyperchromatic

TABLE 7

Hepatocellular Fatty Change Observed in Rats Following Subchronic Exposure to 2,3,7,8-TCDD[a]

Treatment Group[b]	28 Weeks					4-Weeks Recovery					12-Weeks Recovery				
	N	S	Mi	Mo	Ma	N	S	Mi	Mo	Ma	N	S	Mi	Mo	Ma
Male Control	7/10	3/10	NS	NS	NS	2/3	1/3	NS	NS	NS	3/3	NS	NS	NS	NS
Males at 0.1 μg/kg	2/10	1/10	1/10	5/10	1/10	2/3	1/3	NS	NS	NS	2/3	NS	NS	1/3	NS
Males at 1.0 μg/kg	0/10	1/10	2/10	5/10	2/10	NS	1/3	1/3	1/3	NS	2/3	NS	NS	1/3	NS
Female Control	10/10	NS	NS	NS	NS	3/3	NS	NS	NS	NS	3/3	NS	NS	NS	NS
Females at 0.1 μg/kg	4/9	4/9	1/9	NS	NS	3/3	NS	NS	NS	NS	3/3	NS	NS	NS	NS
Females at 1.0 μg/kg	1/10	4/10	4/10	1/10	NS	1/3	1/3	NS	1/3	NS	1/4	3/4	NS	NS	NS

[a]Source: King and Roesler, 1974

[b]Animals were treated twice weekly by gavage with 2,3,7,8-TCDD dissolved in corn oil:acetone (9:1)

N = None
S = Slight: random hepatocyte containing a solitary, large lipid droplet-equivocal
Mi = Mild: several centrilobular hepatocytes contain lipid
Mo = Moderate: most centrilobular hepatocytes contain lipid
Ma = Marked: all centrilobular and some midzonal hepatocytes contain lipid
NS = Not specified

nuclei. All of these lesions were considered to be slight or mild, and less toxicologically relevant than the fatty changes. The data on hepatic steatosis indicated that the liver was a sensitive organ to the toxic effect of 2,3,7,8-TCDD, and although some recovery occurred after termination of treatment, the recovery process was slow and not complete by the time the study was terminated.

The recovery time was also demonstrated to be long in a subchronic study by Goldstein et al. (1982) of 2,3,7,8-TCDD-induced porphyria. Groups of eight female Sprague-Dawley rats were given 2,3,7,8-TCDD in corn oil:acetone (7:1) weekly by gavage for 16 weeks at doses of 0.0, 0.01, 0.1 or 10.0 μg/kg/week and killed 1 week after the last treatment. Additional groups of rats received doses of 0.0 or 1.0 μg/kg/week for 16 weeks and were allowed to recover for 6 months. The high dose level was lethal to all animals within 12 weeks, while the only other gross sign of toxicity was a decrease in body weight gain in the group receiving 1.0 μg/kg/week. After 16 weeks of exposure to 2,3,7,8-TCDD, liver porphyrins were elevated ~1000-fold in 7 of 8 animals receiving 1.0 μg/kg/week, but only 1 of 8 animals in the 0.1 μg/kg/week group had elevated porphyrin levels. No effect was observed in the low dose animals. After a 6-month recovery period the porphyrin level in animals exposed to 1 μg/kg/week was still 100-fold higher than values in the control group. A similar pattern was observed for urinary excretion of uroporphyrin. The rate limiting enzyme in heme synthesis, δ-aminolevulinic acid synthetase, was also elevated at both the time of termination of treatment and at the end of the recovery period; however, other enzymes that were increased after 10 weeks of treatment, cytochrome P-450, aryl hydrocarbon hydroxylase, and glucuronyl transferase, returned to near normal levels by 6 months. It was clear that a

6-month recovery period from subchronic exposure to 2,3,7,8-TCDD at a dose of 1.0 μg/kg/week was not sufficient for complete reversal of 2,3,7,8-TCDD-induced porphyria.

In rats, increased urinary porphyrin was also observed after subchronic exposure to 2,3,7,8-TCDD (Cantoni et al., 1981). Female CD rats were orally administered weekly doses of 2,3,7,8-TCDD at levels of 0.01, 0.1 and 1.0 μg/kg/for 45 weeks. The initial increase was observed in the high dose group at 3 months, and in the other two groups at 4 months, after the start of exposure. Not only did the absolute amount of porphyrin increase, but the relative distribution also changed to compounds containing more carboxyl groups. Only in the high dose group did the livers, at the terminal necropsy, show signs of excess prophyrin under examination by ultraviolet light.

The toxic effects, other than neoplasia, of long-term exposure to 2,3,7,8-TCDD have been studied in rats, mice and monkeys. The primary purpose of many of the studies in rodents was to assess the carcinogenicity of 2,3,7,8-TCDD. (These effects are discussed in detail in the Carcinogenesis section.) The observation of non-neoplastic systemic toxic effects in these studies was often limited, and observations were made near the end of the natural lifespan when conditions associated with aging may have obscured some effects produced by 2,3,7,8-TCDD. Table 8 summarizes the toxic effects of chronic exposure to 2,3,7,8-TCDD and provides information on the exposure levels which result in the observed effects.

Human health effects related to excessive exposures to 2,3,7,8-TCDD have been noted in several instances. However, in many of these cases it is difficult to quantify the exposure to 2,3,7,8-TCDD leading to the observed symptoms. Most of the exposures occurred in relation to the manufacture of

TABLE 8

Effects of Chronic Exposure to 2,3,7,8-TCDD in Laboratory Rodents

Species/ Strain	Sex/No.	Dose	Treatment Schedule	Duration of Study	Parameters Monitored	Effects of Treatment	Reference
Rat/Sprague-Dawley	M/10	0.0 ppt	NA	95 weeks	survival	40% survived until 95 weeks, the first death occurred at week 68	Van Miller et al., 1977a
	M/10	1 ppt	continuous in diet for 78 weeks	95 weeks	survival	80% survived until 95 weeks, the first death occurred at week 86	Same as above
	M/10	5 ppt	continuous in diet for 78 weeks	95 weeks	survival	60% survived until 95 weeks, the first death occurred at week 33	Same as above
	M/10	50 ppt	continuous in diet for 78 weeks	95 weeks	survival	60% survived until 95 weeks, the first death occurred at week 69	Same as above
	M/10	500 ppt	continuous in diet for 78 weeks	95 weeks	survival	50% survived until 95 weeks, the first death occurred at week 17	Same as above
	M/10	1000 and 5000 ppt	continuous in diet for 78 weeks	95 weeks	survival	No animals survived until 95 weeks, the first death occurred at week 31	Same as above
	M/10	50,000, 500,000 and 1,000,000 ppt	continuous in diet for 78 weeks	95 weeks	survival	No animals survived until 95 weeks, the first deaths occurred at weeks 2 and 3	Same as above
Rat/Sprague-Dawley	M&F/50&50	~2193 ppt (0.1 μg/kg/day)	continuous in diet for 2 years	2 years	extensive histopathology, hematology, urine analyses, and clinical chemistry	Cumulative mortality increased (F); Body weight gain decreased (M,F); Red blood cell count decreased (M,F); Packed cell volume decreased (M,F); Hemoglobin decreased (M,F); Reticulocytes increased (M,F); White blood cell count decreased (F); SGPT increased (F); G-Glutamyl transferase increased (F); Alkaline phosphatase increased (F); Urinary coproporphyrin increased (F); Urinary uroporphyrin increased (F); Urinary δ-aminolevulinic acid increased hepatic degeneration increased (M,F)	Kociba et al., 1978, 1979

TABLE 8 (cont.)

Species/ Strain	Sex/No.	Dose	Treatment Schedule	Duration of Study	Parameters Monitored	Effects of Treatment	Reference
Rat/Sprague-Dawley	M&F/50&50	~200 ppt (0.01 μg/kg/day)	continuous in diet for 2 years	2 years	extensive histopathology, hematology, urine analyses and clinical chemistry	Urinary coproporphyrin increased (F); Urinary uroprophyrin increased (f); Hepatic degeneration increased (M,F)	Kociba et al., 1978, 1979
Rat/Sprague-Dawley	M&F/50&50	~22 ppt (0.001 μg/kg/day)	continuous in diet for 2 years	2 years	extensive histopathology, urine analyses and clinical chemistry	No differences from values obtained from control animals	Same as above
Rat/Osborne-Mendel	M&F/75&75	0.0 μg/kg/week	NA	106 weeks	extensive histopathology	Toxic hepatitis; 0/74 (M), 0/75 (F)	U.S. DHHS, 1980b
Rat/Osborne-Mendel	M&F/50&50	0.5 μg/kg/week	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 14/50 (M), 32/50 (F)	Same as above
Rat/Osborne-Mendel	M&F/50&50	0.05 μg/kg/week	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	The incidence of toxic hepatitis was not elevated 0/50 (M), 1/50 (F)	Same as above
Rat/Osborne-Mendel	M&F/50&50	0.01 μg/kg/week	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	The incidence of toxic hepatitis was not elevated 1/50 (M), 0/50 (F)	Same as above
Mice/B6C3F1	M&F/75&75	0.0 μg/kg/week	NA	105-106 weeks	extensive histopathology	Toxic hepatitis; 1/73 (M), 0/73 (F)	Same as above
Mice/B6C3F1	M&F/50&50	0.5 μg/kg/week (M) 2.0 μg/kg/week (F)	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 44/50 (M), 34/47 (F)	Same as above
Mice/B6C3F1	M&F/50&50	0.05 μg/kg/week (M) 0.2 μg/kg/week (F)	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	The incidence of toxic hepatitis was not elevated 3/49 (M), 2/48 (F)	Same as above
Mice/B6C3F1	M&F/50&50	0.01 μg/kg/week (M) 0.04 μg/kg/week (F)	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	The incidence of toxic hepatitis was not elevated 5/44 (M), 1/50 (F)	Same as above

TABLE 8 (cont.)

Species/ Strain	Sex/No.	Dose	Treatment Schedule	Duration of Study	Parameters Monitored	Effects of Treatment	Reference
Mice/Swiss	M/38	0.0 µg/kg/week	NA	588 days	histology on all organs	Dermatitis and amyloidosis; 0/38	Toth et al., 1978, 1979
Mice/Swiss	M/44	0.007 µg/kg/week	administered by gavage weekly for 1 year	649 days	histology on all organs	Dermatitis and amyloidosis; 5/44	Same as above
Mice/Swiss	M/44	0.7 µg/kg/week	administered by gavage weekly for 1 year	633 days	histology on all organs	Dermatitis and amyloidosis; 10/44	Same as above
Mice/Swiss	M/43	7.0 µg/kg/week	administered by gavage weekly for 1 year	424 days	histology on all organs	Early mortality, dermatitis and amyloidosis; 17/43	Same as above

NA = Not applicable

2,4,5-trichlorophenol or 2,4,5-trichlorophenoxyacetic acid. The best known among these may be the release of 2,3,7,8-TCDD due to the 1976 explosion of a malfunctioning 2,4,5-trichlorophenol reactor in Seveso, Italy (Carreri, 1978). The area closest to the plant received exposures of up to 5000 $\mu g/m^2$ of soil; more remote areas received 1-75 $\mu g/m^2$, and within these areas 12 cases of chloracne developed (Reggiani, 1980). Other symptoms included acute dermatitis. All but the most severe cases of chloracne recovered within 26 months. No neurological, visceral or immune effects were noted in these reports.

Pocchiari et al. (1979) reported a more extensive study of the Seveso incident. These authors showed 75 cases of chloracne due to 2,3,7,8-TCDD exposure; 15 of those cases were severe or very severe. After 18-24 months, 19 cases had fully recovered while 1 case was still listed as severe. A subsequent survey in children by these authors uncovered an additional 137 cases of mild to serious chloracne. As indicated in the Pocchiari et al. (1979) report, signs of liver damage were also found after the Seveso incident in Italy. Raised serum transaminase and γ-glutamyl transferase levels were found in ~20% of the people living in or near the area of greatest deposition of 2,3,7,8-TCDD (Bert et al., 1976; Hay, 1976). In addition, some neurological effects were also noted among the exposed people. Immunological and cytogenetic investigations yielded normal results.

The most commonly reported symptom related to 2,3,7,8-TCDD exposure in man has been chloracne (Bauer et al., 1961; Kimmig and Schulz, 1957; Schulz, 1957; Firestone, 1977; Dugois and Colomb, 1956, 1957; Dugois et al., 1958; Bleiberg et al., 1964; Oliver, 1975; Poland et al., 1971; Kimbrough, 1980). The acneform lesions of the skin may develop a few weeks after the exposure and may persist for over 1 year following the cessation of exposure. Other

skin problems which have been reported include hyperpigmentation, hirsutism, increased skin fragility and vesicobullar eruptions on exposed areas of the skin.

Most cases of chloracne have been reported for industrial workers. Bauer et al. (1961) reported that 31 cases of chloracne occurred within a few months after a factory manufacturing 2,4,5-T near Hamburg, Germany, instituted a change in its industrial process. On this occasion, the investigators were able to show that the chloracne was not caused by purified 2,4,5-T, but that it was attributable to 2,3,7,8-TCDD which was a contaminant in the technical grade 2,4,5-T. A similar outbreak of chloracne affected 60 workers at a 2,4,5-T plant in Michigan in 1964 (Firestone, 1977).

In addition to chloracne, some occupational exposures to 2,3,7,8-TCDD have provided evidence for liver damage and for neurological effects. Hyperlipemia and hypercholesterolemia were reported (in addition to chloracne) for 17 workers at a plant producing trichlorophenol in France (Dugois and Colomb, 1956, 1957; Dugois et al., 1958). Among 29 subjects who exhibited chloracne after working in a plant which produced 2,4-D and 2,4,5-T in Newark, New Jersey, 11 had increased excretion of uroporphyrins. Three of these were diagnosed as porphyria cutanea tarda, and two of these also exhibited elevated serum glutamic-oxalacetic transaminase levels (Bleiberg et al., 1964; Firestone, 1977).

Pazderova-Vejlupkova et al. (1981) reported that 80 workers developed chloracne, nausea, fatigue and weakness in the lower extremities while engaged in the production of 2,4,5-sodium trichlorophenoxyacetate and trichlorophenoxyacetate butylester in a plant located in Prague, Czechoslovakia. Prominent clinical symptoms among 55 of the 80 workers included hypercholesterolemia, hyperlipemia and hyperphospholipemia, increased plasma

alpha and gamma globulins, and decreased plasma albumin. Porphyria cutanea tarda was observed in 11 of the 55 workers tested. In some cases, illness subsided while other cases became more severe during a 3 to 4-year follow-up period. Long-term pathological symptoms (remaining evident 5 years after exposure) include deviations in lipid metabolism, abnormal glucose tolerance, and high urinary excretion of uroporphyrins. Polyneuropathy, usually of the lower extremities, occurred during the period of illness and the symptoms remained after 4 years. Singer et al. (1982) also indicated a decrease in nerve conduction velocities of sural nerves in workers exposed to phenoxy acid herbicides (average exposure, 7 years) when compared to a similar group of non-exposed workers (40.3 m/sec in exposed vs. 42.8 m/sec in non-exposed, $p=0.02$). Although the causative agent was not known, dioxin contaminants are suggested.

Poland et al. (1971) re-examined all of the employees of the Newark factory in 1969, after the level of 2,3,7,8-TCDD in the trichlorophenol had been reduced from 10-25 mg/kg to <1 mg/kg. Chloracne was still found in 13 of 73 workers. A number of employees exhibited hyperpigmentation or facial hypertrichosis. No definite diagnoses of porphyria were made, and only one worker had a mild case of uroporphyrinuria. The authors suggested that chloracne and porphyria cutanea tarda are essentially independent syndromes.

In the Newark workers, Poland et al. (1971) noted that serum cholesterol was elevated in 10% of the cases and serum lactic dehydrogenase was elevated in 29% of the cases. Seven persons (10% of the workers) had a white blood cell count of <5000/mm^3. In addition, ~30% of the workers reported suffering from gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pains, blood in the stools); ~10% of the workers had other symptoms, such as weakness of the lower extremities, headaches and decreased auditory acuity.

Some hypomania was observed, with the degree of hypomania (as measured on the Minnesota Multiphasic Personality Inventory Hypomanic Scale) showing an association with the severity of chloracne.

Among the workers exposed in Hamburg, many also showed clinical signs of systemic toxicity, mainly muscular weakness, loss of appetite and weight, sleep disturbances, orthostatic hypotension, abdominal pain and liver impairment. Most of the workers presented psychopathological changes that were interpreted to be a specific syndrome (Bauer et al., 1961; Kimmig and Schulz, 1957; Schulz, 1957).

Telegina and Bikbulatova (1970) reported that the production of 2,4,5-T in the USSR began in 1964. At a specific site, 128 workers showed skin lesions, and among 83 examined, 69 had chloracne. Many, especially those with severe skin lesions, also presented evidence of liver impairment. In addition, 18 workers had a neurasthenic syndrome.

Similar findings were described by Jirasek et al. (1973, 1974), who reported 76 cases of chloracne following exposure to 2,3,7,8-TCDD between 1965 and 1968 in a plant in Czechoslovakia which produced 2,4,5-T and pentachlorophenol. Fifty-five of these cases were followed medically for over 5 years; some had symptoms of porphyria cutanea tarda, uroporphyrinuria, abnormal liver tests (elevated bilirubin levels, increased SGOT or SGPT activities, and elevated bromsulphthalein clearance times) and liver enlargement. The majority of the patients also suffered from neurasthenia and a depressive syndrome. In 17 persons, signs of a peripheral neuropathy, especially in the lower extremities, were confirmed by electromyographic examinations. More than half of the patients showed raised blood levels of cholesterol and total lipids.

Three scientists were poisoned in the course of an experimental preparation of 2,3,7,8-TCDD made by heating potassium trichlorophenate (Oliver, 1975). Two scientists developed typical chloracne 6 and 8 weeks after the exposure. Delayed symptoms, possibly due to 2,3,7,8-TCDD, developed ~2 years after the initial exposure in two of the scientists. These symptoms were personality changes, including loss of energy and drive, impairment of vision, taste and muscular coordination, disturbance of sleep, gastrointestinal symptoms and hirsutism. Hypercholesterolemia (in excess of 300 mg/100 mℓ) occurred in all three patients.

In November 1953, an accident occurred at a factory in Ludwigshafen, Federal Republic of Germany, during the manufacture of trichlorophenol (Goldman, 1972; Hofmann, 1957). As a consequence, 53 workers were affected by chloracne, 42 in a severe form. The son of one of the workers developed chloracne following contact with his father's clothes. Twenty-one of the 42 workers with severe chloracne showed signs of damage to internal organs or disturbances of the nervous system. The most relevant features were polyneuritis, sensory impairment and liver damage.

In a follow-up study of these workers, Theiss and Goldmann (1977) reported that of the 53 workers exposed to 2,3,7,8-TCDD in the 1953 accident, 22 were still working at the factory, 16 had retired and 15 had died (6 while still employed at the factory and 9 while in retirement). Of the 22 workers still employed, 2 still had acne of the face and scrotum, 1 had paralysis of the left leg and 1 had permanent loss of hearing. The remaining workers were well, except for scars left by the chloracne. Of the 15 deaths, 7 were from cardiovascular disease, 2 of which were myocardial infarcts, 1 due to mitral stenosis, 4 from cancer, 2 from suicide, 1 from necrotic pancreatitis and 1 from esophageal hemorrhage. The 16 men who had

retired and were still alive were well. No abortions or miscarriages were reported in the wives of exposed workers still employed at the factory.

2,3,7,8-TCDD has also been identified as the cause of an outbreak of poisoning in humans, horses and other animals in 1971 (Carter et al., 1975; Kimbrough et al., 1977). Exposure was related to the spraying of waste oil contaminated with 2,3,7,8-TCDD on riding arenas for dust control. The most severe effects occurred in a 6-year-old girl who played in the arena soil. Her symptoms included headache, nosebleeding, diarrhea, lethargy, hemorrhagic cystitis and focal pyelonephritis. Three other children and one adult who were frequently in the arena complained of skin lesions. In two of the children, the described lesions were similar to chloracne.

Exposure to 2,3,7,8-TCDD and other dioxins occurring as contaminants in Agent Orange have been associated with many health effects reported in veterans and residents of Vietnam. Symptoms include numbness of extremities, skin rashes and irritation, liver dysfunction, weakness, loss of sex drive, and psychological changes (Holden, 1979).

The toxic effects attributed to 2,3,7,8-TCDD exposure were studied over a 10-month period in a group of 78 Vietnam veterans who claimed to have been exposed to Agent Orange (Bogen, 1979). Symptoms reported by the veterans included gastrointestinal complaints (anorexia, nausea, diarrhea, constipation, abdominal pain), joint pain and stiffness, and neurological complaints (numbness, dizziness, headaches, depression and bouts of violent rage). It is mentioned that these patients had previously been chronically ill and had frequent infections and allergies. This study was apparently based on personal evaluations of health in a survey-type format. No control group was used for comparison and no clinical or medical evaluations of health were made. Most of these complaints were non-specific, judgmental and occur commonly in the general public.

In an effort to evaluate the toxic effects attributed to 2,3,7,8-TCDD as a contaminant of Agent Orange, Stevens (1981) established a minimum toxic dose of 2,3,7,8-TCDD and determined the amount of this contaminant to which veterans may have been exposed during Agent Orange spraying. Based on studies in which rhesus monkeys were fed small amounts of dietary 2,3,7,8-TCDD and analogy with human data on the minimum toxic dose of 2,3,7,8-tetrachlorodibenzo-p-furan (TCDF), the cumulative minimum toxic dose of 2,3,7,8-TCDD in man was calculated to be 0.1 μg/kg. Based on application rates (4.1 g Agent Orange/m²) and 2,3,7,8-TCDD concentration in the herbicide (2 ppm), the average concentration of TCDD on sprayed surfaces of Vietnam was ~8 μg/m². Based on a comparison of the development of toxic systems in humans exposed to 2,3,7,8-TCDD in the Seveso accident and a child exposed in an Eastern Missouri horse arena, the measured environmental levels of 2,3,7,8-TCDD, and estimates of the absorbed dose necessary to produce these symptoms, the author calculated an average intake transfer factor (ratio of absorbed compound to environmentally available compound) of 1:2050 for 2,3,7,8-TCDD. Assuming this absorption-to-exposure ratio and even assuming that a soldier was directly sprayed (exposed to 8 μg/m²) for each day of his 1 year service in Vietnam, his cumulative intake would be only 1.4 μg or 0.02 μg/kg of 2,3,7,8-TCDD. Based on these calculations, Stevens (1981) reports that 5 years of direct daily contact with Agent Orange would be necessary to reach a toxic level of 2,3,7,8-TCDD and feels that claims of illness caused by 2,3,7,8-TCDD in Agent Orange are without merit. Exception is made, however, for certain workers (forest industries) who could be exposed to 2,4,5-T and 2,3,7,8-TCDD for many years.

Synergism and/or Antagonism

2,3,7,8-TCDD elicits diverse toxic and biologic effects and therefore can be expected to alter the activities of other chemicals. For example, many compounds, including 2,3,7,8-TCDD, which induce drug-metabolizing enzymes or act as cancer promoters, can greatly influence the activity of other carcinogens and toxins. This type of interaction can result in non-additive effects which could be called synergism or antagonism. However, when the mechanism of action of the interacting chemical is different (such as initiators and promoters of carcinogenesis), the interaction effects should be called modulation. Thus a cancer promoter modulates the effects of a carcinogen. An example of a synergistic or antagonistic effect would occur when two chemicals that elicit the same toxic effect are coadministered and the resultant magnitude of the toxic response is non-additive. It is clear that 2,3,7,8-TCDD interacts with chemicals via modulation and synergism/ antagonism as indicated below.

Results from several cocarcinogenicity studies appear to give only limited support to the modulating effect of 2,3,7,8-TCDD. The DBA/2N mouse strain, which responds only weakly to the sarcomatogenic action of MC, becomes susceptible after treatment with 2,3,7,8-TCDD (Kouri, 1976; Kouri and Nebert, 1977). As an extension of this study, Kouri et al. (1978) demonstrated in two inbred strains of mice, C57BL/6Cum and DBA/2Cum, that administration of 2,3,7,8-TCDD simultaneously with MC appears to enhance the carcinogenic response. The authors concluded that their results suggest that 2,3,7,8-TCDD acts as a cocarcinogen, possibly as an inducer of AHH at the site of inoculation. In contrast, when mice were painted with 1 μg 2,3,7,8-TCDD prior to 7,12-dimethylbenz(a)anthracene (DMBA) initiation and 12-O-tetradecanoylphorbol-13-acetate (TPA) promotion, tumor formation was

inhibited (Berry et al., 1979). The greatest degree of inhibition (94%) was seen when the 2,3,7,8-TCDD was applied 5 days prior to initiation by DMBA; when pretreatment was at 3 days and 1 day, the inhibition was 86 and 3%, respectively.

2,3,7,8-TCDD did not promote the carcinogenicity of DMBA in a two-stage skin tumorigenesis assay with CD1 female mice (Berry et al., 1979). 2,3,7,8-TCDD was applied twice weekly for 30 weeks at 0.1 μg in acetone to both DMBA treated and untreated control rats. No papillomas were observed in either group.

The recent results from the NCI carcinogenesis testing program (U.S. DHHS, 1980a) indicate that in female Swiss-Webster mice the incidence of fibrosarcoma in the integumentary system is higher when 2,3,7,8-TCDD is applied alone or following DMBA application than in the control. The incidence between the two experimental groups is comparable. This report has been discussed in detail in the carcinogenicity section of this document.

In order to test the potential of 2,3,7,8-TCDD as a promoter of hepatocarcinogenesis, rats which had received a single 10 mg/kg dose of diethylnitrosamine (DEN) following partial hepatectomy were given 2,3,7,8-TCDD (0.14 and 1.4 μg/kg s.c. once every 2 weeks) for 7 months. Animals which received (a) only a single initiating dose of DEN after partial hepatectomy and no further treatment, or (b) 2,3,7,8-TCDD alone with no initiating dose of DEN exhibited relatively few enzyme-altered foci and no hepatocellular carcinomas. However, animals initiated with DEN and then given 2,3,7,8-TCDD had a marked increase in enzyme-altered foci. At the higher dose of 2,3,7,8-TCDD, hepatocellular carcinomas were present in 5 of 7 rats. By means of three different enzyme markers used to evaluate the phenotypes of the enzyme-altered foci, a distinct phenotype heterogeneity of the foci was

noted with a shift towards phenotypes exhibiting a greater deviation from normal liver when 2,3,7,8-TCDD was given following DEN-partial hepatectomy. Quantitation of the numbers of enzyme-altered foci was performed by relating measurements made from two-dimensional tissue sections to the number of foci per unit volume of liver using relationships established in the field of stereology. The total volume of the liver occupied by the enzyme-altered foci, but not their number, increased with the dose of 2,3,7,8-TCDD administered following DEN-partial hepatectomy. These studies demonstrate that 2,3,7,8-TCDD is a potent promoting agent for hepatocarcinogenesis (Pitot et al., 1980).

DiGiovanni et al. (1979) noted an inhibitory effect of 2,3,7,8-TCDD on mice initiated with benzo(a)pyrene [B(a)P] and promoted with TPA. Again, the greatest inhibition of skin tumor formation (65%) was seen when 2,3,7,8-TCDD was applied 5 days prior to B(a)P initiation. Inhibition was 57 and 13% at 3 and 1 days pretreatment, respectively. DiGiovanni et al. (1979) and Berry et al. (1979) suggested that this anticarcinogenic effect was related to the ability of 2,3,7,8-TCDD to induce monooxygenase systems of the skin.

2,3,7,8-TCDD pretreatment has been observed to modify the effects of barbituates and other xenobiotics (Greig, 1972). Adult male Porten rats were given a single oral dose of 200 μg 2,3,7,8-TCDD/kg bw 1-3 days preceding treatment with 100 mg/kg zoxazolamine hydrochloride or 150 mg/kg hexabarbitone sodium. 2,3,7,8-TCDD pretreatment resulted in a 54% decrease in the duration of the paralysis induced by zoxazolamine and a 2-fold increase in the sleeping time produced by hexabarbitone.

The synergistic or antagonistic effects of chemical combinations have not been well documented. A recent report compares the immunotoxicity of 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 2,3,7,8-TCDF plus 2,3,7,8-TCDD (coadministered) (Rizzardini et al., 1983). Seven days after administration of 1.2 μg/kg of 2,3,7,8-TCDD to C57BL/6J mice, sheep red blood cells were injected by i.p. administration and plaque-forming cells (PFC) in the spleen were counted 5 days later. 2,3,7,8-TCDD inhibited antibody production by 80%. In a parallel study, a dose of 2,3,7,8-TCDF was administered (10 μg/kg) and no significant immunotoxic effects were observed. Coadministration of 2,3,7,8-TCDD (1.2 μg/kg) plus 2,3,7,8-TCDF (10 μg/kg) resulted in 50% reduction in antibody production and demonstrates a significant antagonistic effect by 2,3,7,8-TCDF. Coadministration of these two isostereomers resulted in antagonistic effects with respect to the induction of hepatic microsomal cytochrome P-450 and 7-ethoxycoumarin O-deethylase. Sweeney et al. (1979) found that iron deficiency protected mice against the development of hepatocellular damage (including porphyria) normally caused by 2,3,7,8-TCDD exposure. In contrast, the teratogenic and fetoxic data reported by Neubert and Dillmann (1972) and Courtney and coworkers (see Teratogenicity section) suggests that coadministration of phenoxy herbicides and 2,3,7,8-TCDD may also result in synergistic effects.

Teratogenicity

Courtney et al. (1970a,b) were the first to report that 2,4,5-T was capable of causing teratogenic effects in rats and mice. In these studies, rats and two strains of mice were exposed s.c. or orally to 2,4,5-T containing 30 ppm 2,3,7,8-TCDD. The mixture was teratogenic and fetotoxic to mice at ≥46.4 mg/kg. Rats were more sensitive, exhibiting fetotoxic responses at 10 mg/kg for this 2,4,5-T/2,3,7,8-TCDD mixture. Since this initial report,

research has focused on determining the role of 2,3,7,8-TCDD contamination in eliciting the teratogenic response. These studies are summarized in Table 9.

Neubert and Dillmann (1972) conducted a detailed study to determine the significance of 2,3,7,8-TCDD contamination. These investigators assayed three 2,4,5-T samples: a highly purified sample containing <0.02 ppm 2,3,7,8-TCDD (referred to as Sample A), a purified sample identical to that used by Roll (1971) that contained 0.05±0.02 ppm 2,3,7,8-TCDD (Sample B), and a commercial sample containing an undetermined quantity of 2,3,7,8-TCDD (Sample C). All three samples induced cleft palates in NMRI mice at sufficiently high doses. In terms of the number of fetuses with cleft palate/the total number of fetuses, the dose/response pattern observed by Neubert and Dillmann (1972) was similar to that observed by Roll (1971) using a similar grade of 2,4,5-T. In addition to the three 2,4,5-T samples, Neubert and Dillmann (1972) also assayed a sample of 2,3,7,8-TCDD alone and in various combinations with the highly purified sample of 2,4,5-T. This approach allows at least partial quantification of the significance of 2,3,7,8-TCDD contamination in 2,4,5-T-induced cleft palates. When the litter is used as the basic experimental unit, the incidences of cleft palate (number of litters with cleft palate/total numbers of litters) versus the dose can be plotted on log dose/probit response paper, correcting for central response using Abbott's equation. According to this method, the ED_{50} (by eye-fit) for cleft palate induction are:

2,3,7,8-TCDD:	4.6 μg/kg bw
2,4,5-T (Sample A):	115 mg/kg bw
2,4,5-T (Sample B):	46 mg/kg bw

TABLE 9

Studies on the Potential Teratogenic Effects of 2,3,7,8-TCDD-Contaminated 2,4,5-T

Species/Strain	Vehicle	Form of 2,4,5-T	TCDD Level	Daily Dose	Treat-ment Days	Obser-vation Day	Maternal Response	Fetal Response	Reference
Mice/NMRI	Rape-seed oil	acid	<0.02 ppm (Sample A)	0, 15, 30, 45, 60, 90 and 120 mg/kg	6-15	18	No toxic effects; decreased maternal weight at doses of 90 mg/kg and greater	Significant increases in the incidence of cleft palates at doses above 30 mg/kg (see text for additional details). Significantly decreased ($p<0.005$) fetal weight at all dose levels.	Neubert and Dillmann, 1972
Mice/NMRI	Rape-seed oil	acid	0.05±0.02 ppm (Sample B)	30, 60 and 90 mg/kg	6-15	18	No toxic effects; decreased maternal weight at 90 mg/kg	Increases in the incidence of cleft palate at 60 and 90 mg/kg; significantly decreased ($p<0.005$) fetal weight at all dose levels	Same as above
Mice/NMRI	Rape-seed oil	acid	NR (Sample C)	90 mg/kg	6-15	18	No toxic effects but decreased maternal weight	Increase in the incidence of cleft palate; significant ($p<0.005$) decrease in fetal weight	Same as above
Mice/NMRI	Rape-seed oil	butyl ester	NR	12 and 17 mg/kg	6-15	18	No toxic effects	Significant decrease in fetal weight but no effect on mortality; increase in the frequency of cleft palate similar to that seen with acid (see text)	Same as above
Mice/NMRI	NR	acid	0.05±0.02 ppm	20, 35, 60, 90 and 130 mg/kg	6-15	NR	Toxic effects observed at 90 and 130 mg/kg	Increases in the percentage of resorptions and/or dead fetuses at 90 and 130 mg/kg; increases in the incidence of cleft palate and retardation of skeletal development at 35 mg/kg and above	Roll, 1971
Mice/CD-1	Corn oil:acetone (9:1)	acid	<0.05 ppm	115 mg/kg	10-15	18	No significant effect on weight gain or liver-to-body weight ratios	No effect on fetal mortality or fetal weight but an increase in the incidence of cleft palate	Courtney, 1977

TABLE 9 (cont.)

Species/Strain	Vehicle	Form of 2,4,5-T	TCDD Level	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Mice/C57BL/6	Honey:water (1:1)	acid	30 ppm	46.4 and 113 mg/kg	6-14	18	NR	Significant ($p<0.01$) increases in the incidence of cleft palate in the high dose group and cystic kidney in both dose groups; increased fetal mortality also observed in the high dose group	Courtney et al., 1970a,b
Mice/AKR	Honey:water (1:1)	acid	30 ppm	113 mg/kg	6-15	19	Increase in liver-to-body weight ratio	Significant ($p<0.05$) increases in the incidence of cleft palate and fetal mortality	Same as above
Rat/Sprague-Dawley (groups of 25 rats)	Gavage/hydroxy-propyl-methyl-cellulose	acid	0.5 ppm	1, 3, 6, 12 or 24 mg/kg/day	6-15	20	No effect on body weight and no observable signs of toxicity	A slight but statistically significant ($p<0.05$) decrease in implantations and litter size in lowest dose group only; no frank teratogenic effects based on a detailed examination of the control and 24 mg/kg dose group; the only effect noted was an increase in the incidence of 5th partially ossified sternebrae	Emerson et al., 1970, 1971 This appears to be a full publication of the abstract summary by Thompson et al., 1971
Rat/Wistar	Gavage/aqueous gelatin or corn oil	acid	<0.5 mg/kg	25, 50, 100 or 150 mg/kg/day	6-15	22	Some maternal mortality and decreased body weight gain at 150 mg/kg; no signs of toxicity at 100 mg/kg or below	At 100 or 150 mg/kg decreased fetal weight, increased fetal mortality and an increase in the incidence of skeletal anomalies; no significant effect at the two lower dose levels	Khera and McKinley, 1972; Khera et al., 1971
Rat/Wistar	Gavage/aqueous gelatin or corn oil	butyl ester	<0.5 mg/kg	50 or 150 mg/kg/day	6-15	22	NR	No significant effect on fetal mortality, fetal weight, or the incidence of anomalies	Khera and McKinley, 1971; Khera et al., 1971

TABLE 9 (cont.)

Species/Strain	Vehicle	Form of 2,4,5-T	TCDD Level	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Rat/Holtzman	Gavage/1:1 solution of honey and water	acid	30 ppm	4.6, 10.0 and 46.4 mg/kg/day	10-15	20	NR	Significant ($p<0.01$) increases in fetal mortality at the two higher dose levels; dose related increases in the percent of abnormal fetuses per litter; a high incidence of cystic kidneys in treated groups	Courtney et al., 1970a,b
Rat/CD	Gavage/15% sucrose solution	acid	0.5 ppm	10.0, 21.5, 46.4 and 80.0 mg/kg/day	6-15	20	Reduced maternal weight gain at the two higher dose levels ($p<0.05$) and increased liver-to-body weight ratio at the highest dose level ($p<0.05$)	Increase in the incidence of kidney anomalies, but no increase in cleft palate	Courtney and Moore, 1971
Rat/Strain not specified	Gavage/methocel	acid	0.5 ppm	50 mg/kg	6-15	NS	No effect on mortality or body weight gain	No significant effect on fetal mortality or fetal weight; a significant ($p<0.05$) increase in the incidence of delayed ossification	Sparschu et al., 1971a
Rat/Strain not specified	Gavage/methocel	acid	0.5 ppm	100 mg/kg	6-10	NS	Increased mortality and decreased body weight gain	Increase in the incidence of delayed ossification and poorly ossified or malaligned sternabrae ($p<0.05$)	Same as above
Syrian hamster/ *Mesocricetus euratus*	Gavage/acetone, corn oil and carboxymethyl cellulose in ratio of 1:5.8:10	acid	<0.1-4.5 ppm	20, 40, 80 and 100 mg/kg	6-10	14	NS	Dose-related increases in fetal mortality, gastrointestinal hemorrhages and fetal abnormalities; see text for discussion of effect of TCDD level on development	Collins et al., 1971

NS = Not specified; NR = not reported

If the assumption were made that all teratogenic activity in the 2,4,5-T samples were attributable to 2,3,7,8-TCDD contamination, the expected ED_{50} for Samples A and B would be 230,000 mg/kg (0.0046 mg/kg x 0.02 ppm^{-1}) and 92,000 mg/kg (0.0046 mg/kg x 0.05 ppm^{-1}), respectively. Since the observed ED_{50} was lower by a factor of over 1000, 2,3,7,8-TCDD appears not to be the sole factor in 2,4,5-T-induced cleft palate.

The nature of possible interaction between 2,4,5-T and 2,3,7,8-TCDD is more difficult to define. Based on assays of five mixtures of 2,3,7,8-TCDD and the highly purified 2,4,5-T, Neubert and Dillmann (1972) noted a greater than additive effect on the induction of cleft palates. A similar conclusion can be reached if one assumes that Sample A was a "totally pure" sample of 2,4,5-T. According to the assumption simple similar action (Finney, 1971) and by treating Sample B as a mixture of 2,3,7,8-TCDD and 2,4,5-T, the expected ED_{50} for Sample B would be 119.8 mg/kg. The observed value of 46 mg/kg again suggests a greater than additive effect. A more detailed statistical analysis of these data, however, would be required to support the assumptions of simple similar action or independent joint action that are implicit in these analyses. Furthermore, the inability to define precisely the levels of 2,3,7,8-TCDD in the 2,4,5-T samples and the possible significance of other contaminants would preclude an unequivocal interpretation of the results of the analysis.

Nevertheless, three of the studies summarized in Table 6 (Neubert and Dillmann, 1972; Roll, 1971; Courtney, 1977) have demonstrated the induction of cleft palate in mice by using 2,4,5-T samples containing 2,3,7,8-TCDD levels of 0.05±0.02 ppm or less. Although 2,3,7,8-TCDD contamination is undoubtedly a factor in the teratogenic activity of 2,3,7,8-TCDD contaminated 2,4,5-T, the above analysis suggests that 2,3,7,8-TCDD contamination is not

the sole factor, and that some teratogenic activity must be attributed to 2,4,5-T itself or other contaminants in 2,4,5-T.

Effects on reproductive success and fertility have also been studied in four groups of C57BL/6 male mice (25 animals/group) following oral ingestion of mixtures of 2,4-D, 2,4,5-T and 2,3,7,8-TCDD. Daily doses of ~40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T and 2.4 μg/kg 2,3,7,8-TCDD in Group II; 20 mg/kg 2,4-D, 20 mg/kg 2,4,5-T and 1.2 μg/kg 2,3,7,8-TCDD in Group III; and 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T and 0.16 μg/kg 2,3,7,8-TCDD in Group IV animals were given. Vehicle control animals in Group I had corn oil added to the feed. At the conclusion of an 8-week dosing period, treated animals were mated to untreated virgin females. No significant decrease in fertility, reproduction and germ cell toxicity were noted. Survival of offspring and the development of the newborns apparently were not affected (Lamb et al., 1980).

Courtney and Moore (1971) tested a purified sample of 2,3,7,8-TCDD for teratogenic potential. A summary of this study and others assessing the teratogenic potential of purified 2,3,7,8-TCDD are presented in Table 10. CD-1, DBA/2J and C57Bl/6J mice were given s.c. injections of 2,3,7,8-TCDD at 1 or 3 μg/kg/day on days 6-15 of gestation in the study by Courtney and Moore (1971). This dose regime did not result in maternal toxicity, although an increase in the maternal liver/bw ratio was observed in DBA/2J and C57Bl/6J mice. 2,3,7,8-TCDD had no measurable effect on fetal mortality; however, anatomical abnormalities were observed in all strains and at all dose levels, with C57Bl/6J being the most sensitive strain. The abnormalities observed were cleft palate and unspecified kidney anomalies.

TABLE 10

Studies on the Potential Teratogenic Effect of 2,3,7,8-TCDD

Species/Strain	Vehicle	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Mouse/C57BL/6 Mouse/AKR	DMSO or honey:water (1:1)	21.5, 46.4, 113.0 mg/kg	6-14 or 9-17	19[a]	increased liver/body weight ratio	fetocidal, cleft palate, cystic kidney	Courtney et al., 1970b
Mouse/CD-1 Mouse/DBA/2J Mouse/C57Bl/6J	DMSO	0.5, 1, 3 μg/kg	6-15	17[a] or 18	increased liver/body weight ratio	cleft palate, kidney anomalies	Courtney and Moore, 1971
Mouse/C57Bl/6	Acetone: corn oil (1:9)	1, 3 μg/kg	10-13 or 10	18[a]	none reported	cleft palate, kidney anomalies	Moore et al., 1973
Mouse/CD-1	DMSO or corn oil	25, 50, 100, 200, 400 μg/kg	7-16	18[b]	increased liver/ body weight ratio	cleft palate, hydronephrotic kidneys, hydrocephalus, open eyes, edema, petechiae	Courtney, 1976
Mouse/CF-1	corn oil: acetone (98:2)	0.001, 0.01, 0.1, 1.0, 3.0 μg/kg	6-15	18[a]	none reported	cleft palate, dilated renal pelvis	Smith et al., 1976
Mouse/NMRI	rape-seed oil	0.3, 3.0, 4.5, 9.0 μg/kg	6-15	18	no effect observed	fetocidal at the high dose, cleft palate at doses at or above 5 μg/kg	Neubert and Dillmann, 1972
Rat/CD	DMSO	0, 0.5, 2.0 μg/kg	6-15, 9 and 10, or 13 and 14	20[a]	none reported	kidney malformations at both dose levels	Courtney and Moore, 1971
Rat/Sprague-Dawley	corn oil/ acetone	0, 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg	6-15	20[a]	vaginal hemorrhage at 2.0 and 8.0 μg/kg	intestinal hemorrhage at 0.125 and 0.5 μg/kg, fetal death at higher doses, subcutaneous edema	Sparschu et al., 1971b
Rat/Wistar	corn oil/ anisole	0.0, 0.125, 0.25, 1, 2, 4, 8, 16 μg/kg	6-15	22	maternal toxicity observed at or above 1 μg/kg	increased fetal death observed at or above 1 μg/kg, subcutaneous edema and hemorrhages in the 0.25-2 μg/kg groups	Khera and Ruddick, 1973

TABLE 10 (cont.)

Species/Strain	Vehicle	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Rat/Sprague-Dawley	corn oil/ acetone (9:1)	0.1, 0.5, 2.0 μg/kg	1-3	21	decrease in body weight gain in the high dose group	decreased fetal weight in the 0.5 and 2 μg/kg group	Giavini et al., 1982a
Rat/Sprague-Dawley	diet	0.001, 0.01 and 0.1 μg/kg[c]	throughout gestation	post-parturition	low fertility at 0.01 and 0.1 μg/kg decreased body weight at 0.01 and 0.1 μg/kg dilated renal pelvis	low survival at 0.01 and 0.1 μg/kg, decreased body weight at 0.01, slight dilated renal pelvis at 0.001 μg/kg in the F_1 but not succeeding generations	Murray et al., 1979
Rabbit/ New Zealand	corn oil/ acetone (9:1)	0.0, 0.1, 0.25, 0.5 and 1 μg/kg	6-15	28	maternal toxicity at doses of 0.25 μg/kg and above	increases in extra ribs and total soft tissue anomalies	Giavini et al., 1982b

[a]First day of gestation designated day zero

[b]First day of gestation designated day one

[c]The high dose level (0.1 μg/kg/day) was discontinued due to very low fertility in adults

Moore et al. (1973) treated pregnant C57Bl/6 mice with an oral dose of 2,3,7,8-TCDD at 1 or 3 μg/kg/day on days 10-13 of gestation, or 1 μg/kg on day 10 of gestation. At the high dose level, the average incidence of cleft palate was 55.4%. Kidney anomalies (hydronephrosis) were observed on an average of 95.1% of the fetuses/litter, with 83.1% having bilateral kidney anomalies. When the dose was decreased to 1 μg/kg/day, the average incidence of cleft palate dropped to 1.9%; however, the incidence of kidney anomalies remained relatively high, with an average incidence of 58.9%. On the average, bilateral kidney anomalies occurred in 36.3% of the fetuses/litter. A single dose of 1 μg/kg on day 10 of gestation produced kidney anomalies in 34.3% of the fetuses; however, no cleft palates were observed. When C57Bl/6 mice were treated with 1 μg/kg on day 10 of gestation and were then allowed to litter, the detection of kidney lesions on postnatal day 14 was found to depend largely on whether the pups nursed on a 2,3,7,8-TCDD-treated mother. When pups from a 2,3,7,8-TCDD-treated mother nursed on control mice, kidney anomalies were found in only 1/14 litters. In contrast, when pups from control mothers nursed on 2,3,7,8-TCDD-treated mice, kidney anomalies were observed in 4/14 litters. In the pups exposed to 2,3,7,8-TCDD both in utero and during the postnatal period, kidney anomalies were observed in 5/7 litters. Kidney anomalies observed following in utero exposure or exposure through the milk were similar, and these kidney anomalies may not be considered a purely teratogenic response.

Neubert et al. (1973) reviewed what was known of the embryotoxic effects of 2,3,7,8-TCDD in mammalian species. Also reported were their own studies and previous work (Neubert and Dillmann, 1972) using NMRI mice, in which cleft palate was observed to be a common abnormality; however, no kidney

anomalies were reported. Neubert and Dillmann (1972) administered 2,3,7,8-TCDD by gavage to 20 female mice on days 6 through 15 of gestation at doses of 0.3, 3.0, 4.5 and 9.0 μg/kg. At day 18 of gestation, extensive reabsorption was observed in the high dose group with 6/9 litters totally resorbed. In the few surviving fetuses, there was an 81% incidence of cleft palate. At lower doses, there were 9 and 3% incidences at doses of 4.5 and 3.0 μg/kg, respectively, and no cleft palates were observed in 138 fetuses examined in the 0.3 μg/kg group. Fetal mortality was increased at the 9.0 μg/kg dose if animals were treated only on days 9 through 13; however, the incidence of cleft palate remained high at a frequency of 60%. In a series of experiments to determine the time of gestation at which 2,3,7,8-TCDD was effective in inducing cleft palate, mice were treated for a single day between days 7 and 13 of gestation with 2,3,7,8-TCDD at a dose of 45 μg/kg. A maximum number of inducted cleft palates occurred when animals were treated on either day 8 or 11 of gestation, while exposure to 2,3,7,8-TCDD after day 13 of gestation produced no cleft palates in the fetuses.

Courtney (1976) compared the teratogenic potential of 2,3,7,8-TCDD administered orally with 2,3,7,8-TCDD administered s.c. CD-1 mice were dosed with 2,3,7,8-TCDD on days 7 through 16 of gestation at levels of 25, 50, 100, 200 or 400 μg/kg/day; the 400 μg/kg dose was not used in animals treated by s.c. injection. Doses of 200 or 400 μg/kg/day produced vaginal bleeding and high rates of abortion. A dose of 100 μg/kg/day was fetotoxic, resulting in decreased fetal weight and survival. Anatomic abnormalities were observed at all dose levels, with cleft palate and hydronephrotic kidneys being most common. Other abnormalities observed included hydrocephalus, open eye, edema and petechiae. Subcutaneous administration of 2,3,7,8-TCDD produced a greater teratogenic response at a lower dose than

oral administration, with abnormalities observed in 87% of the fetuses following s.c. administration and 42% after oral administration of a dose of 25 μg/kg/day.

The effects of 2,3,7,8-TCDD on the incidence of fetal anomalies were also studied by Smith et al. (1976) in CF-1 mice. The mice were given 0.001-3.0 μg 2,3,7,8-TCDD/kg/day by gavage from day 6 through 15 of gestation. The incidence of cleft palate was found to be significantly increased in 1.0 and 3.0 μg/kg/day dose groups, and the incidence of kidney anomalies was significantly increased at 3.0 μg/kg/day. There were no observable teratogenic effects in the study at 0.1 μg/kg/day; however, some were noted at lower dose levels, although not statistically significantly elevated.

Poland and Glover (1980) compared cleft palate formation by 2,3,7,8-TCDD in the responsive C57BL/6J, the non-responsive DBA/2J and the hybrid $B6D2F_1/J$ strains of mice. Female mice were mated with male mice of the same genetic strain and on day 10 of pregnancy the pregnant mice were given a single s.c. dose of 3.0, 10.0 or 30.0 μg/kg of 2,3,7,8-TCDD dissolved in p-dioxane or the solvent (control) alone (0.4 mℓ/kg). On day 18 the animals were killed and the number of cleft palates and resorbed fetuses was determined. At doses of 3.0 and 10.0 μg/kg of 2,3,7,8-TCDD, cleft palates (3% incidence among live fetuses) were only observed in the C57BL/6J mice at the higher dose level. At a dose of 30 μg/kg, the incidence of cleft palates among live fetuses for the C57BL/6J, $B6D2F_1/J$ and DBA/2J mice was 54, 13 and 2%, respectively. This study also reported that cleft palate formation was significantly higher in several other responsive mouse strains compared with non-responsive mice. At a dose level of 30 μg/kg of

2,3,7,8-TCDD, the incidence of cleft palates among live fetuses for the responsive C57BL/6J, A/J, BALB/cByJ and SEC/1REJ mice was 54, 73, 65 and 95%, respectively. The only responsive mouse (CBA/J) strain that was resistant to 2,3,7,8-TCDD-mediated cleft palate was also resistant to the teratogenic effects of cortisone. In contrast, the incidence of cleft palates in the non-responsive DBA/2J, RF/J, AKR/J, SWR/J and 129/J mice was between 0-3% at the 30 μg/kg dose level. Thus the responsive mice, containing high levels of the Ah receptor, are highly susceptible to the effects of 2,3,7,8-TCDD in producing cleft palate, whereas the non-responsive mice, which contain low (or 0) levels of the Ah receptor protein, are resistant to this teratogenic effect of 2,3,7,8-TCDD. These data and other results (Hassoun and Dencker, 1982) suggest that cleft palate formation elicited by 2,3,7,8-TCDD segregates with the Ah locus.

In an early study, Courtney and Moore (1971) tested the teratogenic potential of 2,3,7,8-TCDD in pregnant rats (CD) injected s.c. on a daily basis with 2,3,7,8-TCDD (0.5 or 2 μg/kg) in dimethyl sulfoxide on days 6 through 15, days 9 and 10, or days 13 and 14 of gestation. The only remarkable anomaly was kidney malformations in fetuses exposed to 2,3,7,8-TCDD. In the group exposed transplacentally at a dose of 0.5 μg/kg, 4/6 litters had fetuses with kidney malformations (average number of kidney defects/litter was 1.8). An 11 and 34% incidence of kidney anomalies occurred in groups exposed to 2,3,7,8-TCDD on days 9 and 10, and 13 and 14, respectively. In addition, six hemorrhagic gastrointestinal tracts were observed in the treated group (these data were not enumerated with respect to dose); however, this was considered a primary fetotoxic effect of 2,3,7,8-TCDD and not a malformation.

2,3,7,8-TCDD was administered by gavage to groups (10-14 animals/group) of pregnant Sprague-Dawley rats at dose levels of 0, 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg/day on days 6 through 15 of gestation (Sparschu et al., 1971b). No adverse teratogenic effects were reported in fetuses exposed transplacentally at the 0.03 μg/kg level. At the 0.125 μg/kg level, three dead fetuses were reported, fetal weights were slightly depressed, and intestinal hemorrhage was noted in 18 of 127 examined fetuses. In the group given doses of 0.5 μg/kg, the number of viable fetuses was reduced, resorptions were increased, 6 dead fetuses were reported, and 36 of 99 fetuses suffered an intestinal hemorrhage. In the 2.0 μg/kg group, only 7 live fetuses were reported (occurring in only 4/11 litters), 4 having intestinal hemorrhage. Early and late resorptions were prevalent. No live fetuses, but many early resorptions, were reported in the group exposed to 8.0 μg 2,3,7,8-TCDD/kg/day. Subcutaneous edema appeared dose-related, occurring in a considerable number of fetuses from the higher dose groups. Male fetuses appeared to be more susceptible to 2,3,7,8-TCDD exposure; however, there was no significant difference in the sex ratio of live fetuses.

Khera and Ruddick (1973) tested a wide range of doses of 2,3,7,8-TCDD for teratogenic and fetotoxic potential. Groups of 7-15 Wistar rats were intubated with 2,3,7,8-TCDD at doses of 0.125, 0.25, 1, 2, 4, 8 and 16 μg/kg on days 6 through 15 of gestation. At day 22 of gestation, there were no live fetuses in groups exposed to $\geq$4 μg/kg, and reduced litter size was observed in the 1 and 2 μg/kg group. Unspecified maternal toxicity was reported in all groups where there was fetal mortality. In groups exposed to 0.25-2 μg/kg, there were fetal anomalies observed as either gross or microscopic lesions consisting of subcutaneous edema of the head and neck, and hemorrhages in the intestine, brain and subcutaneous tissue.

The incidences of grossly observed lesions were 0/18, 2/11, 7/12 and 11/14 in the control, 1, 1 and 2 μg/kg dose groups, respectively (the study was conducted in two parts, and the 1 μg/kg dose was repeated). With regard to the other dose levels tested, the table enumerating the results had an entry of "not done". The incidence of microscopically observed lesions for the control, 0.25, 0.5, 1, 1 and 2 μg/kg groups was 0/10, 1/33, 3/31, 3/10, 3/6 and 3/7, respectively. There were no effects of treatment observed in the 0.125 μg/kg group.

Khera and Ruddick (1973) also exposed dams to 2,3,7,8-TCDD at doses of 0.125, 0.25, 0.5 and 1 μg/kg on days 6 through 15 of gestation and allowed the dams to litter and wean the pups. In this experiment, maternal toxicity was reported in the 0.5 and 1 μg/kg group. At birth, there were fewer viable pups, and the pups had lower body weights in all but the 0.125 μg/kg group. At weaning on day 21 after birth, there were no surviving pups in the 1 μg/kg group, and 40% of the pups in the 0.5 μg/kg group did not survive. Fostering pups from dams exposed to 2,3,7,8-TCDD at 1 μg/kg onto control dams did not appreciably increase survival, while fostering control pups onto dams exposed to 2,3,7,8-TCDD, did not increase pup mortality. These data suggest that poor pup survival was a result from delayed toxicity from in utero exposure to 2,3,7,8-TCDD.

Giavini et al. (1982a) assessed the effect of small doses of 2,3,7,8-TCDD administered during the preimplantation period in Sprague-Dawley rats. The animals, in groups of 20, were treated by gavage with 2,3,7,8-TCDD at doses of 0.0, 0.1, 0.5 and 2 μg/kg on days 1-3 of gestation. (The legends to the tables in this paper indicated that the low dose was 0.125 μg/kg.)

At day 21 of gestation, no toxic effects were observed in the dams except for a decrease from 19.3-12.9 g in average maternal weight gain in the high dose animals as compared to controls. In the fetuses, weight was significantly reduced ($p<0.05$) in the 0.5 and 2 μg/kg groups. Malformed litters and malformation/fetuses examined were 2, 5, 5 and 6, and 2/270, 8/260, 5/255 and 8/253, respectively, in the control 0, 0.1, 0.5 and 2 μg/kg groups; however, these increases in the treated animals were not statistically significant. The anomalies observed were restricted to cystic kidney. This exposure to 2,3,7,8-TCDD early in pregnancy did not affect implantation frequency, and the decrease in fetal weight was considered a result of 2,3,7,8-TCDD delayed implantation.

In a second study, Giavini et al. (1983) administered the same doses of 2,3,7,8-TCDD (0.0, 0.125, 0.5 and 2 μg/kg) daily to 15 female CRCD rats per group by gavage in corn oil:acetone (9:1) for 2 consecutive weeks prior to mating. Females that did not become pregnant during three estrous cycles were necropsied to determine signs of toxicity, while pregnant animals were allowed to proceed to day 21 of gestation at which time necropsies were performed with particular emphasis on reproductive organs and reproductive success. At the lowest dose tested (0.125 μg/kg), there were no overt clinical signs of toxicity in the dams or adverse effects in any of the fetal parameters examined. At the 0.5 and 2 μg/kg levels, average maternal weight was decreased. Also, one animal in each of these groups did not become pregnant, although necropsy did not reveal any obvious dysfunctions. The only other overt sign of toxicity was listlessness during the treatment period in the animals of the high-dose group. The only significant ($p<0.01$) fetal effect observed in the 0.5 μg/kg group was an increase in postimplantation losses from 2.9% in the control group to 10.2%. In the

high-dose group, there were decreases in corpora lutea and implantations (averages of 17.6% in control and 14.9% in treated animals, and 15.5% in control and 12.0% in treated animals, respectively), and increases in both pre- and postimplantation losses of 11.7% for controls and 19.5% ($p<0.05$) in treated animals, and 2.9% in control and 30.3% ($p<0.001$) in treated animals, respectively. In addition to these signs of fetal toxicity, 9 of 10 litters in the high-dose group contained at least one malformed fetus as compared with 1/13, 2/13 and 2/13 in the control, 0.125 and 0.5 μg/kg groups. The predominant fetal malformations were cystic kidney and dilated renal pelvis, which have been observed in other studies in which 2,3,7,8-TCDD was administered during gestation.

The reproductive effects of 2,3,7,8-TCDD were also examined in a 3-generation study using Sprague-Dawley rats (Murray et al., 1979). Throughout the study, animals were continuously maintained on diets providing doses of 0, 0.001, 0.01 or 0.1 μg 2,3,7,8-TCDD/kg/day. The parental group (f_0) was maintained for 90 days on the test diets prior to mating. The f_0 rats were mated twice, producing the filial generations (f_{1A} and f_{1B}). Selected f_{1B} and f_2 rats were mated at ~130 days of age to produce the f_2 and f_3 litters, respectively. In later generations, the high dose group (0.1 μg 2,3,7,8-TCDD/kg/day) was discontinued because few offspring were produced in this group. At the intermediate dose (0.01 μg/kg/day), 2,3,7,8-TCDD caused lower body weights in exposed rats of both sexes (f_1 and f_2). At the low dose, no toxic effects were discerned.

Fertility was greatly reduced in the f_0 generation exposed to 0.1 μg 2,3,7,8-TCDD/kg/day. At 0.01 μg 2,3,7,8-TCDD/kg/day, fertility was significantly ($p<0.05$) reduced in the f_1 and f_2 rats. Fertility in rats (of any generation) exposed to 0.001 μg 2,3,7,8-TCDD/kg/day was not

different from that of control rats. Decreases in litter size were noted in the f_{1A} group exposed to 0.1 μg/kg/day and the f_2 and f_3 litters exposed at 0.01 μg/kg/day. Statistically significant decreases in fetal survival throughout gestation were noted in f_2 and f_3 litters of the 0.01 μg 2,3,7,8-TCDD/kg/day exposed dams. At 0.001 μg 2,3,7,8-TCDD/kg/day, a decreased gestational survival was reported for the f_2 litters, but not for other generations. Decreased neonatal survival was noted among f_{1A} and f_2 pups exposed to 0.01 μg 2,3,7,8-TCDD/kg/day, but not among f_{1B} or f_3 pups. Postnatal body weights of the f_2 and f_3 litters at 0.01 μg 2,3,7,8-TCDD/kg/day were significantly depressed. At the low dose (0.001 μg 2,3,7,8-TCDD/kg/day) necropsy of 21-day-old pups revealed a statistically significant ($p<0.05$) increase in dilated renal pelvis in the f_1 generation. Subsequent generations at this dose level or any at the intermediate dose (0.01 μg 2,3,7,8-TCDD/kg/day) did not have a significant increase in this abnormality. Significantly decreased thymus weight and increased liver weight were reported in the f_3 generation, but not in the f_1 generation (f_2 generation data not obtained) of the intermediate dose group. Murray et al. (1979) concluded that 2,3,7,8-TCDD ingested at 0.01 or 0.1 μg/kg/day impaired reproduction among rats, and NOAELs were associated with 0.001 μg 2,3,7,8-TCDD/kg/day. Nisbet and Paxton (1982) reevaluated the primary data of Murray et al. (1979) using different statistical methods. From this reevaluation it was concluded that 2,3,7,8-TCDD significantly reduced the gestational index, decreased fetal weight, and increased liver-to-body weight ratios and the incidence of dilated renal pelvis in both lower dose groups. Nisbet and Paxton (1982) concluded that the dose of 0.001 μg/kg/day was not a NOAEL in this study.

A single report by Giavini et al. (1982b) describes the effects of exposure to 2,3,7,8-TCDD on fetal development in rabbits. Groups of 10-15 New Zealand rabbits were administered 2,3,7,8-TCDD by gavage at doses of 0.0, 0.1, 0.25, 0.5 and 1 μg/kg on days 6 through 15 of gestation. The dams were examined for implantation sites, resorptions, and live fetuses, and the fetuses were examined for malformations on day 28 of gestation. Decreased maternal weight gain and unspecified signs of maternal toxicity occurred in dams exposed to 2,3,7,8-TCDD at doses of ≥0.25 μg/kg. At doses of 0.5 and 1 μg/kg, there were 2 and 4 deaths, respectively, among the dams. There were increases in abortions and resorptions at a dose of ≥0.25 μg/kg with no live fetuses detected in the high dose group. In the fetuses, the most common observation was a significant increase in extra ribs from 33.3% in the controls to 82, 66.6 and 82% in the 0.1, 0.25 and 0.5 μg/kg dose groups. Although there was no significant increase in specific soft tissue anomalies, there was an increase from 0/87 to 3/78, 2/33 ($p<0.05$) and 2/28 ($p<0.05$) in total soft tissue anomalies in the control, 0.1, 0.25 and 0.5 μg/kg groups. The most prevalent soft tissue anomaly was hydronephrosis, which the authors point out was a common finding in rat fetuses exposed to 2,3,7,8-TCDD *in utero*. These effects were considered to be signs of embryotoxicity rather than a teratogenic effect.

In addition to the fetotoxic effects of prenatal exposure to 2,3,7,8-TCDD, Norman et al. (1978) demonstrated that 2,3,7,8-TCDD could induce liver microsomal enzymes following *in utero* exposure. Pregnant New Zealand rabbits were given s.c. injections of 2,3,7,8-TCDD at a dose of 30 nmol/kg (9.6 μg/kg) on day 24 of gestation, and the livers of newborns were examined for enzyme activity within 12 hours after birth. While this treatment increased the liver cytochrome P-450 levels in the adults ~2-fold, from

1.8-3.7 nmol/mg protein, the increase in the newborns was ~5-fold, from 0.3-1.6 nmol/mg protein. SDS-polyacrylamide gel electrophoresis revealed that 2,3,7,8-TCDD induced a single form (form 6) of cytochrome P-450, and that this form was one of the two that were also induced by 2,3,7,8-TCDD in the adult liver. The identity of form 6 was confirmed by immunologic reaction and its peptide fingerprint. It was shown that induction of cytochrome P-450 in newborns resulted in levels of benzo(a)pyrene hydroxylase and 7-ethoxyresorufin-O-deethylase activity similar to adult levels. The consequence to the newborn of these changes in the development of liver microsomal enzymes has not been established.

Dougherty et al. (1975) failed to find evidence for teratogenicity or embryotoxicity in rhesus monkeys which were given on days 22-38 of gestation daily oral doses (in gelatin capsules) of up to 10 mg/kg/day of 2,4,5-T containing 0.05 ppm 2,3,7,8-TCDD. The 2,3,7,8-TCDD dose at the highest dose level of 2,4,5-T administered (10 mg/kg/day) would correspond to 0.5 μg 2,3,7,8-TCDD/kg/day. However, it should be noted that palate closure in the monkey occurs on gestational days 42-44 and the kidney is also a late developing organ.

Adverse effects of exposure to 2,3,7,8-TCDD on reproductive success in monkeys have also been described. Schantz et al. (1979) fed a diet containing 50 ppt 2,3,7,8-TCDD to rhesus monkeys for 20 months. Seven months into the study the female monkeys were bred to control males. There were four abortions and one stillbirth, two monkeys did not conceive even though they were mated repeatedly, and two monkeys carried their young to term. The total 2,3,7,8-TCDD intake over the seven months was estimated by the authors to be 0.35 μg/kg, corresponding to a calculated daily dose of 0.0015 μg 2,3,7,8-TCDD/kg/day.

Allen et al. (1979) fed adult female rhesus monkeys on diets containing 50 or 500 ppt of 2,3,7,8-TCDD for 7 months. These exposure levels correspond to total doses per animal at the end of 7 months of 1.8 and 11.7 μg 2,3,7,8-TCDD, respectively. Although menstrual cycles were not affected in either treatment group, 5/8 animals in the high dose group had decreased serum estradiol and progesterone levels. Hormone levels were normal in the low dose animals. At 7 months, the females were bred to non-exposed males, and 6/8 and 3/8 females in the low and high dose groups, respectively, were impregnated. Of the impregnated animals, 4/6 and 2/3 had spontaneous abortions, while the remaining impregnated animals had normal births.

McNulty (U.S. EPA, 1980b) treated pregnant rhesus monkeys by gastric gavage to 2,3,7,8-TCDD in a vehicle of corn oil:acetone solution. Group I animals were administered a total dosage of 5 μg/kg bw (two animals), 1 μg/kg bw (four animals) and 0.2 μg/kg bw (four animals) in nine divided doses, 3 times/week during weeks 4, 5 and 6 (days 20 through 40) after conception. Group II, consisting of 12 animals, received single doses of 1 μg/kg bw of 2,3,7,8-TCDD on days 25, 39, 35 and 40 after conception. Three animals were exposed in each of these 4 days. The vehicle control group, consisting of 11 animals, was treated with corn oil:acetone only, on the same schedule as Group I animals. Both the females, who received the highest dose (5 μg/kg), had fetal losses. In the next lower dosed animals (1 μg/kg in both groups) 12 of 16 females had fetal losses; and in the lowest dosed animals (0.2 μg/kg in Group I) one abortion occurred in four pregnancies. Maternal toxicity was observed in many of these treated females. The difference in frequency of fetal loss between all pregnant animals given 1 μg/kg and the rate of historical abortion in the author's breeding colony was found to be significant. The author concluded that

short exposure to 1 μg/kg bw of 2,3,7,8-TCDD during early pregnancy results in fetal loss in rhesus monkeys and the results appear to be related to the adverse effects of 2,3,7,8-TCDD on the fetus (U.S. EPA, 1980b).

A positive association between 2,4,5-T exposures and increases in birth defects or abortions has been reported in human populations in Oregon (U.S. EPA, 1979), New Zealand (Hanify et al., 1981) and Australia (Field and Kerr, 1979). A lack of any such association has been reported in human populations in Arkansas (Nelson et al., 1979), Hungary (Thomas, 1980), New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977) and Australia (Aldred, 1978). Almost all of the reports are geographic correlation studies, and because of the uncertainties inherent in this type of epidemiologic investigation, as well as the difficulties in distinguishing the effects of 2,4,5-T from those of 2,3,7,8-TCDD contamination, none of the reportedly positive associations unequivocally identify either 2,4,5-T or 2,3,7,8-TCDD as the causative agent. Similarly, the reportedly negative associations do not rule out 2,4,5-T or 2,3,7,8-TCDD as potential teratogens or abortifacients in humans.

Based on a report of a high incidence of abortions in a small group of women living around Alsea, Oregon, who may have been exposed to the herbicide 2,4,5-T from aerial spraying (Smith, 1979), the U.S. EPA (1979) initiated a study, often referred to as the "Alsea II study", to determine if spontaneous abortion rates differed between the exposed and unexposed population, if spontaneous abortion rates evidenced seasonal variation in these two groups, and if such seasonal variations were associated with 2,4,5-T spray application.

Spontaneous Abortion Rate Index, as defined by the U.S. EPA, is "basically the ratio of the number of hospitalized spontaneous abortions to

the number of births corresponding to the spontaneous abortions, based on the residence zip code of the women contributing to each event." Upon completion of the study, the EPA concluded that (1) the 1972-1977 Spontaneous Abortion Rate Index for the study area was significantly higher than in the Rural Control Area or the Urban area; (2) there was a statistically significant seasonal cycle in the abortion index in each of the areas with a period of about 4 months. In particular there was an outstanding peak in the study area in June; and (3) there was a statistically significant correlation between the Spontaneous Abortion Rate Index and spray patterns in the study area when a lag-time of 2 or 3 months was included. The EPA concluded however, "This analysis is a correlational analysis, and correlation does not necessarily mean causation."

Milby et al. (1980), citing three critiques of the Alsea II study that were not published in the open literature, state that the statistical method and basic design of the Alsea II study were sufficiently flawed to make this study of no use in human risk assessment. The Alsea II study has also been reviewed by a panel of epidemiologists who, in a published report of their meeting, also concluded that the basic design of the study was inadequate to demonstrate either an effect or absence of an effect of exposure to 2,4,5-T (Coulston and Olajos, 1980). The major inadequacies of the study were that the data collection methods were biased and would likely result in the underestimation of abortions, particularly in the urban area (the incidence of abortions in all three groups was within the expected background rate of 8-15%); only a small portion of the area from which the exposed subjects were selected was actually sprayed with 2,4,5-T; and the study was not controlled for other factors such as age, smoking habits and alcohol consumption, which may affect the spontaneous abortion rate. Based on a new report

by Smith (1979), the U.S. EPA is attempting or has attempted to correlate 2,3,7,8-TCDD levels in the affected areas with the observed rate of abortion. No published reports have been encountered on the outcome of this effort.

In the only other report encountered on a population in the United States, Nelson et al. (1979) noted a general increase in the reported incidence of facial cleft in both high and low exposure groups in Arkansas from 1948 to 1974. In this study, exposure estimates were based on average rice production in different areas of Arkansas, and the incidence of cleft palate was determined by screening birth certificates and checking records of the Crippled Children's Services. No consistent exposure/effect correlations were noted, and the general increase with time in the incidence of facial clefts was attributed to better reporting procedures; however, there does not have to be a direct correspondence of malformations in human beings and experimental animals.

Of the four reports available from New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977; Hanify et al., 1981; Smith et al., 1982a), the report by the Dept. of Health is essentially anecdotal, involving two women who gave birth to malformed children (one with an atrial septal defect and a malformation of the tricuspid valve of the heart and the other with biliary atresia). In both cases, exposure to 2,4,5-T could not be ruled out. Based on an analysis of spraying records, the time course of the pregnancies and plant damage near the women's homes, however, the Department of Health, New Zealand (1980) concluded that there was insufficient evidence to implicate 2,4,5-T spraying as a causative factor. Even if the spraying had been implicated, a lack of information on 2,3,7,8-TCDD levels in the spray and the absence of any monitoring data on 2,4,5-T or 2,3,7,8-TCDD would limit the usefulness of this report.

The study by McQueen et al. (1977) is not published in the open literature but is summarized by Milby et al. (1980). According to the summary, McQueen et al. (1977) "...examined the epidemiology of neural-tube defects in three areas in New Zealand and concluded 'there is no evidence to implicate 2,4,5-T as a causal factor in human birth defects.'" No additional details are provided.

Hanify et al. (1981) performed an epidemiologic study in Northland, New Zealand, in areas where spraying of 2,4,5-T was carried out by various companies for a number of years. The rate of birth defects was obtained from an examination of hospital records in seven mutually exclusive areas on a monthly basis over a period extending from 1959-1977. The rate of birth defects from 1959-1965 represented the rate for a non-exposed population since this was prior to the use of 2,4,5-T, while the incidence of birth defects from 1972-1976 represented the rate for the exposed population. During the time of the survey there were 37,751 births, 436 stillbirths, 264 deaths shortly after birth, and 510 congenital anomalies. Three categories of birth defects, heart abnormalities, hypospadias and epispadias, and talipes, had elevated rate ratios of >1 ($p=0.05$) in comparisons between the exposed (1972-1976) and control (1959-1965) populations. Exposure estimates were made for the seven areas and for different years using company records of aerial spraying and a model that factored in assumed fractional removal rates/month (this factor was assumed to be either 1.0 or 0.25). Comparisons of the rate of specific malformations with exposure demonstrated a statistically significant association between the occurrence of talipes and exposure when the fractional removal rate was assumed to be 0.25. There was, however, no statistically significant association where 1.0 was used as the fractional removal rate.

Smith et al. (1982a) investigated the outcome of pregnancy in families of professional 2,4,5-T applicators and agricultural contractors in New Zealand. Agricultural contractors were chosen as the control population since both sprayers and contractors were of the same economic group with similar outdoor occupations. The survey was conducted by mail with 89% of the chemical applicators responding and 83% of the agricultural contractors responding to questions asking whether they used 2,4,5-T and its temporal relationship to reproductive histories regarding birth, miscarriages, still-births and congenital defects. The relative risks of congenital defects and miscarriages were 1.19 (0.58-2.45% confidence limits) and 0.89 (0.61-1.30% confidence limits) for the wives of chemical sprayers as compared to the wives of agricultural contractors. These data indicate that exposure of fathers and mothers (e.g., while cleaning clothes) had no effect on the outcome of pregnancy. Biases that may have affected the results, such as the age of the mother at childbirth, smoking habits and birth to Maori parents were investigated and eliminated as possible confounders.

The two reports from Australia (Aldred, 1978; Field and Kerr, 1979) also present apparently conflicting results. The report by Aldred (1978) is not published in the open literature, but the following summary is taken from Milby et al. (1980): "The report concluded that birth defects in a group of babies born in the [Yarram] district in 1974 and 1976 could not be attributed to exposure to 2,4,5-T or 2,4-D." Additional details that might be useful in assessing the rationale for this statement are not provided in the summary. The report by Field and Kerr (1979) plotted the incidence of neural-tube defects (anencephaly and meningomyelocele) in New South Wales, Australia, over the years 1965-1975, and the previous years usage of 2,4,5-T in all of Australia. The authors noted a decrease in the incidence of

neural-tube defects expected on the basis of the plotted line in 1975 and 1976, when Australia instituted monitoring of 2,4,5-T to ensure a 2,3,7,8-TCDD level <0.1 ppm. The data were not tested for significance, although Field and Kerr (1979) indicate that they consider the epidemiological data on neural-tube defects to be "relatively complete," they do not comment on the increasing incidence of neural-tube defects with time and whether or not an increase in the thoroughness of reporting neural-tube defects could have contributed to the apparent correlation of 2,4,5-T exposure with these defects. A visual replotting of the data suggests that the incidence of cleft palate correlates better with 2,4,5-T usage than with time. Nonetheless, the appropriateness of correlating 2,4,5-T usage in all of Australia with the incidence of defects in one area of Australia is questionable.

Thomas (1980) used an approach similar to that of Field and Kerr (1979) on data from Hungary. One major difference, however, is that Thomas (1980) compared the incidence of stillbirths, cleft lip, cleft palate, spina bifida, anencephalus and cystic kidney disease in all of Hungary between 1976 and 1980 with 2,4,5-T use in 1975 in all of Hungary. Because Hungary requires compulsory notification of malformations diagnosed from birth to age 1 year, because a relatively large percentage (55%) of the Hungarian population lives in rural areas where 2,4,5-T exposure may be expected to be greatest, and because annual use of 2,4,5-T in Hungary had risen from 46,000 kg in 1969 to 1,2000,000 kg in 1975, Thomas (1980) considered Hungary to be "...probably the best country in which to examine possible health effects of this herbicide." In any event, all indices of birth defect rates decreased or remained stable over the period of study.

In addition to contamination of 2,4,5-T being a potential source of 2,3,7,8-TCDD exposure, 2,3,7,8-TCDD is also an inadvertant contaminant of

2,4,5-trichlorophenol (TCP). Chronic exposure to 2,3,7,8-TCDD may occur during the manufacture of TCP and high level acute exposure to 2,3,7,8-TCDD has occurred after an accident in July, 1976 at the ICMESA TCP chemical factory in Seveso, Italy (Bonaccorsi et al., 1978). In this accident, the reaction used to produce TCP became uncontrolled, producing conditions favorable for 2,3,7,8-TCDD formation prior to venting the contents of the chemical reactor into the atmosphere. The resulting cloud of chemicals settled over a heavily populated area. Although the amount of 2,3,7,8-TCDD released was not known, the reported cases of chloracne, a symptom of acute exposure to 2,3,7,8-TCDD, indicated that exposure to 2,3,7,8-TCDD had occurred. Some preliminary results are available from epidemiologic studies of reproductive events in the inhabitants of Seveso, and recently a study has become available on the reproductive history of men employed in the chemical manufacturing industry with possible chronic exposure to 2,3,7,8-TCDD (Townsend et al., 1982).

Epidemiologic studies to determine the reproductive effects in individuals exposed to 2,3,7,8-TCDD and TCP following the accidental contamination of a populated area around Seveso, Italy, are not completed. The incidence of spontaneous abortions occurring between March 1976 and January 1978 have been reported for inhabitants in the area around Seveso by Bonaccorsi et al. (1978), Reggiani (1980) and Bisanti et al. (1980). The spontaneous abortion rate in the contaminated area for the three trimesters following the accident was 13.1, 11.0 and 13.05%, which was similar to the worldwide 15-20% frequency of spontaneous abortion. Subdividing the contaminated area into highly, moderately, and least contaminated, and examining the rates for each area individually, also failed to demonstrate any change in the spontaneous abortion rate. The incidence rates of malformations also were examined;

however, the numbers were too small for meaningful assessment. There are several reasons why these studies would not indicate that the effect of 2,3,7,8-TCDD exposure in this accident had no effect on human reproduction. The authors note that there are many difficulties in interpreting these data. The incidence rates of spontaneous abortions and birth defects were not adequately available for the region prior to the accident as a result of suspected under-reporting. There was inadequate reporting even after the accident due to political turmoil with regard to the management of health services. Also, an unknown number of pregnancies were surgically aborted for fear of 2,3,7,8-TCDD induced birth defects. In a recent review of the progress of epidemiologic investigations of the Seveso accident, Tognoni and Bonaccorsi (1982) indicated that the data on spontaneous abortions and malformation rates still needed verification, and that these data were too preliminary to allow for conclusions.

Townsend et al. (1982) investigated the reproductive history of wives of employees potentially exposed to 2,3,7,8-TCDD during chlorophenol production. A total of 930 potentially exposed males were identified who had worked for ≥1 month between January 1939 and December 1975 in a job with potential 2,3,7,8-TCDD exposure. Exposure estimates of low, moderate and high were made by an industrial hygienist primarily from job description and surface contamination data; however, the high potential exposure group was reserved for process workers during 1963-1964 when changes in operations resulted in a number of cases of chloracne. The control population was an equal number of male employees not involved in any process that might cause exposure to 2,3,7,8-TCDD and matched for date of hire. In these groups, 586 wives were identified and 370 agreed to participate as the exposed group,

while 345 wives in the control group agreed to participate. After identification of the participants, a personal interview was conducted with the wives to determine pregnancy outcome. Of the total of 737 conceptions in the exposed category and 1785 conceptions in the control category (conception which occurred in the exposed group prior to work records indicating potential exposure to 2,3,7,8-TCDD were placed in the control group), there was no statistically significant increase in spontaneous abortions, stillbirths, infant deaths or selected congenital malformations. Sample sizes were too small to provide meaningful data if the populations were subdivided by extent of exposure. It was suggested that many confounding factors could account for these negative results, such as the inappropriate selection of the populations, unidentified covariables and insufficient power; however, it was maintained that these results were consistent with animal data, which report that paternal exposure to 2,3,7,8-TCDD does not affect the conceptus.

Poole (1983), in testimony before the House Committee on Science and Technology, described a re-analysis of the primary data used by Towsend et al. (1982). In this re-analysis, the rate of cleft palate and cleft lip were reported to be elevated by 1.9 (90% confidence intervals of 1.0-3.6) in the years 1971-1974 for both the control and exposed groups (the comparison population was not described). At the same House Committee hearing, Houk (1983) presented data from the Birth Defect Monitoring Program of the Center for Disease Control on the yearly rate of cleft palate alone or cleft lip with or without cleft palate for births in Midland County, Michigan (the site of a chlorophenol production facility) during the years 1970-1981. The data indicated an increased rate for these defects of between 50 and 100% in the years 1971-1975, with the rate returning to expected from 1976-1981.

The observed increase was only statistically significant if the rates for cleft palate alone and cleft lip with or without cleft palate were combined; however, it was the opinion of Houk (1983) that these defects should not be combined since the causal mechanism may be different. The Michigan Department of Public Health (1983a) also reported these results and, in addition, demonstrated that the same results occurred if the comparison was made with other counties in Michigan as well as with the general population of the United States. It was noted in this report that "runs" of increases in oral cleft for successive years have occurred in six other counties with no obvious potential for chemical exposure described. The Michigan Department of Public Health (1983a) interpreted the data to indicate that a more detailed case control study was necessary to determine if any common factors may exist, such as exposure to chemicals contaminated with 2,3,7,8-TCDD.

Mutagenicity

Short-term *in vitro* test systems have been developed to assess the biologic, toxic and genotoxic effects of chemicals. These assays have proven to be useful indicators of potential activity of diverse industrial chemicals, a broad range of drugs and xenobiotics, carcinogens and crude environmental extracts. The most widely used short-term test system, the Ames test for bacterial mutagenesis, employs several strains of *Salmonella typhimurium* which are highly susceptible to the effects of mutagenic chemicals. Despite the obvious utility of the Ames test and related short-term assays, their predictive capabilities (i.e., the correlation between bacterial mutagenicity and carcinogenicity) have not been fully assessed (Bartsch et al., 1982).

Mutagenicity assays in microorganisms have been used to assess the genotoxic effects of 2,3,7,8-TCDD; however, the results of most of these assays have indicated little potential for mutagenic effects (Table 11).

TABLE 11

The Results of Mutagenicity Assays for 2,3,7,8-TCDD in *Salmonella typhimurium*

Type of Assay	Strains of *Salmonella typhimurium*														Reference
	S-9	TA98	TA1530	TA1535	TA1537	TA1538	TA1532	TA1950	TA1975	TA1978	G46	TA100	TA1531	TA1534	
Spot test	+/-	NT	NT	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	McCann, 1978
Plate incorporation	+/-	NT	NT	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	McCann, 1978
Plate incorporation*	+/-	0	0	0	0	0	0	0	0	0	0	0	NT	NT	Gilbert et al., 1980
Fluctuation test	+/-	0	0	0	0	0	0	0	0	0	0	0	NT	NT	Gilbert et al., 1980
Spot test	-	NT	0	NT	NT	NT	+	NT	NT	NT	0	NT	QR	QR	Seiler, 1973
Plate incorporation	+	0	NT	0	0	0	NT	NT	NT	NT	NT	0	NT	NT	Geiger and Neal, 1981
Plate incorporation	-	NT	NT	NT	0	NT	NT	NT	NT	NT	NT	NT	NT	NT	Geiger and Neal, 1981
Suspension assay	-	NT	0	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT	Hussain et al., 1972
Suspension assay	+/-	0	NT	0	0	NT	NT	NT	NT	NT	NT	0	NT	NT	Zeiger, 1983

*The assay was performed under both aerobic and anaerobic conditions.

NT = Not tested; QR = Questionable response

Hussain et al. (1972) exposed Salmonella typhimurium histidene-dependent strains TA1530 and TA1532 in liquid suspension to 2,3,7,8-TCDD followed by plating into selective medium to observe reversion to prototypes. No increase in the reversion rate was observed with strain TA1530 at exposure levels of 1 and 10 μg/mℓ. These exposures resulted in cell survivals of 90 and <1%, respectively. In strain TA1532 increased reversion frequency was not observed at 2,3,7,8-TCDD concentrations of 2-3 μg/mℓ, which resulted in a 0-50% decrease in survival; however, at 2,3,7,8-TCDD levels which resulted in a 99% decrease in survival, there was an increased number of revertant colonies/surviving cells. The dose levels were not specified. The source of the 2,3,7,8-TCDD sample studied in this paper was the Food and Drug Administration, and its reported purity was 99%. Also, Seiler (1973) observed a positive mutagenic response in a spot test of 2,3,7,8-TCDD performed in the absence of a metabolic activation system. However, the purity of the sample studied was not provided. In tester strains G46 and TA1530, the ratio of revertants/10^8 cells in the treated plates divided by spontaneous revertants/10^8 cells was ≤ 1. In strains TA1531 and TA1534, the ratio was between 1 and 2, which was considered a "doubtful" mutagenic response, while in strain TA1532, the ratio was >10. There was no mention of the 2,3,7,8-TCDD levels tested in this assay. The positive controls, diethylsulfate, 2-aminopurine and 2-aminofluorene, produced ratios of 2 to 5, ≤ 1 and 5 to 10, respectively, in strain TA1532. In both the study by Hussain et al. (1972) and the study by Seiler (1973), 2,3,7,8-TCDD produced a positive mutagenic response only in the S. typhimurium strain TA1532, which is sensitive to frameshift mutagens.

Hussain et al. (1972) also performed a mutagenicity test of 2,3,7,8-TCDD in two other microbial test systems. A positive response was observed in

Escherichia coli Sd-4 as indicated by a reversion to streptomycin independence. In this assay, cells were treated in suspension for 1 hour with 2,3,7,8-TCDD at 0.5-4 μg/mℓ. The greatest mutation frequency (256 mutants x 10^{-8}, as compared to the control frequency of 2.2 mutants x 10^{-8}) occurred at a dose level of 2 μg/mℓ. The absolute number of colonies/plate was 7 for the control and 46 for the treated plate. The dose of 2 μg/mℓ caused an 89% decrease in cell survival. In the second test system, the ability of 2,3,7,8-TCDD to increase prophage induction in E. coli K-39 cells was examined. The vehicle control, DMSO, inhibited prophage induction as compared to the untreated controls, while the most effective dose level of 2,3,7,8-TCDD (0.5 μg/mℓ) resulted in an increased prophage induction as compared to vehicle control but not as compared to the untreated controls. Hussain et al. (1972) concluded that 2,3,7,8-TCDD was capable of causing increases in the reverse mutation rate in E. coli Sd-4 and that 2,3,7,8-TCDD had a weak ability to induce prophage in E. coli K-39 cells.

The studies which followed these two early reports of Hussain et al. (1972) and Seiler (1973) failed to detect mutagenic activity of 2,3,7,8-TCDD in S. typhimurium. Wasson et al. (1978) reported on a personal communication from McCann (1978) that 2,3,7,8-TCDD was inactive in both the spot test and plate incorporation assay with S. typhimurium strains TA1532, TA1535, TA1537 and TA1538. Doses and other experimental protocols were not mentioned except that the tests were performed both with and without metabolic activation. Gilbert et al. (1980) reported that 2,3,7,8-TCDD gave "substantially negative results" with S. typhimurium strains TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, TA1532, TA1950, TA1975 and TA1978. Both the standard plate incorporation assay and the bacterial fluctuation test were used, and both were performed with and without S-9 prepared from the livers

of Aroclor 1254 pretreated rats. In the plate incorporation assay the test compound was tested at 1-2000 μg/plate under both aerobic and anaerobic conditions. Details were not provided for the fluctuation assay. It is difficult to assess possible reasons for the conflicting results between the earlier studies and these later mutagenicity assays, since information on experimental conditions was limited in the negative studies.

In an attempt to resolve the conflicting results and observe a mutagenic response, Geiger and Neal (1981) tested 2,3,7,8-TCDD in the standard plate incorporation assay using S-9 prepared from different sources. In order to maximize the amount of compound tested, dioxane, a better solvent for 2,3,7,8-TCDD than the commonly employed DMSO, was used. Even with the use of dioxane, the limited solubility of 2,3,7,8-TCDD allowed only 20 μg/plate to be tested, a dose which was shown to be non-toxic to the cells. The S-9 used in these assays was prepared from the livers of Aroclor 1254 pretreated male Sprague-Dawley rats and male Golden Syrian hamsters, and from 2,3,7,8-TCDD induced male hamsters. In all assays at 2,3,7,8-TCDD concentrations of 0.2, 2, 5 or 20 μg/plate, and regardless of the source of the S-9, there was no observed mutagenic response. In further attempts to duplicate the previous positive results, Geiger and Neal (1981) tested the same concentrations of 2,3,7,8-TCDD in strain TA1537, a more sensitive direct descendent of strain TA1532, for mutagenic activity in the absence of S-9. Again, no increase in the number of revertants was observed. In assays either with or without S-9, positive controls had predictable increases in the number of revertant colonies. The authors concluded that 2,3,7,8-TCDD was not active under the conditions of this assay; however, testing at higher concentrations may elicit a positive response. It was

also noted that many other polychlorinated aromatic compounds are not mutagenic in the Ames test, even though there is positive evidence of carcinogenicity.

Mutagenic effects of 2,3,7,8-TCDD in yeast were observed by Bronzetti et al. (1983). Positive results for reversion and gene conversion were obtained in vitro and in the host-mediated assay. The in vitro experiments yielded small dose-related increases in trp^+ convertants and ilv^+ revertants. An S10 metabolic activation system was required. Exposure of the yeast to 2,3,7,8-TCDD at the highest level tested (10 μg/mℓ) resulted in 16% survival and yielded 4-fold increases in reversion and gene conversion.

In the host-mediated assay, male mice were exposed to 25 μg of 2,3,7,8-TCDD/kg (Bronzetti et al., 1983). After 5, 10, 20 or 30 days, 0.2 mℓ of a yeast culture ($4x10^8$ cells) was instilled retroorbitally. Four hours later, the liver and kidneys were removed and the yeast cells in these organs were assayed for mutagenic responses. Increases (4- to 6-fold) in reversion and gene conversion were observed in yeast cells obtained from the livers and kidneys. The toxic response of the animals to an exposure of 25 μg/kg was not described in this report. Toxicity should be expected at this high dose. The positive results described in this paper may suggest that 2,3,7,8-TCDD is mutagenic in yeast, but more definitive studies are needed before a firm conclusion can be drawn.

Hay (1982) has found that 2,3,7,8-TCDD dissolved in DMSO transformed baby hamster kidney cells (BHK) in vitro. The dioxin isomers 2,8-dichloro- and 1,3,7-trichlorodibenzo-p-dioxin also transformed BHK cells but the response was weak. The unchlorinated dibenzo-p-dioxin and the fully chlorinated octachlorodibenzo-p-dioxin were both negative in the BHK assay (i.e.,

there was no cell transformation). More recently, Rogers et al. (1982) reported that 2,3,7,8-TCDD induced mutations in the excess thymidine, thioguanine and methotrexate selective systems in L5178Y mouse lymphoma cells in culture.

The National Toxicology Program (Zieger, 1983) provided data on TCDD from four assay systems: the S. typhimurium (strain TA98, TA100, TA1535 and TA1537) histidine reversion assay, the sex-linked recessive lethal test in Drosophila, and cytogenetic studies (sister chromatid exchange and chromosome aberrations) in Chinese hamster ovary cells. Negative results were obtained in all these assays. However, these studies cannot be evaluated because the procedures used to obtain the data were not described.

The solubility of 2,3,7,8-TCDD in water is only 0.2 μg/ℓ (Crummett and Stehl, 1973). Therefore, negative in vitro results must be viewed with caution unless precise descriptions of the preparation of each test sample are supplied (e.g., were the samples predissolved and, if so, in what solvent).

In vitro reactions of 2,3,7,8-TCDD with bacteriophage QB RNA were evaluated by Kondorosi et al. (1973). Active RNA was purified from QB phage followed by incubation for 1 hour at 37°C with 0.0, 0.2, 2.0 or 4.0 μg/mℓ of 2,3,7,8-TCDD. At all concentrations tested, 2,3,7,8-TCDD had no effect on the transfectivity of QB RNA. Other compounds tested included the alkylating agents methyl, ethyl and isopropyl methanesulfonate, and diethyl pyrocarbonate, all of which inactivated QB RNA under the same experimental conditions. The authors suggested that 2,3,7,8-TCDD inactivity in this assay indicated that 2,3,7,8-TCDD was an intercalating agent, and hence would require double stranded DNA in order to interact. The data presented in this study, however, were insufficient to support this conjecture.

In vivo binding of radiolabeled 2,3,7,8-TCDD to liver macromolecules was studied in Sprague-Dawley rats by Poland and Glover (1979). Both male and female animals were administered [1,6-^{3}H]2,3,7,8-TCDD by i.p. injection at a dose of 7.5 μg/kg. This dose corresponded to a tritium level of 0.87 mCi/kg. The animals were killed either 12, 48 and 168 hours after treatment, or 24 hours after treatment when the animals were pretreated with the enzyme inducers phenobarbital or unlabeled 2,3,7,8-TCDD. Following sacrifice, isolation of macromolecules, and removal of free labeled 2,3,7,8-TCDD, the amount of label bound to protein, RNA and DNA was determined. The greatest non-extractable binding of labeled 2,3,7,8-TCDD occurred to protein; however, the amount of label bound was small and only amounted to 0.03-0.1% of the total radioactivity administered. The total amount of label associated with RNA and DNA was, respectively, only 50 and 4 cpm above background. Time after exposure, sex, or prior enzyme induction had no significant effect on 2,3,7,8-TCDD binding. As a result of the extremely low levels of radioactivity associated with RNA and DNA, it is uncertain whether 2,3,7,8-TCDD truly binds covalently to these macromolecules and if so, whether there is any biological significance to this low level of apparent binding.

The effects of 2,3,7,8-TCDD exposure on the extent of chromosomal aberrations in the bone marrow of male rats were reported in an abstract by Green and Moreland (1975). In the initial experiment, no increase in chromosomal aberration was observed after 5 daily gavage treatments at a 2,3,7,8-TCDD dose of 10 μg/kg. In the second portion of this study, rats were exposed by a single i.p. injection of 2,3,7,8-TCDD at 5, 10 or 15 μg/kg or a single gavage treatment at 20 μg/kg. The animals at the two highest exposure levels were killed 24 hours post-treatment, while the

remaining animals were killed 29 days post-treatment. Again, no increase in chromosomal aberrations was observed, except in the positive control group exposed to triethylenemelamine.

In a later report, a small but significant increase in chromosomal aberrations was observed in the bone marrow cells of male and female Osborne-Mendel rats (Green et al., 1977). Bone marrow cells for cytogenetic analysis were obtained from Osborne-Mendel rats used in a range-finding study preliminary to a chronic bioassay (Green et al., 1977). The animals in groups of 8 males and 8 females received twice weekly intubations of 2,3,7,8-TCDD at respective doses of 0.25, 1.0, 2.0 and 4.0, or 0.25, 0.5, 2.0 and 4.0 μg/kg for 13 weeks. Because it was not required for the range-finding study, a control group was not included. Bone marrow cells were analysed for abnormalities and cells in mitosis in the animals which survived to the end of the study (4-8 animals/group). The only significant increases in chromosomal aberrations in comparison to the low dose group were in males at 2 and 4 μg/kg and females at 4 μg/kg. The greatest incidence observed was 4.65% of the cells with chromosomal breaks in the high-dose males, and this was considered only weakly positive. The weak response, as well as the lack of data from control animals and the reported difficulty of obtaining cells from the high-dose animals as a result of 2,3,7,8-TCDD toxicity, makes the conclusion from this study that 2,3,7,8-TCDD produced chromosomal breaks tenuous.

Czeizel and Kiraly (1976) reported an increased incidence ($p<0.001$) of chromatid-type and unstable chromosome aberrations in the peripheral lymphocytes of workers exposed to the herbicides 2,4,5-trichlorophenoxyethanol (2,4,5-TCPE) and Buminol. The 2,3,7,8-TCDD levels in the final product were <0.1 mg/kg; however, the exposure levels for individual workers were not available.

Mulcahy (1980) reported in a letter no increased incidences of chromosomal aberrations in lymphocytes of 15 soldiers exposed to "Agent Orange". The exposure was for 6-15 months and all subjects complained of symptoms, including skin eruptions, which they associated with "Agent Orange". The analyses were performed with lymphocytes obtained ~10 years after the last exposure, and comparisons were made with eight subjects who had no history of exposure to 2,3,7,8-TCDD. Neither sister chromatid exchange nor structural aberrations including both gaps and breaks were increased. The authors note that the long time between exposure and analysis may have accounted for the negative results.

Also, both Reggiani (1980) and Mottura et al. (1981) have studied inhabitants in Seveso, Italy, exposed to 2,3,7,8-TCDD from an accident in a trichlorophenol manufacturing plant. Reggiani (1980) examined 4 adults and 13 children (3-13 years) for chromosomal aberrations within 2 weeks of the accident. These 17 individuals were examined to support claims of, and determine extent of, injury before an inquest judge. Although burnlike skin lesions in these 17 individuals indicated chemical exposure, no increase in chromosomal aberrations was detected. The methods of performing the analyses and the actual number of aberrations detected were not described. Similar negative results were reported in an abstract by Mottura et al. (1981). In this study, subjects were chosen from the area of heavy contamination following the accident (acute high level exposure), from the working population of the plant (chronic low level exposure) and a non-exposed control population. The number of subjects in each group was not enumerated. The specimens were examined by three independent laboratories and no laboratory reported an increase in chromosomal aberrations, although there was a significant difference in the reported scores between laboratories. There

was no information in this abstract on the extent of individual exposure or the length of time that elapsed between the accident and obtaining samples for analyses of chromosomal aberrations.

DiLernia et al. (1982) conducted additional studies on lymphocytes prepared in 1976 and 1979 from 8 persons considered acutely exposed to 2,3,7,8-TCDD in the Seveso accident, 8 ICMESA factory workers (considered chronically exposed), and 14 control subjects (8 had chromosomes prepared in 1976 and 6 in 1979). Cells were examined for average number of satellite associations (SAs) (evidence for functional ribosomal genes), both on a cell basis and for the large acrocentric chromosomes (D group chromosomes). There was no change in the frequency of SAs on a per cell basis in any of the groups as compared to control values, nor in D group chromosomes from acutely exposed subjects examined immediately after the accident. There was, however, a decrease in the average frequency of SAs in group D chromosomes of acutely exposed subjects examined in 1977 and in ICMESA workers at both the 1976 and 1979 examinations. Although the biologic relevance of these observations has not yet been confirmed, DiLernia et al. (1982) observed a similar decrease in SAs after exposure of lymphocytes to x-irradiation. It was concluded that the decrease in SAs may have resulted from mutagenic damage to functional nucleolar organizing regions.

The potential of exposure to 2,3,7,8-TCDD to result in chromosomal damage has been studied in experimental animals and humans. Most of the studies in experimental animals gave no evidence that 2,3,7,8-TCDD may result in chromosomal aberrations; however, there is a report of a single positive response which was weak and little detail was provided in the report to assess the quality of the results. In the studies of humans, exposure occurred to chemicals which contain 2,3,7,8-TCDD as a contaminant.

In two of these studies involving individuals exposed in the Seveso accident, there was no observed increase in the incidence of chromosomal aberrations. In a third report of individuals exposed at Seveso, there were changes observed in lymphocyte chromosomes from exposed workers which were suggested to have risen from mutation in functional nucleolar organizing regions; however, this bioassay has yet to be validated. In the only positive study, workers in a chemical plant were exposed to the herbicides, 2,4,5-TCPE and Buminol, as well as 2,3,7,8-TCDD. The participation of the herbicides in the resulting increase in the workers of chromosomal aberrations cannot be excluded. At present, the data from experimental animals and humans are too limited to designate 2,3,7,8-TCDD as a clastogenic agent.

In summary, a limited number of initial studies of the mutagenicity of 2,3,7,8-TCDD in bacteria reported positive results in S. typhimurium strain TA1532 in the absence of a mammalian metabolic activation system (Hussain et al., 1972; Seiler, 1973). More recent attempts to repeat these results with strain TA1532 or related strains have failed (Geiger and Neal, 1981; Gilbert et al., 1980; McCann, 1978). These authors have also reported no increase in mutation rate when 2,3,7,8-TCDD was tested in the presence of a mammalian metabolic activation system. In other in vitro assays, 2,3,7,8-TCDD has produced a positive response in reversion to streptomycin independence in E. coli Sd-4 cells and questionable positive response with prophage induction in E. coli K-39 cells (Hussain et al., 1972). Also, 2,3,7,8-TCDD has been reported to be mutagenic in the yeast S. cerevisiae in both the in vitro assay with S-10 and the host-mediated assay (Bronzetti et al., 1983). Rogers et al. (1982) have also reported positive mutagenicity results in the mouse lymphoma assay. In the E. coli studies, the poor survival of the cells or the interference of the vehicle solvent, DMSO, with the assay makes

the evaluation of the studies difficult. With the data available, it is not possible to resolve the conflicting reports on the mutagenic potential of 2,3,7,8-TCDD.

Overall, the data indicate little potential for the interaction of 2,3,7,8-TCDD with nucleic acids or the ability of 2,3,7,8-TCDD to produce chromosomal aberrations. Kondorosi et al. (1973) demonstrated that 2,3,7,8-TCDD did not react with RNA in vitro in the absence of a metabolic activation system. In vivo studies using radiolabeled 2,3,7,8-TCDD indicated some association of non-extractable label with RNA and DNA (Poland and Glover, 1979); however, the very low level of bound label observed suggest that the "binding" may have been merely an artifact. Similar marginal data were available on the clastogenic effect of 2,3,7,8-TCDD. Although one in vivo study in rats (Green and Moreland, 1975) failed to demonstrate any treatment-related chromosomal aberration, a second study by the same authors (Green et al., 1977) using a longer exposure period reported a small increase in the number of aberrations. In humans exposed to 2,3,7,8-TCDD during the manufacture of 2,4,5-TCPE and Buminol, Czeizel and Kiraly (1976) reported an increase in the number of chromosomal aberrations, while no increase was detected in individuals exposed to 2,3,7,8-TCDD following an industrial accident in Seveso, Italy (Reggiani, 1980; Mottura et al., 1981). The studies of the clastogenic effect of 2,3,7,8-TCDD were presented with little or no experimental detail to assist in evaluating the merits of the reports. The data available are too limited to indicate whether 2,3,7,8-TCDD can interact with nucleic acids or produce chromosomal aberrations.

The differences among the results described above could be due to several factors, such as treatment protocols, solubility problems, purity of

the samples tested and the high toxicity of 2,3,7,8-TCDD. This chemical may be a weak mutagen, but because it is very toxic, the dose range for detecting a positive genetic effect may be very narrow. Therefore, additional experimentation is necessary before any conclusive determination can be made. Suggested further testing includes the ability of 2,3,7,8-TCDD to induce forward mutations in mammalian cells in culture, additional yeast and bacterial studies and the sex-linked recessive lethal test in Drosophila.

Carcinogenicity

Epidemiological Studies

Case Reports. Observations of an unusual occurrence of relatively rare soft-tissue sarcomas were first made by Hardell (1977). Of some 87 patients seen from 1970-1976 at the Department of Oncology, University Hospital, Umea, Sweden, seven individuals with soft-tissue sarcomas were identified. All seven had had occupational exposure to phenoxy acids 10-20 years earlier. The tumors were 2 leiomyosarcomas, 1 liposarcoma, 1 rhabdomyosarcoma, 1 myxofibrosarcoma and 2 additional sarcomas of which the histopathology was uncertain but was probably a neurofibrosarcoma in one and a rhabdomyosarcoma in the other. The clustering of this rare tumor type among these patients prompted the author to suggest that epidemiological studies be done to determine if exposure to phenoxy acids and the impurities they contain are related to the occurrence of soft-tissue sarcomas.

Zack and Suskind (1980) reported the finding of a soft-tissue sarcoma death in a cohort study of workers exposed to 2,3,7,8-TCDD in a trichlorophenol process accident in Nitro, West Virginia. This tumor, a fibrous histiocytoma, was noted by the author as a rare event. This study, referred to as the Nitro study, is discussed later.

Cook et al. (1980) in a cohort mortality study of 61 male employees of a trichlorophenol manufacturing area, who acquired chloracne following a 1964 incident, noted four deaths by the end of his study period, but one of the four was a fibrosarcoma. The authors did not seem to attribute any special significance to this finding at the time.

Ott et al. (1980) in a cohort mortality study of 204 employees exposed to 2,4,5-T during its manufacture from 1950 to 1971, revealed no soft-tissue sarcomas among 11 deaths that had occurred by 1976. But only 1 of these 11 was a malignant neoplasm.

In a discussion of the cohort studies of Zack and Suskind, Cook, a third unpublished study by Zack (in which a liposarcoma was found), and a fourth study by Ott et al. (1980), Honchar and Halperin (1981) noted 3 (2.9%) soft-tissue sarcomas in a total of 105 deaths, compared roughly to 0.07% deaths in U.S. males aged 20-84 years (ICD 171, 8th Revision, 1975)* indicating an unusual excess of such tumors. This may be somewhat of an underestimate due to the possiblity that some soft-tissue sarcomas may be coded to categories other than ICD 171. Separately none of the reported case studies reported a significant excess of soft-tissue sarcomas. The number of soft-tissue sarcomas noted by Honchar and Halperin was increased by a fourth when Cook (1981a) found a malignant fibrous histiocytoma after a later review of the medical records from his earlier cohort study. Cook, who was familiar with the earlier three cases, went on to say that frank chloracne occurred previously in two cases of the 4 having a diagnosis of

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*Department of Health, Education, and Welfare. U.S. Public Health Service. National Center for Health Statistics of the United States, 1974. Vol. II. Mortality, Part A.

malignant fibrous histiocytoma. A third case diagnosed as a fibrosarcoma worked in a trichlorophenol (TCP) process area contaminated with 2,3,7,8-TCDD. This individual exhibited facial dermatitis, but no diagnosis of chloracne was made. The last case was diagnosed as a liposarcoma, and the individual had been employed earlier in a plant producing 2,4,5-T. Cook noted that although chloracne was not reported, it could not be discounted. He also noted that all four were smokers and suggested that smokers with chloracne caused by 2,3,7,8-TCDD exposure may be subject to an increased risk of fibrous soft-tissue sarcomas.

Hardell and Eriksson (1981) discounted this hypothesis by citing that only one of Hardell's seven cases exhibited chloracne prior to the appearance of the soft-tissue saromcas, and that in his later case control study, he found no difference in smoking habits between his cases and controls.

Moses and Selikoff (1981) reported discovering a fifth soft-tissue sarcoma in a worker employed at the Monsanto Chemical Company at a time when trichlorophenol and 2,4,5-T were being produced. He died of a retroperitoneal neurogenic sarcoma (malignant schwanoma) in 1980 at the age of 58. The employee, prior to his death, in a detailed occupational history said that he was potentially exposed to these chemicals while he was a truck driver, hauler and maintenance worker, but that he did not work in the production of either chemical. He was a non-smoker and did not have a history of chloracne.

Johnson et al. (1981) treated a father and son with soft-tissue sarcomas (the 33-year-old son was diagnosed as having a fibrosarcomatous mesothelioma, while the 53-year-old father had a liposarcoma). Both were exposed to halogenated phenol derivatives. The author noted that 2,4-dichlorophenol

can be a precursor of 2,4-D and 2,4,5-T. The father had had prolonged exposure prior to his disease. The son supposedly had a shorter latency, according to the author. In neither case is the follow-up time given.

Sarma and Jacops (1981) reported three cases of thoracic soft-tissue sarcoma in individuals who were exposed to Agent Orange while serving in Vietnam. The diagnoses were fibrous histiocytoma, mediastinal fibrosarcoma, and a pleural/diaphragmatic leiomyosarcoma. All three served in areas where defoliants were used at the time. One was drenched with the material in one spraying.

Bishop and Jones (1981) found two cases of non-Hodgkin's lymphomas of the scalp in a related clinical study of 158 employees of a pentachlorophenol manufacturing plant in Wales. Homologues of 2,3,7,8-TCDD occurred as contaminants at up to 300 ppm at intermediate manufacturing stages and 5 ppm in the final products. Mild, moderate and severe cases of chloracne were seen in many employees, including the two men who subsequently developed lymphomas. Both men worked in processes where exposure to other chemicals occurred, including exposure to aromatic hydrocarbons. The authors reported that only 0.28 tumors of this type could be expected to occur in a group of 158 workers (ICD 200 and 202), although the basis for the computation of expected numbers is not stated.

Olsson and Brandt (1982) noted that of 123 male patients seen at his clinic in Sweden with a recent diagnosis of non-Hodgkin's lymphoma (NHL), 5 had cutaneous lesions as the only clinically detectable manifestation of NHL. Four of the five had repeatedly sprayed large areas with phenoxy acid herbicides. In the remaining 118 NHL patients, only seven had a similar occupational exposure to phenoxy acids. The authors reported this to be significant at $P<0.001$. Olsson and Brandt suggested that a relationship

exists between cutaneous presentation of NHL and occupational exposure to phenoxy acids, and believed their observations were similar to those of Bishop and Jones.

Adding these case studies together, the total number of workers exposed to phenoxy acids and/or chlorophenols is small, but considering the rarity of this cancer, it is unusual that so many cases of soft-tissue sarcomas have occurred. A Lancet editorial (Anonymous, 1982) calls this phenomenon "disturbing." It is suggestive of an association of cancer with exposure to phenoxy acids and/or chlorophenols, and consequently with the dioxin impurities found in these herbicides.

Soft-Tissue Sarcomas. Soft-tissue sarcomas (STS) constitute a collection of heterologous lesions that include both malignant and non-malignant tumors. Not all of them have their origin in primordial mesenchymal cells. Some exceptions are tumors of peripheral nerves, and neuroectodermal tumors which are classified as STS, but are derived from non-mesenchymal cells. Classification, grading and staging of STSs is difficult because of the capacity of such cells to differentiate into many different tissues. Fairly precise histogenetic classification of such tumors is accomplished through consideration of growth patterns and cell morphology and evaluation of intracellular and extracellular products of tumor cells. There are a dozen distinctly different classes of mesenchymal cells that develop into the following six well-defined tissue complexes: fibrous tissue, tendosynovial tissue, adipose tissue, muscle, vessels and bone. STSs can be induced in any of these tissue types (Hajdu, 1983). The classification of STSs for cause of death coding in the ninth and latest revision of the International Classification of Diseases (ICD, 1975) places STSs into one of several categories. But chiefly, they fall into "malignant neoplasms of connective

and other soft-tissue" (ICD 171). Lymphosarcomas, retroperitoneal sarcomas and extra skeletal STSs of the bone are coded elsewhere. In some instances, if site is mentioned, it is coded to the site, e.g., leiomyosarcoma of the stomach (ICD 151.9), neurofibroma of the chest wall (215.4).

Questions have been raised concerning the appropriateness of lumping together malignant tumors of different sites and tumor types in order to derive risk estimates. It may not be scientifically appropriate to do so because an elevated risk cannot readily be ascribed to a particular site or type as is usual with most carcinogenic chemicals and substances. Unfortunately, with respect to STSs, tallies of deaths due to STSs of particular sites and types are not maintained separately by the vital statistics offices because of their rarity, and therefore, it is impossible to derive risk estimates for particular types at given sites. Altogether, ~2000 deaths/year can be attributed to STSs in the United States, most of which are coded to ICD category 171 for purposes of developing incidence and mortality rates for this composite cause. Within ICD 171, individual types that may be correlated with exposure cannot be identified.

A separate problem that potentially could arise from assigning STSs to multiple ICD codes is that incidence and death rates due to STSs may be underestimated. Furthermore, risk estimates derived from dividing observed cases (or deaths) by expected cases (or deaths) could be biased upward. This could happen when observed STSs classified to ICD codes other than ICD 171 are lumped together while expected STSs are based upon ICD 171 only. Thus, action of this sort, especially with respect to cohort studies of individuals exposed to dioxin-containing herbicides and/or chlorophenols,

could lead to risk estimates that may be biased upward by the inclusion of STSs in the observed category for risk estimation that should be coded to categories other than 171.

Prompted by clinical observations over a 7-year period of malignant sarcomas in seven men with previous occupational exposure to phenoxyacetic acid herbicides (Hardell, 1977), researchers at the Department of Oncology, University Hospital, Umea, Sweden, initiated epidemiologic studies to test the hypothesis of an etiologic association (Hardell and Sandstrom, 1979). The investigators elected to conduct case-control studies, a type of epidemiologic research particularly well suited for rare diseases with long periods of induction (Cole, 1979). Cases were defined as male patients with sarcomas of soft connective tissue, such as smooth muscle (leiomyosarcoma) and fat (liposarcoma). The distribution of tumor types in the two studies is shown in Table 12. Sarcomas of harder connective tissues, such as bone and cartilage, were excluded. According to the authors, these tumors may have a different etiology and there occurred a different age-distribution in patients with these tumors as compared to that of STS (Hardell, 1983).

Two case-control studies were conducted, the first in northern Sweden (referred to below as Study A), and the second in the southern part of the country (Study B). The frequencies of exposure to the substances of primary interest are shown in Table 13. In the north, occupational exposure to phenoxyacetic acids took place in both forestry and agricultural work. In the south, these exposures were predominantly agricultural. The phenoxyacetic acids to which exposure occurred consisted predominantly of 2,4,5-T and 2,4-D in both studies. Exposure to 2,4,5-T in the absence of 2,4-D was rarely reported in either study. Exposure to chlorophenols, which contain

TABLE 12

Distribution of Tumor Types in Two Case-Controls Studies of Soft-Tissue Sarcoma

Diagnosis	Tissue of Origin	Percent of Cases	
		Study A[a] (n=52)	Study B[b] (n=110)
Leiomyosarcoma	Smooth muscle	30	23
Fibrous histiocytoma	Subcutaneous connective tissue	17	25
Liposarcoma	Fat tissue	14	6
Neurogenic sarcoma	Nerve tissue	10	4
Angiosarcoma	Blood vessels	8	2
Myxosarcoma	Primitive connective tissue	6	8
Fibrosarcoma	Fibrous tissue	4	8
Other sarcomas		11	24
Total		100	100

[a]Unpublished information supplied by Hardell to EPA (Hardell and Sandstrom, 1979)

[b]Eriksson et al., 1979, 1981

TABLE 13

Exposure Frequencies in Two Case-Control Studies of Soft-Tissue Sarcoma

Substance(s)	Percent Exposed			
	Study A		Study B	
	Cases (n=52)	Controls (n=206)	Cases (n=110)	Controls (n=219)
Phenoxyacetic acids only	23.1	6.3	12.7	2.3
Chlorophenols only	11.5	2.4	10.0	3.6
Both	1.9	0.5	0	0
Total	36.5	9.2	22.7	5.9

*Sources: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981

chlorinated dibenzodioxin impurities (Levin et al., 1976) occurred mostly in sawmill work and paper pulp production. Very few persons reported exposure both to phenoxyacetic acid and chlorophenols in these studies. Of the two predominant phenoxyacetic acids, only 2,4,5-T is known to be contaminated with 2,3,7,8-TCDD. In Study B, a relative risk of 4.9 (90% confidence intervals 1.6-11.1) was found in relation to exposure to phenoxyacetic acid herbicide other than 2,4,5-T (2,4-D, MCPA, mecoprop, dichloroprop).

Relative risks in relation to the three major categories of exposure are shown in Table 14.* Studies A and B indicate a risk of developing STSs among workers exposed to phenoxyacetic acids only, chlorophenols only, or phenoxyacetic acids and/or chlorophenols several times higher than among persons not exposed to these chemicals. In each comparison, the point estimate of relative risk is high and unlikely to have resulted by chance alone.

Since little is known of the etiology of STSs, the consideration of confounding in these studies was largely a hypothetical matter. The authors prevented the effects of age, sex, and place of residence as possible confounding factors in the selection of controls.† Because of the high correlation between exposure to the substances of interest and employment in agriculture and forestry, a reasonable hypothesis could be developed that some other unknown factor present in these occupations was responsible for the elevated relative risks.

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*In the analyses considering phenoxyacetic acids only and chlorophenols only, persons exposed to the other categories of substances were excluded. In Study A, the three persons exposed to both chlorophenols and phenoxyacetic acids were included in all comparisons.

†Controls were matched individually to cases on the basis of these factors. Unmatched analyses are presented in Table 24 for the sake of simplicity. The matched-method relative risks for exposure to phenoxyacetic acids and/or chlorphenols were 6.2 ($P<0.001$) in Study A and 5.1 ($P<0.001$) in Study B.

TABLE 14

Relative Risks of Soft-Tissue Sarcoma in Relation to Exposure to Phenoxyacetic Acids and Chlorophenols in Two Case-Control Studies[a]

	Phenoxyacetic Acids Only		Chlorophenols Only		Phenoxyacetic Acids and/or Chlorophenols	
	Study A	Study B	Study A	Study B	Study A	Study B
Relative risk[b]	5.3	6.8	6.6	3.3	5.7	4.7
90% Confidence interval[c]	2.7-10.2	3.1-14.9	2.8-15.6	1.6-7.0	3.2-10.2	2.7-8.3
Significance level[d]	<0.001	<0.001	<0.001	<0.005	<0.001	<0.001

[a]Source: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981

[b]Unmatched odds ratio

[c]Test-based method of Miettinen, 1976

[d]Chi square statistic, no continuity correction, one-tailed test

To test this hypothesis, it is possible to calculate the relative risk in relation to the phenoxyacetic acid exposure in Study B, restricting the analysis to workers within agriculture and forestry. The result is a relative risk of 6.1 (90% confidence interval 2.4-15.4). This finding strongly suggests that some confounding risk factor for STS distributed throughout agriculture and forestry work was not responsible for the overall increase in risk found in relation to phenoxyacetic acid exposure.

Because exposure histories were obtained by means of questionnaires and interviews, the major potential source of bias in these studies stems from the need to rely upon the personal recollection of cases and controls for exposure histories. The published papers indicate that the researchers paid a great deal of attention to this potential problem and state that they took all reasonable precautions to avoid it during the conduct of the study.

In addition, the relative risk calculated by considering the agriculture and forestry workers who did not report exposure to phenoxyacetic acids or chlorophenols and comparing them to unexposed persons in other occupations was 0.9 (90% confidence interval 0.3-2.4) in Study B. This suggests that a great deal of recall bias was not present (Axelson, 1980).

In an update of his earlier study, Eriksson et al. (1981) obtained information on the effects of phenoxy acids in the absence of the impurities--polychlorinated dibenzodioxins and dibenzofurans. The risk ratio given exposure to phenoxy acids free of polychlorinated dibenzodioxins and dibenzofurans equaled 4.2 based upon 7 out of 14 respondents who indicated exposure to phenoxy acid herbicides. When consideration was given to only phenoxy acids that contain such impurities, the risk was 17.0. A description of the basis for the determination of exposure or non-exposure to dioxins is not well presented in this study.

The author concluded that exposure to phenoxy acids and chlorophenols "might constitute a risk factor in the development of soft-tissue sarcomas." This risk relates not only to 2,4,5-trichlorophenoxy acids containing dioxin impurities, but to other phenoxy acids as well. Some doubt was raised concerning the possible misclassifications of individuals who were exposed to phenoxy acids free of polychlorinated dibenzodioxins (i.e., in particular, "dichoroprop" in the Eriksson study). In a recent communication from Hardell (1983), Eriksson recalculated his risk estimates after reclassifying his dichoroprop-exposed cases and controls into the category of probable exposure to phenoxy acids contaminated with polychlorinated dibenzodioxins and removing them from the non-exposed category. His new estimates were 4.0 based upon 5 out of 8 respondents who were exposed to phenoxy acids free of contamination and 10.9 for those exposed to contaminated phenoxy acid. The first estimate was of only borderline significance utilizing the Mietinen test based statistic, thus, weakening any finding that the risk of STS extends to phenoxy acids free of dioxin.

In a cohort mortality study (Cook et al., 1980a) of 61 males involved in a 1964 chloracne incident, employees in a trichlorophenol manufacturing area were found to have chloracne due to skin absorption of 2,3,7,8-TCDD. The skin lesions characterizing chloracne ranged from a few comedones on the back of one employee (predating his entry into the process area where exposure could occur) to severe cysts and comedones over the faces, scalps, ears, necks and backs of the remaining employees of the group. Since the main route of exposure was not through the respiratory tract, no measurements of dioxin in the air were provided by the author. On the other hand, the author did subjectively divide the cohort of 61 males into potentially "high" vs. "low" exposure by place of work based upon dermal exposure,

although not stated. Vital status was traced from the data of the incident through 1978. Altogether only 4 deaths were observed by the end of the follow-up, vs. 7.8 expected. Of these, 3 were cancer vs. 1.6 expected. The remaining death was hypersensitive heart disease vs. 3.8 expected. The histopathologic causes of death of the three cancer victims were 1) fibrosarcoma, 2) glioma with metastases, and 3) adenocarcinoma. The authors report that all three victims smoked a minimum of one pack of cigarettes a day for "many years."

Cancer mortality is slightly elevated in this cohort despite its relative low sensitivity, the lack of a sufficient latent period, and the presence of the healthy worker effect. This increased mortality was not attributable to any particular cause. Additionally, the authors state that only one of the cancer deaths possessed "documented" evidence of chloracne, although this appears to be at variance with the definition of the cohort, which was reported by the authors to consist of males who reported to the medical department with skin conditions subsequently "diagnosed as chloracne." The authors furthermore concluded that the latency period was sufficient to "allow the identification of a potent human carcinogen," since it "exceeded 14 years." Orris (1981) criticized this conclusion with a reference to the Hardell and Sandstrom (1979) study in which the authors noted that the latent period for soft-tissue tumors may be as long as 27 years and for many, over 14 years. Cook (1981b) countered that the Hardell and Sandstrom (1979) conclusions were based upon questionable data in that the self-administered questionnaires used in that study provided neither valid quantitative nor qualitative estimates of exposure. Therefore, it could not be used to determine latent periods. In any case, Hueper and Conway (1964) noted that the latent period for the chemical induction of solid malignant tumors in man exceeds 15 years and is probably <30 years.

Although the Hardell and Sandstrom (1979) study has some deficiencies, the Cook et al. (1980a) study provides little evidence to support the premise advanced by the authors that dioxin "cannot be considered to be a potent human carcinogen with organ or tissue specificity." There is a distinct likelihood that the latent period for the development of STSs and related tumors due to exposure to dioxin may not have been achieved within the 14-year follow-up period specified in the study. Furthermore, a much larger cohort may be needed in order to detect a significantly increased cancer risk.

Smith et al. (1982b) conducted a case-control study of 102 males identified from the New Zealand Cancer Registry as having STSs (ICD 171) between 1976 and 1980. For each case, three controls each with another form of cancer were matched by age and year of registration. The selection of cancer controls from the same registry was done to eliminate recall bias and/or interviewer bias. The distribution of histological types in the cases is given in Table 15. The interview to elicit occupational history information was accomplished via the telephone either with the next of kin to the patient or the patient himself if he was well enough. Anxiety was alleviated by the mailing of a letter prior to the interview, the purpose of which was to inform the person of the intention of the interviewer to ask some questions about his occupational history.

Apparently, the questions asked were <u>not</u> specific enough to identify definite exposure to phenoxy herbicides and/or chlorophenols. The authors asked only about current occupation or last occupation if retired. Comparisons between cases and controls were accomplished by use of occupational groupings according to the Standard Classification System of New Zealand focusing on those occupational groups with a potential for exposure to

TABLE 15

Distribution of Histological Types of Soft-Tissue Sarcomas

Cell Type	Number of Cases	Percent
Fibrosarcoma	25	24
Liposarcoma	20	20
Rhabdomyosarcoma	9	9
Leiomyosarcoma	7	7
Malignant Histiocytoma	6	6
Other	22	21
Unspecified	13	13
Total	102	100

phenoxy herbicides and chlorophenols. Expected cases for each major occupational classification were derived based upon the occupational distribution of the controls. The authors found no unusual excess of cases of STS in any major occupational category. In agriculture, forestry and fishing, 14 cases were observed vs. 14.0 expected. In laborers, production and transport workers, 35 cases were observed vs. 37.0 expected. A further breakdown of these two broad categories into finer subcategories within the major occupational categories revealed no significant excesses. The study, however, is not useful in assessing the risk of STS from exposure to phenoxy acids and/or chlorophenols for several reasons. First, as was pointed out by the authors, but subsequently dismissed by them as having not much of an influence, is the possibility that switching from one major occupational category to another over the time period involved for latent conditions to manifest themselves could introduction a negative bias into any estimates of relative risks. The latency for STS is felt to be a minimum of 15 years (Hueper and Conway, 1964).

The finding of no switching from one occupational category to another that was noted in the "first 20 interviews" in which a change could be noted is not necessarily indicative of fidelity to the same job over long periods in all 408 cases and controls. Information identifying a switch may be lacking in those cases and controls in which a switch did occur only because the switch resulted in separation of the earlier work history from the latter. Besides the "first 20 interviews" where a change could be noted is not representative of the entire cohort in any case.

Furthermore, the authors do not know absolutely that any of their cases and controls were exposed to phenoxy acids and/or chlorophenols since

apparently no effort was made to confirm "potential" exposures. Only differences in occupational classification were noted where "potentially" cases or controls could have had exposure to the dioxin-containing herbicides. It was pointed out that the risk estimates noted do not "preclude" the possibility that an association may be found in this study when the cases and controls (or surviving kin) are interviewed for chemical spraying at a later time. The authors themselves conclude that the preliminary study results "should not be taken as substantial evidence against the hypothesis that phenoxy herbicides and chlorophenols may cause human cancer."

It should be noted that the distribution of tumor types differed considerably from the Hardell and Eriksson study to the Smith study. Leiomyosarcomas, malignant histocytomas, neurogenic sarcomas and myxosarcoma seem to predominate in the Hardell and Eriksson study, whereas fibrosarcomas and liposarcomas appear prominently in the Smith study. More attention should be devoted to the study of the disbributions of STS types in registry data everywhere in order to determine if such variations in the reporting of STS types are random occurrences. It is possible that the cancer effect of exposure to phenoxy herbicides may be narrowed to just certain types of STSs, the predominant ones in the Swedish studies.

In a later study of STSs, Smith et al (1983a) conducted a case-control study of STSs in males that were reported to the New Zealand Cancer Registry by Public Hospitals between 1976 and 1980. The author matched one cancer control randomly chosen from the registry with each case, initially starting with 112 of each. Controls were matched for year of registration and by date of birth $\pm$ 2 years. Inquiries were made by the authors with the hospital consultant, family doctor, and finally the next-of-kin or patient if alive. Telephone interviews were conducted by only one interviewer who had

no knowledge of the patients cancer history and were completed on 80 cases and 92 controls. Because some 32 potential cases (14 ineligible) and 20 controls were excluded or lost from the study for various reasons, it raises a question whether control of confounding by age and year of registration was maintained in the final group of 172 cases and control included in the analysis. Presumably the corresponding "matched" case or control to each of the 52 lost members of the total study group were not excluded.

Patients were classified as having had potential exposure to phenoxyacetic acids if they had definite, probable or possible exposure to phenoxyacetic acid through spraying or hand contact. The actual chemical was identified only in some instances. The authors concluded in all remaining situations that if the member sprayed "gorse" and/or "blackberries" this was tantamant to potential exposure to phenoxyacetic acid. Smith calculated elevated but non-significant relative risks of exposure to phenoxyacetic acid ranging from 1.3 in those individuals who were "probably exposed" for a minimum of 5 days not in the previous 10 years prior to cancer registration to 1.6 in individuals "probably exposed" for a minimum of 1 day not in the previous 5 years prior to cancer registration. When risk ratios were calculated after stratifying by year of birth and whether or not the patient or a relative was interviewed, the rates increased to 1.7 (from 1.6) in the latter and 1.4 (from 1.3) in the former calculation, although still nonsignificant. It would be of interest to repeat the above calculations excluding only those with potential exposure occurring only within the 15-year period just prior to cancer registration. Furthermore, the categories of exposure "probably or definitely" exposed for $\geq$1 day or even 5 days raises a question whether any of the cases or controls could really be said to have ever come in contact with enough phenoxyacetic acid to justify such a

designation. It could be that, in fact, potentially exposed individuals in New Zealand have had little or no contact with the herbicide.

The authors did conclude that the finding of a relative risk of 1.7 in individuals with ≥1 day exposure not in the last 5 years cannot be entirely discounted. But then the authors state that if exposures of ≥5 days prior to 10 years before cancer registration are not included they would expect an increase, and since they do not see an increase, there is no evidence of a "real causal link." One might question whether this is a suitable criterion for providing evidence of a causal association. Perhaps a more valid group for study would be one where the potential exposure was considerably longer than "5 days" and >15 years prior to initial cancer registration. As kind of a subtle justification for the finding of no significant risk in workers exposed in phenoxy acids, the author alludes to the fact that there are currently 500 full-time workers registered in New Zealand who do full time ground spraying and altogether some 2000 workers who were at some time professionally involved in phenoxyacetic acid herbicide spraying from the air or ground with exposure "very much greater" than that of patients in this study. This kind of argument has appeal if these workers could be shown to have had their exposure sufficiently far in the past that latency considerations could be adequately addressed. However, the real question again remains how much real exposure did those patients in the study really have 10-15 years earlier, and in what numbers. The author remarks that it is surprising that he found no STS victims who had ever worked full-time in phenoxyacetic acid herbicide spraying. Perhaps they have not yet been observed for a long enough period. However, as was pointed out by the author, the findings do not support the hypothesis that exposure to phenoxyacetic

acid herbicides causes STS. But neither do they support a negative finding without better documentation regarding actual exposure and time of actual exposure.

Pazderova-Vejlupkova et al. (1981) studied 80 workers involved in the production of 2,4,5-sodium trichlorophenoxyacetate and butylester of trichlorophenoxyacetic acid who subsequently became ill from exposure to 2,3,7,8-TCDD during the period 1965-1968. Only 55 members of this group were followed for 10 years. The remaining 25 either refused participation or moved leaving no forwarding address. Most patients developed chloracne while 11 developed porphyria cutanea tarda. Chief chemical signs were metabolic disturbances, pathologically elevated lipids with abnormalities in the lipoprotein spectrum, and "pathological" changes in glucose tolerance. Other symptoms noted were biochemical deviations consistent with "a mild liver lesion," light steatosis, periportal fibrosis or activation of Kupffer cells, or nervous system focal damage (peripheral neuron lesion in lower extremeties). Altogether six patients were reported to be deceased during this 10-year period, 2 from bronchogenic carcinoma, 1 from cirrhosis, 1 atherosclerosis precipue cerebi and 2 in auto accidents. No STSs or lymphomas were found. Since there was no comparison population with which to estimate relative risk for cancer, the study must be classified at best as clinical with respect to cancer. The six deaths that occurred during the 10-year observation period in the 55 cannot be construed to be associated with exposure to the 2,4,5-T. Because of the small number of cases and the short follow-up period, nothing can be said concerning the association of exposure with cancer, especially specific types of cancer such as STS or non-Hodgkin's lymphoma.

Riihimaki et al. (1982, 1983) studied a cohort 1926 herbicide applicators formed in 1972 from personnel records of four Finnish employers (i.e., the Forestry Authority, Highway Authority, State Railways and a state-owned electric power company). Chlorinated phenoxyacids had been used since the 1950's in Finland for spraying. They constituted 2:1 mixtures of emulsified esters of 2,4-D and 2,4,5-T dissolved in water. Analyses from old herbicide formulations dating back to the 1960's revealed that these mixtures contained 0.1-0.9 mg/kg of 2,3,7,8-TCDD).

This cohort of male workers was exposed a minimum of 2 weeks during at least one growing season from 1955-1971. Follow-up continued 9 years through 1980 for mortality but only until 1978 for morbidity. Fifteen individuals could not be traced by 1980. Expected deaths were generated based upon cause- and age-specific national Finnish death rates for 1975. Expected cases were similarly calculated based upon national incidence rates of 1975.

By 1980, 144 deaths had occurred vs. 184.0 expected, a deficit of 22% in observed mortality. Only 26 cancer deaths had occurred vs. 36.5 expected, a 29% deficit. The authors separated out "natural" deaths from the total. The observed residual deaths equaled 39 while the expected deaths equaled 28.7. This excess was of borderline significance. The authors also considered 10-year and 15-year latent periods. Even after 15 years, the deficit of deaths continued to manifest itself both in categories of all causes and total cancers; 35 observed vs. 53.6 expected and 5 observed vs. 11.3 expected, respectively. Similarly, the 7-year follow-up of cancer morbidity revealed 26 cases of cancer vs. 37.2 expected. After 10 years latency, 16 cancer cases were observed vs. 20.1 expected. None of the 26 cancer deaths

or 26 cancer cases were of the STS or lymphoma type. (However, only 0.1 STS and 0.5 lymphomas were expected.) In no instance was cancer of any site significantly elevated.

The authors note that this unusual deficit of mortality and morbidity of between 70-82% (even after 15 years from initial exposure) is probably a consequence of the "healthy worker effect" in that only able-bodied and healthy individuals were selected into the industry. The fact that the cohort was assembled in 1972 from records of persons who were exposed as early as 1955 (17 years prior) raises the likelihood that in 1972 a "survivor" population remained (45 deaths prior to 1972 were eliminated from the cohort) that was relatively healthy. Furthermore, the unusually large number of not "natural" expected and observed deaths (probably accidents and external causes) occurring to this cohort indicate a relatively youthful population was under scrutiny. The leading cause of death to persons under 35 years is from accidents, based on national vital statistics.

The authors correctly note that, because of limitations in the study material, only powerful carcinogenic effects could be detected. Risk ratios higher than 1.5 for all cancers, 4.0 for lymphomas and 10.0 for STS could be excluded based on this data set from the authors own calculations. More follow-up is needed in order to provide a stable assessment of the relationship between exposure and cancer. The authors concluded that this study will allow no assessment of STS because "the number of persons having a sufficiently long latency period is too small." It was suggested that more valid conclusions could be made only with the passage of time.

Recently, the Michigan Department of Public Health (1983b), produced an ecological study of soft and connective tissue cancer mortality rates in Midland and other selected Michigan counties. They found that mortality

rates for this cause were 3.8-4.0 times the national average for the periods 1960-1969 and 1970-1978, respectively, for white females in Midland. These estimates are based upon 5 deaths and 7 deaths, respectively, and are listed in Table 16. No excess risk was reported among white males, however. The Michigan Department of Health concluded that because of the occurrence of these two successive elevated rates, it is unlikely to be a chance happening. At the same time the age-adjusted male and female cancer mortality rates for Midland were below that of the State of Michigan in the period 1970-1979. Midland County is the home of a major chemical company that produced phenoxyacetic acid herbicides until recently. The authors state that a detailed review of death certificates, hospital records, residency and occupational histories of the 20 male and female cases revealed no "commonalities" suggesting a "single causative agent" although a majority or their spouses had worked at this chemical facility. They recommend that a case-control study should be instituted to evaluate possible influences, such as lifestyle, occupation or location of residence on the risk of STS.

In a separate review of the epidemiological evidence for STS from exposure to 2,4,5-T-containing herbicides, the United Kingdom Ministry of Agriculture, Fisheries and Food (1983) concluded that there was no evidence to recommend altering their earlier conclusion that formulations of phenoxy acid herbicides and related wood preservatives as "presently cleared" are safe and may continue to be used. This report too readily discounts the positive studies of Hardell and Eriksson as being biased, and it makes no reference to the later validity study by Hardell (1981) of his own work utilizing colon cancer controls (see Section on Malignant Lymphoma). In this report Hardell effectively answered these early criticisms that were reiterated by the British in their report. At the same time, the British

TABLE 16

Midland County Soft and Connective Tissue Cancer Deaths 1960-1981*

Identification			Type of Malignancy			
Year of Death	Sex	Age	Type	Primary Site	Metastases	Month and Year Diagnosed
1961	F	24	Hemangiosarcoma	Face	Skull and upper lobe of lung	5-58
1963	F	75	Liposarcoma	Right gluteal	Unknown	Unknown
1964	F	51	Leiomyosarcoma	Uterus	Widespread	11-63
1968	F	37	Liposarcoma	Spine	Lungs, pelvis	1-66
1969	F	45	Fibrosarcoma Leiomyosarcoma	Right thigh Uterus	Lung, liver Adrenal gland and skin	10-68
1970	F	59	Kaposi sarcoma	Right leg	Lymph nodes	8-68
1970	F	56	Fibrosarcoma Leiomyosarcoma	Right thigh Abdominal wall	Spine Lung	1960 1967
1974	F	1	Rhabdomyosarcoma	Inguinal area	Unknown	8-73
1976	F	77	Liposarcoma	Right thigh	Buttock, lung, rib, lymph nodes	12-74
1978	F	64	Leiomyosarcoma	Left knee	Liver, lymph nodes, lung, bone	7-70

TABLE 16 (cont.)

Identification			Type of Malignancy			
Year of Death	Sex	Age	Type	Primary Site	Metastases	Month and Year Diagnosed
1978	F	26	Rhabdomyosarcoma	Rectum	Lung, neck, inguinal region	6-76
1978	F	88	Fibrosarcoma	Right cheek	Facial area	6-78
1979	F	27	Leiomyosarcoma	Left thigh	Lung	3-78
1962	M	63	Rhabdomyosarcoma	Left lower leg	Lung and right outer chest wall	8-61
1967	M	77	Mesothelioma	Lung	Lung, peritoneum and diaphragm	6-67
1967	M	20	Rhabdomyosarcoma	Pharynx	Periorbital area and liver	1-67
1969	M	32	Liposarcoma	Left arm	Perineum and buttock	6-64
1971	M	76	Leiomyosarcoma	Small intestine	Liver	10-69
1972	M	89	Leiomyosarcoma	Retro-peritonal region	Hepatic system	7-72
1976	M	53	Fibrosarcoma	Peritioneum	Lung, liver	3-75

*Source: Michigan Department of Public Health, 1983b

report appears to put undue emphasis on non-positive studies that do not demonstrate a risk, although most of them have methodological limitations (i.e., low power, insufficient latency and inappropriate study method). In short, the British review appears to be overly optimistic about the safety of 2,4,5-T herbicides.

In summary, the associations reported in the two Swedish soft-tissue sarcoma studies are great enough to make it unlikely that they have resulted entirely from random variation bias or confounding, even though the possibility cannot be dismissed that bias or confounding was present. Therefore, the studies provide a strong suggestion that phenoxyacetic acid herbicides, chlorophenols or their impurities are carcinogenic in humans.

Malignant Lymphoma. A separate series of clinical observations at the Department of Oncology in Umea, Sweden (Hardell, 1979), led the researchers to conduct a case-control study of malignant lymphoma in relation to phenoxyacetic acid, chlorophenols, and other organic compounds (Hardell et al., 1980, 1981). Approximately 33% of the cases in this study were patients with Hodgkin's disease; the remainder of the lymphomas were non-Hodgkin's forms.

This study employed essentially the same methods and produced results closely comparable to these from the STS studies: statistically significant 5-fold to 6-fold relative risks in relation to phenoxyacetic acids and chlorophenols. In addition, an elevated relative risk was found in connection with exposure to organic solvents, such as benzene, trichloroethylene, and styrene. In the published report, the methods and results were incompletely documented, especially the possibility of confounding by exposure to the organic solvents.

In the update of the earlier 1980 study, Hardell et al. (1981), utilizing the same basic data source, found that 36.1% of the cases had been exposed to phenoxy herbicides or chlorophenols, while only 9.6% of their controls were so exposed. The estimated relative risk was 6.0 when matching was considered and 5.3 when matching was eliminated. When cases and controls who were exposed to chlorophenols only were excluded, the relative risk of lymphoma from phenoxy acids alone was 4.8 (95% C.I. 2.9-8.1). On the other hand, if phenoxy acids are excluded and consideration is given to just chlorophenols (which includes combined exposure to phenoxy acids and chlorophenols), then the relative risk equaled 4.3 (95% C.I. 2.7-6.9). The author further subdivided this group into "low-grade" vs. "high-grade" exposures to chlorophenols. A continuous exposure of not more than 1 week or repeated intermittent exposures totaling not more than 1 month was classified as low-grade. The relative risk for high-grade exposure was 8.4 (95% C.I. 4.2-16.9), while that for low-grade exposure equaled 9.2 (95% C.I. 1.6-5.2). If exposure to organic solvents is examined, given that cases and controls exposed to only phenoxy acids and/or chlorophenols were excluded except for combined exposure to organic solvents, it is found that high-grade and low-grade relative risks were 2.8 (95% C.I. 1.6-4.8) and 1.2 (95% C.I. 0.5-2.6), respectively. However, the author notes that exposure to phenoxy acids and high-grade organic solvents (exposure to chlorophenols excluded) produced a relative risk of 11.2 (95% C.I. 3.2-39.7) based upon a few cases and controls with exposure to both. The authors concluded that "exposure to organic solvents, chlorophenols and/or phenoxy acids constitutes a risk factor for malignant lymphoma."

This latter study is still subject to the same methodological criticisms to which the earlier study was subjected. Chief among those is the possibility of observational and/or recall bias creeping into the responses that are elicited from self-administered questionnaires on kind and length of exposure. *Secondly, confounding by exposure* to potentially carcinogenic organic solvents and other agents could have had an effect, although the author assures the reader that they did not.

Other research has tentatively suggested that lumberjacks may be at increased risk of lymphoma (Edling and Granstam, 1979). The Nitro study found three deaths from cancers of the lymphatic and hematopoietic system, against only 0.88 expected ($P = 0.06$, one-tailed Poisson test).

The lymphoma case-control study (Hardell et al., 1980, 1981) is consistent with the two STS studies discussed above. On the other hand, the consistency could also reflect an as-yet unidentified methodologic flaw in all these studies.

The two Swedish case control studies on STSs and a later case control study of malignant lymphoma (Hardell et al., 1981) were subjected to a validity analysis with respect to the assessment of exposure by Hardell and Eriksson (1981). To answer the question raised regarding the recall of occupation in a forestry/agriculture job, secondary to the recall of exposure to phenoxy acids and/or chlorophenols, the cases and controls were divided into three groups: those who worked their entire time since 1950 in an agriculture/forestry job, those who worked some time in an agriculture/forestry job but not exclusively, and the remainder who never worked in a forestry/agriculture job. The study found that the risk ratio was still 8.2 for STS in exclusively agriculture/forestry workers who were exposed to phenoxy acids compared to workers found in other occupations having no apparent exposure to phenoxy acids or chlorophenols. Even when comparing

phenoxy acid and/or chlorophenol exposed agricultural/forestry workers exclusively with non-exposed agricultural/forestry workers, the risk ratio was still 7.1. This argument seems to answer effectively questions regarding recall of occupation secondary to exposure.

On the other hand, the relative risk remains 5.4 when comparing phenoxy acid and/or chlorophenol exposed workers exclusively in occupations other than agriculture/forestry with non-exposed workers in those same occupations, thus, suggesting the presence of either recall bias or still another occupation with potential exposure to phenoxy acids and/or chlorophenols (Table 17).

When woodworkers are separated out (possible exposure to chlorophenols in treatment of wood) the risk ratio becomes 9.7 (Table 18). These data suggest the presence of some recall bias.

Another focus of this study was to determine if observational bias on the part of the investigators could explain the significantly high risk estimates. To answer the question, the study compared the exposure data derived from the interviewee's returned questionnaires only with the combined information from both the phone interviews and questionnaires. The study found no substantial differences in the frequency of reporting exposure.

Still a third consideration of possible bias involves recall of exposure to phenoxy acids and/or chlorophenols because of subject knowledge of having cancer in the cases versus no knowledge of cancer in the referent population. The study chose as a referent group for the 52 STS cases (Hardell and Sandstrom, 1979) and the 169 malignant lymphomas (Hardell et al., 1981) a group of 154 colon cancer cases from the same population source and compared their exposure to phenoxy acids and/or chlorophenols by broad age groupings, and by rural vs. urban residence.

TABLE 17

Other Occupations (Minus Forestry/Agriculture)*

Group	Phenoxy Acids/Chlorophenols	Non-exposed
Cases	11	68
Referents	5	167
	RR = 5.4	X^2 = 11.01 ($P<0.01$)

*Source: Hardell and Erikkson, 1981

TABLE 18

Other Occupations (Minus Forestry/Agriculture/Woodworkers)*

Group	Phenoxy Acids/Chlorophenols	Non-exposed
Cases	4	66
Referents	1	160
	RR = 9.7	X^2 = 5.98 ($P<0.05$)

*Source: Hardell and Erikkson, 1981

Utilizing a Mantel-Haenszel rate ratio, the study found the risk of exposure to phenoxy acids remaining significantly high at 5.5 and to chlorophenols 5.4 in the STS cases compared to the colon cancer controls. Similarly, with the malignant lymphomas, the identically derived risk ratios remain significantly high at 4.5 with respect to phenoxy acids and/or chlorophenol exposure in the cases, hence, the study concludes, no "substantial observational bias" exists. If the study is assuming that recall bias was and is the same as observational bias, then such a conclusion may not be entirely warranted from the comparison. Certainly, it appears that no recall bias existed because of subject "knowledge of having cancer" based on the authors analysis. But it does not rule out the possibility that recall bias can still be present in their data for other reasons. Hardell refers to an intense "debate about phenoxy acids and their presumptive risk" in Sweden at the time the colon cancer study was conducted. But, there is no reason to think that colon cancer victims would assume their disease was brought about from exposure to dioxin containing chemicals if no connection was suggested.

It seems plausible that STS and/or non-Hodgkin's lymphoma patients would either learn at the time of their diagnosis that exposure to dioxin containing chemicals was the likely cause of this rare type of tumor or quickly learn from other sources, such as the news media, that exposure to herbicides containing dioxin could cause their rare form of cancer. Whereas, colon cancer victims (a rather common form of cancer) would not necessarily be led to believe that exposure to the same dioxin containing chemicals caused their disease. Hence, it is not difficult to imagine that such unusual victims of cancer could better "remember" exposure to such chemicals than could colon cancer patients.

Therefore, although this study may explain any biases introduced from secondary recall of occupation, observational bias introduced from the telephone interviewer and recall bias due to subject knowledge of cancer, it does not adequately answer questions of recall bias introduced through the acquired awareness on the part of the victim of STS or non-Hodgkin's lymphoma that his condition may have been caused by exposure to dioxin containing herbicides.

Stomach Cancer. Studies of two of the oldest cohorts of workers known to have been exposed to phenoxyacetic acid herbicides and/or 2,3,7,8-TCDD report stomach cancer mortality rates significantly higher than expected, but the results in each study were based on small numbers of deaths. In one study (Axelson et al., 1980), 348 Swedish railroad workers with at least 46 days of herbicide exposure between 1955 and 1972 were followed through October 1978. The workers were grouped on the basis of their primary herbicide exposures: those primarily exposed to phenoxyacetic acids (2,4-D and 2,4,5-T) only, to amitrole (aminotriazole) only, and to both types of herbicides. After a 10-year latency was achieved, 3 stomach cancer deaths were observed vs. 0.71 expected ($P<0.05$). None were attributable to amitrol alone, but two were assigned to phenoxy acids alone while the remaining stomach cancer death occurred in a worker exposed to both amitrol and phenoxy acids in combination. The excess was more pronounced (3 observed vs. 0.57 expected, $P<0.05$) among those with early exposure (1957-1961) to phenoxy acids and/or amitrol. If persons who were exposed to just amitrol alone are excluded, thus leaving individuals exposed to phenoxy acid alone and amitrol in combination, the excess is enhanced further (3 observed vs. 0.41 expected, $P<0.01$).

Axelson et al. (1980) also notes an excess in total "tumors" after 10 years latency as well (15 observed vs. 6.87 expected, P<0.005). This is pronounced in those exposed early to phenoxy acids alone (6 observed vs. 2.60 expected, P<0.01) and phenoxy acids in combination with amitrol (5 observed vs. 1.34 expected, P<0.05). Presumably, "tumors" in Sweden are analogous to malignant neoplasms in the United States. The author states that no specific type of tumor predominates and no breakdown by tumor type is provided.

The other study showing increased stomach cancer mortality is the follow-up of 75 workers exposed to 2,3,7,8-TCDD during and after a 1953 run-away reaction at a trichlorophenol manufacturing facility in Ludwigshafen, Federal Republic of Germany (Theiss and Frentzel-Beyme, 1977). Two sources were used to calculate expected deaths: national mortality rates for the period 1971-1974, and 1972-1975 rates for Rhinehessen-Palatinate, the region in which Ludwigshafen is located.*

The results, shown in Table 19, indicate an increased rate of stomach cancer mortality that also is not likely to have been due to chance alone.

Two aspects of the methodology used should be noted that could have influenced these results. First, the available report does not include an analysis allowing for a minimum period of cancer induction. It is known that all three stomach cancer deaths in the Ludwigshafen cohort occurred more than 10 years after initial exposure. Employing a 10-year restriction to follow-up (as in the Swedish cohort study) would result in a higher relative risk estimate by reducing the number of expected deaths.

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*The report originally included expected deaths using rates for the city of Ludwigshafen, which were later shown to be inaccurate.

TABLE 19

Analysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

Source for Expected Deaths	Stomach Cancer Deaths		Relative Risk	Significance Level
	Observed	Expected		
Federal Republic of Germany 1971-1974	3	0.559	5.4	0.02
Rhinehessen-Palatinate 1972-1975	3	0.495	6.1	0.01

*Source: Theiss and Frentzel-Beyme, 1977

Secondly, national and regional mortality rates from the 1970's were used to generate expected deaths to compare with observed mortality over a much longer period (1953-1977). Strong temporal trends in stomach cancer mortality in West Germany during the late 1950's and 1960's would make these expected figures inaccurate. Without knowledge of such trends, the direction and magnitude (if any) of this possible source of bias cannot be estimated.

The researchers also used an internal control group which does not raise the second concern discussed above. This group consisted of 75 men, each matched to study group members by age and date of entry into employment, and selected at random from a list of over 10,000 persons who had been included in previous cohort studies by the same investigators. No stomach cancer deaths occurred in this control group during the follow-up period. Thus, use of the internal control groups also indicates an excess of stomach cancers in the exposed workers.

In an update of this earlier study, Theiss et al. (1982) continued the follow-up of his cohort through 1979 by adding 2 additional years of follow-up and apparently reducing the size of his cohort from 75 to 74. Altogether 21 deaths (4 more than from the earlier study) occurred vs. 18 and 19 deaths in the 2 matched (1 to 1) internal comparison groups. With respect to cancer deaths, the numbers were respectively 7, 5 and 5. The first control group was manually matched from the total number of persons (5500 included in the cohort until the end of 1976) and the second, at random, by computer for some 8000 employees. In addition, 19 expected total deaths were estimated based on 1970-1975 mortality statistics of Rhinehessin-Palatinate, 18 expected deaths based on 1970-1975 mortality statistics of Ludwigshafen, and 20 expected deaths based upon 1971-1974 mortality statistics of the Federal

Republic of Germany. Just as in the earlier study, the three stomach carcinomas noted earlier appear to be significantly elevated irregardless of which external comparison group is used (Table 20).

On the other hand, one stomach cancer appeared in the randomized internal control group. None appeared in the manually matched internal control. No other elevated risks for any other cause were evident and no STSs appeared. When latency was considered only, the risk of stomach cancer remained significantly elevated after a lapse of 10 years (3 observed, 0.52 expected, P<.016) and then after a lapse of 15 years (2 observed, 0.23 expected, P<.02) based upon death rates of Rhinehessin-Palatinate, 1970-1975.

Again, these study conclusions are limited by the small size of the study group and the very few cancer deaths noted at any particular site. Thus, it is insensitive to the detection of a significantly elevated risk for most causes of cancer, especially STS and lymphomas. Although, stomach cancer is elevated significantly, it is based only upon three deaths and since one stomach cancer death has been noted in an internal control group in the updated version, it appears that this finding has been weakened somewhat. Furthermore, as was pointed out earlier, trends in stomach cancer mortality during the 1950's, 1960's and 1970's could make the comparison of stomach cancer mortality with expected deaths less valid based upon 1970-1975 rates.

In summary, the evidence that phenoxyacetic acids and/or 2,3,7,8-TCDD might increase the risk of stomach cancer consists of two studies, each of which reports a statistically significant excess that is based on only three stomach cancer deaths. Further follow-up of these and similar cohorts is warranted, but firm conclusions cannot be made on the basis of the available data.

TABLE 20

Reanalysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

Source for Expected Deaths	Stomach Cancer Deaths		Relative Risk	Significance Level
	Observed	Expected		
Federal Republic of Germany 1971-1974	3	0.7	4.3	0.034
Rhinehessin-Palatinate 1970-1975	3	0.64	4.7	0.027
Ludwigs-Shafen 1970-1975	3	0.61	4.9	0.024

*Source: Theiss et al., 1982

Four additional cohort studies have reported results that do not show increased stomach cancer mortality rates in groups of workers exposed to phenoxyacetic acids and/or 2,3,7,8-TCDD. These are studies of 2,4,5-T production workers in Midland, Michigan (Ott et al., 1980), Finnish phenoxyacetic acid herbicide applicators (Riihimaki et al., 1978), the Nitro study in which workers were exposed to 2,3,7,8-TCDD (Zack and Suskind, 1980) and trichlorophenol manufacturing workers (Cook et al., 1980a).

As previously mentioned, the Nitro study included a single death from STS and a weakly suggestive increase in lymphatic and hematopoietic system cancer mortality. The Midland study included only one cancer death, a tumor in the respiratory system. In the Finnish study, histologic information on tumor types was not provided; however, there were no deaths from lymphoma.

The results pertinent to stomach cancer mortality in the three studies are shown in Table 21. Neither the Midland study nor the Nitro study contradicts the findings of the Swedish and West German investigations previously discussed. This can be shown in two ways. First, the upper 95% confidence limits for the relative risk estimates from these two "negative" studies exceed even the highest point estimates of relative risk (6.1) from the two "positive" studies (see Tables 14 and 19).

This indicates that the relative risk estimates from the Midland and Nitro studies, even though equal to zero, are nevertheless not significantly different from the estimates of 6.1, given the sample sizes, follow-up periods, age distribution and comparison group rates.

TABLE 21

Stomach Cancer Mortality in Three Studies of Workers Exposed to Phenoxyacetic Acid Herbicides and/or 2,3,7,8-TCDD

Stomach Cancer Deaths		Relative Risk	95% Confidence Interval	Reference
Observed	Expected			
0	0.14[a]	0	0-26.3	Ott et al., 1980
5	6.9[a,b]	0.7	0.2-1.7	Riihimaki et al., 1978
0	0.5[b]	0	0-7.4	Zack and Suskind, 1980

[a]Estimated from total cancer expected deaths (see footnote in text).

[b]Entire follow-up period without regard for minimum time for cancer induction (Ott et al., 1980 used a 10-year minimum induction period).

In addition, the smallest relative risk in the Midland study (α = 0.05, φ = 0.2 one-tailed Poisson test) was 21.4 (3 observed deaths, 0.14 expected).* Similarly, the smallest detectable relative risk in the Nitro study (α = 0.05, φ = 0.2, one-tailed Poisson test) was 10.0 (5 observed deaths, 0.5 expected). This calculation, however, was based on results for the entire follow-up period. If, as in the Midland study, a minimum period of cancer induction had been employed, the expected deaths would have been fewer and the smallest reasonably detectable relative risk would have been greater. This analysis of statistical power indicates that the Nitro and Midland studies had very low probabilities of detecting the ~6-fold increases in risk suggested by the Swedish and West German investigations.

Statistically, the study of Finnish herbicide applicators is inconsistent with the results of the Swedish and West German cohort studies. The smallest reasonably detectable relative risk (α = 0.05, φ = 0.2, one-tailed Poisson test) was only 3.1 (11 observed deaths, 3.6 expected).† The study, therefore, appears powerful enough to detect relative risks even smaller than those seen in the Swedish and West German studies. A partial explanation for this apparent inconsistency could lie in the fact that the

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*Ott et al. (1980) did not report expected deaths from stomach cancers. The figure 0.14 was obtained by multiplying the numbers of expected deaths from all cancers (2.6, allowing a 10-year minimum induction period) by the percentage of stomach cancers among the expected deaths in the Nitro study (0.5/9.04 = 5.5%). The two studies used United States white male mortality rates and covered similar calendar years in follow-up (1949-1978 in Nitro and 1950-1976 in Midland), but a similarity in age distributions cannot be established from the published reports.

†The expected stomach cancer deaths were estimated in the same manner as for the Midland study. A proportion of 20% of all cancer deaths was applied because Finnish male mortality rates are known to be very high.

Finnish study set the minimum period of herbicide exposure for membership in the cohort at 10 days (2 working weeks) and noted that the "total strength of exposure has, in most cases, been a few weeks only." The Swedish study of herbicide applicators set the minimum exposure at 46 days (>1 spraying season).

There are also certain inconsistencies in the data from the Finnish study which the authors note but find difficult to explain. In particular, no cancer deaths occurred during the latter part of the study period among Forestry Authority workers (1 of 4 groups included in the cohort), even though 9.0 deaths were expected. This finding strongly suggests some deficiency in follow-up or in the source records from which vital status was determined.

In summary, four cohort studies of workers exposed to phenoxyacetic acid herbicides and/or 2,3,7,8-TCDD do not report increased risks of stomach cancer. Only one of these, however, was statistically powerful enough to be inconsistent with the two studies that tentatively suggest an increase in stomach cancer risk. The available report of this study of Finnish herbicide applicators contains methodologic questions that require clarification.

Summary of Epidemiological Studies. The net result of adding together the number of workers exposed to phenoxy acids and/or chlorophenols from all case studies was an unusually high number of STSs, considering the rarity of the disease. It is suggestive of an association of cancer with exposure to phenoxy acids and/or chlorophenols, and consequently, with the impurities found in these herbicides, including 2,3,7,8-TCDD.

Two Swedish case-control studies report highly significant association of STS with exposure to phenoxy acid and/or chlorophenols. They do not pinpoint the risk to the dioxin contaminants, however. In fact, in one study,

the risk was found to extend to phenoxy acids free of dioxin impurities. In that study, the risk increases to 17 when phenoxy acids known to contain dioxin impurities (polychlorinated dibenzodioxins and dibenzofurans) are considered. The extent of observer bias and recall bias introduced into these studies by the employment of an undesirable methodology (self-administered questionnaires) is probably not of sufficient magnitude to have produced the highly significant risks found in the studies. However, the possibility exists that these biases could have played a role in the determination of these risks, and consequently the data must be considered limited for the carcinogenicity of phenoxy acid herbicides and/or chlorophenols in the absence of confirmatory studies.

Later studies that did not reveal a significant excess risk of STS have severe limitations with their methodologies. These problems make these latter studies inadequate to evaluate the risk of STSs from exposure to phenoxy acids and/or chlorophenols and, consequently, 2,3,7,8-TCDD.

Therefore, the Swedish case-control studies provide limited evidence for the carcinogenicity of phenoxy acids and/or chlorophenols in humans. However, the evidence for the human carcinogenicity for 2,3,7,8-TCDD based on the epidemiologic studies is only suggestive due to the difficulty of evaluating the risk of 2,3,7,8-TCDD exposure in the presence of the confounding effects of phenoxy acids and/or chlorophenol.

Substantially weaker evidence exists incriminating 2,4,5-T and/or 2,3,7,8-TCDD as the cause of malignant lymphoma and stomach cancer in humans.

Studies in Animals

When outbred Swiss mice were given weekly doses of 2,3,7,8-TCDD by gavage, an increase in liver tumors was observed (Toth et al., 1979).

Animals receiving 0.007 μg/kg/week for 1 year showed an elevated tumor incidence over vehicle-treated mice; in the 0.7 μg/kg/week group the increase was statistically significant ($P<0.005$). Mortality was sufficiently high at 7.0 μg/kg/week as to interfere with carcinogenicity evaluation.

DiGiovanni et al. (1977) reported a mouse skin painting study. The authors indicated that 2,3,7,8-TCDD was a weak initiator on the skin.

A bioassay of 2,3,7,8-TCDD for possible carcinogenicity was conducted by the Illinois Institute of Technology Research, Chicago, Illinois, on a contract with the NCI Carcinogenesis Testing Program by dermal administration of the test material in Swiss-Webster mice for 104 weeks (U.S. DHHS, 1980a). Thirty male and female Swiss-Webster mice were dermally treated with an acetone suspension of 2,3,7,8-TCDD for 3 days/week for 104 weeks. Similar groups were pretreated with 1 application of 50 μg dimethylbenzanthracene (DMBA) in 0.1 mℓ acetone 1 week before 2,3,7,8-TCDD administration began. Female mice received 0.005 μg 2,3,7,8-TCDD/application, and the male mice received 0.001 μg 2,3,7,8-TCDD. As vehicle controls, 45 mice of each sex received 0.1 mℓ acetone 3 times/week. Thirty animals of each sex were used as untreated controls (Tables 22 and 23).

Throughout the bioassay, mean body weights of the male or female groups of mice administered 2,3,7,8-TCDD, or 2,3,7,8-TCDD following DMBA, were essentially the same as those of the corresponding vehicle-control group. Mean body weights of dosed and vehicle-control groups of the females were less than those of the untreated control group throughout the study, and for the males were less than mean body weights of untreated controls during the first 80 weeks.

In female mice, the incidence of fibrosarcoma in the integumentary system in groups dosed with 2,3,7,8-TCDD and 2,3,7,8-TCDD following DMBA was significantly higher than that in the corresponding controls (see Table 22).

TABLE 22

Incidence of Primary Tumors in Female Swiss-Webster Mice by Dermal Application of 2,3,7,8-TCDD or 2,3,7,8-TCDD Following DMBA[a]

Tissue: Types of Neoplastic Growth	Vehicle Control	2,3,7,8-TCDD	2,3,7,8-TCDD plus DMBA
Integumentary System:			
Fibrosarcoma	2/41 (5%)	8/27 (30%)[b]	8/29 (28%)[c]
Lung:			
Alveolar/Bronchiolar Adenoma	4/41 (10%)	1/25 (4%)	3/28 (11%)
Alveolar/Bronchiolar Carcinoma	5/41 (12%)	1/25 (4%)	3/28 (11%)
Alveolar/Bronchiolar Carcinoma or Adenoma	9/41 (22%)	2/25 (8%)	6/28 (21%)
Hematopoietic System:			
Lymphoma	14/41 (34%)	10/27 (37%)	8/29 (28%)
All Sites:			
Hemangioma	2/41 (5%)	0/27 (0%)	1/29 (3%)
Hemangioma or Hemangiosarcoma	3/41 (7%)	0/27 (0%)	1/29 (3%)

[a]Source: U.S. DHHS, 1980a

[b]$p<0.007$

[c]$p<0.010$

TABLE 23

Incidence of Primary Tumors in Male Swiss-Webster Mice by Dermal Application of 2,3,7,8-TCDD or 2,3,7,8-TCDD Following DMBA*

Tissue: Types of Neoplastic Growth	Vehicle Control	2,3,7,8-TCDD	2,3,7,8-TCDD plus DMBA
Integumentary System:			
Fibrosarcoma	3/42 (7%)	6/28 (21%)	6/30 (20%)
Lung:			
Alveolar/Bronchiolar Adenoma	6/41 (15%)	1/28 (4%)	5/29 (17%)
Alveolar/Bronchiolar Carcinoma	1/41 (2%)	1/28 (4%)	2/29 (7%)
Alveolar/Bronchiolar Carcinoma or Adenoma	7/41 (17%)	2/28 (7%)	6/29 (21%)
Hematopoietic System:			
Lymphoma or Leukemia	4/42 (10%)	2/28 (7%)	5/30 (17%)
All Sites:			
Hemangiosarcoma	1/42 (2%)	4/28 (14%)	0/30 (0%)

*Source: U.S. DHHS, 1980a

It was concluded that, under the conditions of this bioassay, 2,3,7,8-TCDD applied to the skin was carcinogenic for female Swiss-Webster mice, inducing increased incidences of fibrosarcoma in the integumentary system (U.S. DHHS, 1980a).

Van Miller et al. (1977a,b) administered 0, 1, 5, 50, 1000 and 5000 ppt of 2,3,7,8-TCDD in the diet of male Sprague-Dawley rats. Higher concentrations (50, 500 and 1000 ppb) were also administered, but all of the rats fed at those three highest concentrations died early in the test. After 65 weeks, all surviving animals underwent laparotomies, and all tumors were biopsied. The rats were kept on the 2,3,7,8-TCDD diet for a total of 78 weeks and then placed on the control diet. After a total of 95 weeks all surviving animals were sacrificed and necropsied. The results of the study are summarized in Table 24.

Although the study by Van Miller et al. (1977a,b) demonstrated the occurrence of neoplasms upon 2,3,7,8-TCDD exposure, the study has a number of shortcomings. The protocol was unusual and only a small number of animals were used. The occurrence of tumors did not follow a clear-cut dose-response relationship. Furthermore, the complete absence of tumors in the controls is a highly unusual finding.

Kociba et al. (1978) reported a more extensive carcinogenicity study on 2,3,7,8-TCDD. Groups of 100 Sprague-Dawley rats (Spartan substrain, 50 males and 50 females/group) were maintained for up to 2 years on diets supplying 0.1, 0.01 or 0.001 μg 2,3,7,8-TCDD/kg/day. The control group consisted of 86 males and 85 females. The terminal necrospy examination was conducted at the end of 2 years of treatment. Females recieving 0.1 μg 2,3,7,8-TCDD/kg/day experienced a greater mortality than controls during the second half of the study. Extensive clinical chemistry data were reported as part of the study.

TABLE 24

Summary of Neoplastic Changes After TCDD in Rats[a]

Concentration of 2,3,7,8-TCDD in Diet (ppt)	Approximate Daily Dose µg/kg	No. of Animals with Neoplasms[b]	No. of Neoplasms	Diagnosis
0	0.0	0	0	NA
1	0.00004	0	0	NA
5	0.0001	5	6	1 ear duct carcinoma
				1 lymphocytic leukemia
				1 adenocarcinoma (kidney)
				1 malignant histiocytoma (peritoneal)
				1 angiosarcoma (skin)
				1 Leydig cell adenoma
50	0.0014	3	3	1 fibrosarcoma (muscle)
				1 squamous cell tumor (skin)
				1 astrocytoma (brain)
500	0.014	4	4	1 fibroma (muscle)
				1 carcinoma (skin)
				1 adenocarcinoma (kidney)
				1 sclerosing seminoma (testes)

TABLE 24 (cont.)

Concentration of 2,3,7,8-TCDD in Diet (ppt)	Approximate Daily Dose µg/kg	No. of Animals with Neoplasms[b]	No. of Neoplasms	Diagnosis
1000	0.057	4	5	1 cholangiocarcinoma (liver)
				1 angiosarcoma (skin)
				1 glioblastoma (brain)
				2 malignant histiocytoma (peritoneal)
5000	0.29	7	10	4 squamous cell tumors (lung)
				4 neoplastic nodules (liver)
				2 cholangiocarcinomas (liver)

[a]Source: Van Miller et al., 1977a

[b]10 animals per group

NA = Not applicable

A portion of the data for the histopathologic lesions found is summarized in Table 25. The only lesions that are listed are those which were statistically different from control levels for at least one dose and in one sex. The following neoplastic lesions were found to be increased above control levels ($P<0.05$):

Hepatocellular hyperplastic (neoplastic) nodules	- females only
Hepatocellular carcinomas	- females only
Stratified squamous cell carcinoma of palate or nasal turbinae	- males and females
Keratinizing squamous carcinoma of the lung	- females only
Stratified squamous carcinoma of the tongue	- males only
Adrenal cortical ademona	- males only

Dr. Robert Squire, pathologist at the Johns Hopkins University Medical School and consultant to the U.S EPA Carcinogen Assessment Group (CAG), evaluated the histopathologic slides from 2-year rat feeding studies on 2,3,7,8-TCDD by Kociba et al. (1978). Squire and his associates examined all livers, tongues, hard palates and nasal turbinates, and lungs available from the 2,3,7,8-TCDD study. His histopathological findings, as well as Kociba's histopathological evaluations, are summarized in Tables 26 and 27. Although there are some differences between the diagnoses of Kociba and Squire, the conclusions about the target organ for cancer induction and the dose levels at which induction occurred are the same whether Squire's or Kociba's diagnoses are considered.

A bioassay of 2,3,7,8-TCDD for possible carcinogenicity was conducted by administering the test material by gavage to Osborne-Mendel rats and $B6C3F_1$ mice for 104 weeks (U.S. DHHS, 1980b). Fifty rats and mice of each

TABLE 25

Summary of Neoplastic Lesions Produced by 2,3,7,8-TCDD in Sprague-Dawley Rats, Spartan Substrain that are Statistically Significant in at Least One Sex[a]

	Males				Females			
Dose µg/kg/day	0	0.001	0.01	0.1	0	0.001	0.01	0.1
Number of Animals	85	50	50	50	86	50	50	50
Hepatocellular hyperplastic nodule	6	0	2	3	8	3	18[b]	23[b]
Hepatocellular carcinomas	2	0	0	1	1	0	2	11[b]
Stratified squamous carcinoma-palate (including nasal turbinate tumors)	0	0	0	4[b]	0	0	1	4[b]
Lung-keratinizing squamous carcinoma	0	0	0	1	0	0	0	7[b]
Benign tumor-uterus	-	-	-	-	28	12	11	7[b]
Subcutaneous fibroma/fibroadenoma/lipoma	10	1[b]	5	6	1	1	0	0
Benign mammary neoplasm	0	0	1	2	73	35	36	24[b]
Mammary carcinoma	0	0	0	2	8	4	4	0
Stratified squamous carcinoma-tongue	0	1	1	3[b]	1	0	0	2
Pituitary adenoma	26	6	11	13	43	18	13	12[b]
Acinar adenoma pancreas	14	7	5	2[b]	0	1	0	1
Adenoma-adrenal cortex	0	0	2	5[b]	9	6	2	5
Pheochromocytoma	28	6	10	4[b]	7	2	1	3

[a]Source: Kociba et al., 1978

[b]Significantly different ($p<0.05$) from control by the Fisher exact text

TABLE 26

2,3,7,8-TCDD Oral Rat Study by Dr. Kociba, with Dr. Squire's Review (8/15/80)
Female Sprague-Dawley Rats - Spartan Substrain (2 years)

Tissues and Diagnoses*	Dose Levels (μg/kg/day)							
	0 (control)		0.001		0.01		0.1	
	S	K	S	K	S	K	S	K
Lung squamous cell carcinomas	0/86	0/86	0/50	0/50	0/49	0/49	8/47 ($P<10^{-3}$)	7/49 ($P<10^{-3}$)
Nasal turbinate/ hard palate squamous cell carcinomas	0/54	1/54	0/30	0/30	1/27	1/27	5/22 ($P<10^{-2}$)	5/24 ($P<10^{-2}$)
Liver Neoplastic nodules/ hepatocellular carcinomas	16/86	9/86	8/50	3/50	27/50 ($P<10^{-4}$)	18/50 ($P<10^{-3}$)	33/47 ($P<10^{-8}$)	34/48 ($P<10^{-12}$)
Total combined (1,2, or above) (each animal had at least one tumor above)	16/86	9/86	8/50	3/50	27/50 ($P<10^{-4}$)	18/50 ($P<10^{-3}$)	34/47 ($P<10^{-8}$)	34/49 ($P<10^{-11}$)

*Where result is significantly different than the appropriate control, probability (P) of incorrectly rejecting the null hypothesis is approximated in parentheses.

S = Dr. Squire's histopathologic analysis; K = Dr. Kociba's histopathologic analysis

TABLE 27

2,3,7,8-TCDD Oral Rat Study by Dr. Kociba, with Dr. Squire's Review (8/15/80)
Male Sprague-Dawley Rats - Spartan Substrain (2 years)

Tissues and Diagnoses*	Dose Levels (μg/kg/day)							
	0 (control)		0.001		0.01		0.1	
	S	K	S	K	S	K	S	K
Nasal turbinate/ hard palate squamous cell carcinomas	0/55	0/51	1/34	1/34	0/26	0/27	6/30 ($P<10^{-2}$)	4/30
Tongue squamous cell carcinomas	0/77	0/76	2/44	1/49	1/49	1/49	3/44 ($P<0.05$)	3/42 ($P<10^{-3}$)
Total - 1 or 2 above (each rat had at least one tumor above)	0/77	0/76	2/44	2/49	1/49	1/49	9/44 ($P<10^{-4}$)	7/42

*Where result is significantly different than the appropriate control, probability (P) of incorrectly rejecting the null hypothesis is approximated in parentheses.

S = Dr. Squire's histopathologic analysis; K = Dr. Kociba's histopathologic analysis

sex were administered 2,3,7,8-TCDD suspended in a vehicle of 9:1 corn oil:acetone 2 days/week for 104 weeks at doses of 0.01, 0.05 or 0.5 μg/kg/week for rats and male mice and 0.04, 0.2 or 2.0 μg/kg/week for female mice. Seventy-five rats and 75 mice of each sex served as vehicle controls. One untreated control group containing 25 rats and 25 mice of each sex was present in the 2,3,7,8-TCDD treatment room, and one untreated control group containing 25 rats and 25 mice of each sex was present in the vehicle control room. All surviving animals were killed at 105-107 weeks.

In rats, a dose-related depression in mean body weight gain became evident in the males after week 55 of the bioassay and in the females after week 45. In mice, the mean body weight gain in the dosed groups was comparable with that of the vehicle-control groups, but it was lower than that of the untreated controls.

In male rats, increased incidences of follicular-cell adenoma or carcinomas in the thyroid were dose related and were significantly higher in the low-, mid- and high-dose groups (P=0.001 for high dose) than in the vehicles controls (Table 28). A significant increase in the subcutaneous tissue fibroma was found for the high-dose group (P=0.048).

In female rats, the incidences of follicular-cell adenomas of the thyroid and hepatocellular carcinomas or neoplastic nodules of the liver were dose-related, and the incidence of hepatocellular carcinomas in the high-dose group was significantly higher (P=0.001) than that in the vehicle controls (Table 29), as were the incidences of subcutaneous tissue fibrosarcoma (P=0.023) and adrenal cortical adenoma (P=0.039).

In both male and female mice, incidences of hepatocellular adenomas or carcinomas were dose related and the incidences in the high-dose groups were higher (P=0.001 male, P=0.002 female) than those in the corresponding vehicle controls (Tables 30 and 31).

TABLE 28

Incidence of Primary Tumors in Male Osborne-Mendel Rats
(2,3,7,8-TCDD Administered by Gavage)[a,b]

Tissue: Types of Neoplastic Growth	Vehicle Control	Dose (μg/kg/wk)[c]		
		0.01	0.05	0.5
Subcutaneous Tissue:				
Fibroma or Fibrosarcoma	12/75(16)	4/50(8)	5/50(10)	10/50(20)
Fibroma	3/75(4)	1/50(2)	3/50(6)	7/50(14)[d]
Fibrosarcoma	9/75(12)	3/50(6)	3/50(6)	3/50(6)
Circulatory System:				
Hemangioma or Hemangiosarcoma	7/75(9)	3/50(6)	1/50(2)	4/50(8)
Hemangiosarcoma	4/75(5)	3/50(6)	0/50(0)	4/50(8)
Liver:				
Neoplastic Nodule or Hepatocellular Carcinoma	0/74(0)	0/50(0)	0/50(0)	3/50(6)
Neoplastic nodule	0/74(0)	0/50(0)	0/50(0)	3/50(6)
Pituitary:				
Adenoma or Chromophobe Adenoma	2/61(3)	1/43(2)	3/43(7)	3/40(8)
Adenoma	0/61(0)	1/43(2)	2/43(5)	3/40(8)
Adrenal:				
Cortical Adenoma	6/72(8)	9/50(18)	12/49(24)	9/49(18)
Pheochromcytoma	5/72(7)	0/50(0)	1/49(2)	1/49(2)
Thyroid:				
Follicular Cell Adenoma or Carcinoma	1/69(1)	5/48(10)[e]	8/50(16)[f]	11/50(22)[g]
Follicular Cell Adenoma	1/69(1)	5/48(10)	6/50(12)	10/50(20)
C-Cell Adenoma	2/69(3)	2/48(4)	4/50(8)	4/50(8)
C-Cell Adenoma or Carcinoma	2/69(3)	2/48(4)	5/50(10)	4/50(8)
Parathyroid:				
Adenoma	0/20(0)	2/41(5)	1/40(3)	1/36(3)
Pancreatic Islets:				
Adenoma	2/70(3)	2/49(4)	3/48(6)	1/50(2)

TABLE 28 (cont.)

Tissue: Types of Neoplastic Growth	Vehicle Control	Dose (μg/kg/wk)[c]		
		0.01	0.05	0.5
Mammary Gland:				
Adenocarcinoma	0/75(0)	0/50(0)	3/50(6)	1/50(2)
Fibroadenoma	5/75(7)	0/50(0)	1/50(2)	0/50(0)

[a]Source: U.S. DHHS, 1980b

[b]Values in parentheses indicate percent response.

[c]P = Values calculated using the Fisher Exact test.

[d]P = 0.048

[e]P = 0.042

[f]P = 0.004

[g]P < 0.001

TABLE 29

Incidence of Primary Tumors in Female Osborne-Mendel Rats
(2,3,7,8-TCDD Administered by Gavage)[a,b]

Tissue: Types of Neoplastic Growth	Vehicle Control	Dose (μg/kg/wk)[c] 0.01	0.05	0.5
Subcutaneous Tissue:				
Fibroma or Fibrosarcoma	4/75(5)	2/50(4)	3/50(6)	5/49(10)
Fibroma	4/75(5)	0/50(0)	0/50(0)	1/49(2)
Fibrosarcoma	0/75(0)	2/50(4)	3/50(6)	4/49(8)[d]
Liver:				
Neoplastic Nodule or Hepatocellular Carcinoma	5/75(7)	1/49(2)	3/50(6)	14/49(29)[e]
Neoplastic nodule	5/75(7)	1/49(2)	3/50(6)	12/49(24)
Pituitary:				
Adenoma	1/66(2)	5/47(11)	2/44(5)	3/43(7)
Chromophobe Adenoma	5/66(8)	0/47(0)	0/44(0)	1/43(2)
Adrenal:				
Cortical Adenoma or Adenoma	11/73(15)	8/49(16)	4/49(8)	14/46(30)[f]
Cortical Adenoma or Carcinoma or Adenoma	11/73(15)	9/49(18)	5/49(10)	14/46(30)
Thyroid:				
Follicular Cell Adenoma	3/73(4)	2/45(4)	1/49(2)	6/47(13)
Follicular Cell Adenoma or Carcinoma	5/73(7)	2/45(4)	1/49(2)	6/47(13)
C-Cell Adenoma	7/73(10)	1/45(2)	8/49(26)	6/47(13)
C-Cell Adenoma or Carcinoma	7/73(10)	3/45(7)	8/49(16)	6/47(13)
Mammary Gland:				
Adenocarcinoma	3/75(4)	3/50(6)	2/50(4)	1/49(2)
Fibroadenoma	27/75(36)	20/50(40)	21/50(42)	17/49(35)
Brain:				
Astrocytoma	0/75(0)	3/47(6)	0/49(0)	0/48(0)

[a]Source: U.S. DHHS, 1980b

[b]Values in parentheses indicate percent response.

[c]P = Values calculated using the Fisher Exact test.

[d]P = 0.0023; [e]P = 0.001; [f]P = 0.039

TABLE 30

Incidence of Primary Tumors in Female B6CF1 Mice
(2,3,7,8-TCDD Administered by Gavage)[a,b]

Tissue: Types of Neoplastic Growth	Vehicle Control	Dose (μg/kg/wk)[c] 0.01	0.05	0.5
Subcutaneous Tissue:				
Fibrosarcoma	1/74(1)	1/50(2)	1/48(2)	5/47(11)[d]
Lung:				
Alveolar/Bronchiolar Adenoma	2/74(3)	3/49(6)	4/48(8)	1/46(2)
Alveolar/Bronchiolar Adenoma or Carcinoma	2/74(3)	3/49(8)	4/48(8)	2/46(4)
Hematopoietic System:				
Lymphocytic Lymphoma	5/74(7)	6/50(12)	4/48(8)	6/47(13)
Histiocytic Lymphoma	9/74(12)	4/50(8)	8/48(17)	14/47(30)[e]
All Lymphoma	18/74(24)	11/50(22)	13/48(27)	20/47(43)[f]
Lymphoma or Leukemia	18/74(24)	12/50(24)	13/48(27)	20/47(43)[f]
Liver:				
Hepatocellular Adenoma or Carcinoma	3/73(4)	6/50(12)	6/48(13)	11/47(23)[g]
Hepatocellular Adenoma	2/73(3)	4/50(8)	4/48(8)	5/47(11)
Hepatocellular Carcinoma	1/73(1)	2/50(4)	2/48(4)	6/47(13)
Pituitary:				
Adenoma	0/62(0)	2/39(5)	0/38(0)	2/33(6)
Thyroid:				
Follicular-Cell Adenoma	0/69(0)	3/50(6)	1/47(2)	5/46(11)[h]

[a]Source: U.S. DHHS, 1980b

[b]Values in parentheses indicate percent response.

[c]P = Values calculated using the Fisher Exact test.

[d]P = 0.032; [e]P = 0.016; [f]P = 0.029; [g]P = 0.002; [h]P = 0.009

TABLE 31

Incidence of Primary Tumors in Male B6CF1 Mice
(2,3,7,8-TCDD Administered by Gavage)[a,b]

Tissue: Types of Neoplastic Growth	Vehicle Control	Dose (μg/kg/wk)[c] 0.01	0.05	0.5
Subcutaneous Tissue:				
Fibrosarcoma or Fibroma	9/73(12)	6/49(12)	5/49(10)	3/50(6)
Fibrosarcoma	8/73(11)	5/49(10)	4/49(8)	3/50(6)
Lung:				
Alveolar/Bronchiolar Adenoma or Carcinoma	10/71(14)	2/48(4)	4/48(8)	13/50(26)
Alveolar/Bronchiolar Adenoma	7/71(10)	2/48(4)	4/48(8)	11/50(22)
Hematopoietic System:				
Histiocytic Lymphoma	5/73(7)	0/49(0)	3/49(6)	0/50(0)
Lymphoma or Leukemia	8/73(11)	3/49(6)	4/49(8)	6/50(12)
Circulatory System:				
Hemangiosarcoma	1/73(1)	2/49(4)	1/49(2)	3/50(6)
Liver:				
Hepatocellular Adenoma or Carcinoma	15/73(21)	12/49(24)	13/49(27)	27/50(54)[d]
Hepatocellular Adenoma	7/73(10)	3/49(6)	5/49(10)	10/50(20)
Hepatocellular Carcinoma	8/73(11)	9/49(18)	8/49(16)	17/50(34)
Thyroid:				
Follicular-Cell Adenoma	0/69(0)	3/48(6)	0/48(0)	0/49(0)
Eye/Lacrimal Glands:				
Adenoma	0/73(0)	1/49(2)	1/49(2)	3/50(6)

[a]Source: U.S. DHHS, 1980b

[b]Values in parentheses indicate percent response.

[c]P = Values calculated using the Fisher Exact test.

[d]$p<0.001$

In female mice, follicular-cell adenomas in the thyroid and histocytic lymphomas in the hematopoietic system occurred at dose-related incidences, and the incidences were significantly higher in the high-dose groups than those in vehicle controls. The high-dose group of females also showed a significantly higher incidence of subcutaneous fibrosarcomas (P=0.032) and lymphoma or leukemia (P=0.029) (see Table 31).

It was concluded that, under the conditions of this bioassay, 2,3,7,8-TCDD was carcinogenic for Osborne-Mendel rats, inducing significant dose-related increased incidences of follicular-cell thyroid tumors in males and liver tumors in females. 2,3,7,8-TCDD was also carcinogenic for $B6C3F_1$ mice, inducing significant dose-related increased incidences of liver tumors in males and females and of thyroid tumors in females (U.S. DHHS, 1980b).

CRITERION FORMULATION

Existing Guidelines and Standards

The National Academy of Sciences Committee on Drinking Water and Health (NAS, 1977) suggested an acceptable daily intake (ADI) of 10^{-4} µg 2,3,7,8-TCDD/kg/day. At that time, 2,3,7,8-TCDD was not considered to be a carcinogen, and the ADI was based on a 13-week feeding study in rats by Kociba et al. (1976).

The FDA has issued a health advisory stating that fish with residues of 2,3,7,8-TCDD ≥50 ppt should not be consumed, but fish with residues of <25 ppt pose no serious health concern (FDA, 1981, 1983; Cordle, 1981). Federal legal limits for Great Lakes fish distributed in interstate commerce were deemed unnecessary because most samples analyzed by the FDA contained <25 ppt. Canada has established a 20 ppt concentration limit for 2,3,7,8-TCDD in Lake Ontario commercial fish exported into the United States to comply with the levels believed by FDA to be safe. No tolerances have been established for 2,3,7,8-TCDD on food crops. A tolerance of 0.05 ppm hexachlorophene in or on cottonseeds (used as livestock feed), with a proviso that the technical grade of hexachlorophene shall not contain >0.1 ppm 2,3,7,8-TCDD, was published in 40 CFR 180.302.

The Ministry of Labour of Canada has set a tentative Ambient Air Quality criterion for PCDDs of 30 pg/m³ (Harding, 1982).

Current Levels of Exposure

The extent of human exposure to 2,3,7,8-TCDD that can be directly attributed to the water route cannot be readily determined. While 2,3,7,8-TCDD does not appear to occur naturally in the environment, it can be produced with low efficiency from the combustion of 2,4,5-T-containing mate-

rials (Stehl and Lamparski, 1977); it may also be produced in a large variety of normal combustion processes (Anonymous, 1978; Bumb et al., 1980), but it is not produced during all combustion processes (Kimble and Gross, 1980). The impact of these processes on human exposure is unknown. The high affinity of 2,3,7,8-TCDD for soils with significant organic content would seem to reduce the likelihood of groundwater contamination; however, as the organic content of soil declines the likelihood of groundwater contamination by 2,3,7,8-TCDD increases.

Contaminated beef fat samples have been found to have concentrations as high as 60 ppt of 2,3,7,8-TCDD in one sample (Ross, 1976). 2,3,7,8-TCDD residues also have been detected in the edible portions of fish from the Tittabawassee, Grand and Saginaw Rivers, Lake Michigan and the Saginaw Bay in Michigan at concentrations ranging from 4-695 ppt (Harless and Lewis, 1980).

The reports of incidents of 2,3,7,8-TCDD exposures in industrial plants and of accidents where 2,3,7,8-TCDD was more widely disseminated are useful in identifying some of the effects of 2,3,7,8-TCDD exposure in man. Unfortunately, the existing human data can only roughly estimate the extent and duration of 2,3,7,8-TCDD exposure which produced the toxic symptoms.

Special Groups at Risk

The most obvious groups at risk are those employed in the manufacture of chemicals in which 2,3,7,8-TCDD may occur as an unwanted by-product. The spraying of herbicides containing traces of 2,3,7,8-TCDD has become less of a problem because of restrictions on the use of such agents. Considering the reproductive toxicity of 2,3,7,8-TCDD, women of child-bearing age, and especially the fetus, are at high risk from exposures to 2,3,7,8-TCDD.

Basis and Derivation of Criterion

2,3,7,8-TCDD is an unusually toxic compound with demonstrated acute, subacute and chronic effects in animals and man. Acute or subchronic exposures to 2,3,7,8-TCDD can adversely affect the skin, the liver, the nervous system and the immune system.

2,3,7,8-TCDD displays an unusually high degree of reproductive toxicity. It is teratogenic, fetotoxic and reduces fertility. In a 3-generation reproductive study, Murray et al. (1979) reported a reduction in fertility after daily dosing at 0.1 or 0.01 μg 2,3,7,8-TCDD/kg in the F_1 and F_2 generations of Sprague-Dawley rats. Although Murray et al. (1979) considered the lowest dose tested, 0.001 μg/kg, to be a no-observed-effect level (NOEL), a re-evaluation of these data by Nisbet and Paxton (1982), using different statistical methods, indicated that there was a reduction in the gestation index, decreased fetal weight, increased liver to body weight ratio, and increased incidence of dilated renal pelvis at the 0.001 μg/kg dose. The re-evaluated data would suggest that equivocal adverse effects were seen at the lowest dose (0.001 μg/kg/day) and that this dose should, therefore, represent a lowest-observed-adverse-effect level (LOAEL). Schantz et al. (1979) found reductions in fertility and various other toxic effects in rhesus monkeys fed a 50 ppt 2,3,7,8-TCDD diet for 20 months. This corresponds to a calculated daily dose of 0.0015 μg 2,3,7,8-TCDD/kg/day. These results suggest that monkeys may be somewhat more sensitive than rats, since the effects in monkeys were more severe and not equivocal.

A toxicity-based criterion has been calculated for comparison with the cancer-based criterion in accordance with public comments. Since the data from the limited study by Schantz et al. (1979) are supportive of the findings by Murray et al. (1979), it seems reasonable to determine an ADI based on the LOAEL. If one selects an uncertainty factor of 100 based on the

existence of lifetime animal studies and knowledge of effects in man as per NAS (1977) guidelines, and then an additional 10 because a LOAEL is used as the basis of this calculation,* then the ADI would be:

$$ADI = \frac{10^{-3}\ \mu g/kg/day\ (LOAEL)}{100 \times 10} = 1 \times 10^{-6}\ \mu g/kg/day.$$

Thus, the acceptable daily intake for a 70 kg man would be 7.0×10^{-5} µg 2,3,7,8-TCDD/day. Using a BCF of 5000 and assuming a daily consumption of 6.5 g of fish, the water concentration corresponding to this ADI would be:

$$\text{water concentration} = \frac{7.0 \times 10^{-5}}{2 + (5000 \times 0.0065)} = 2.0 \times 10^{-6}\ \mu g/\ell.$$

However, this concentration may not be sufficiently protective of human health since it does not take into account the demonstrated carcinogenic effects of 2,3,7,8-TCDD in animals and the probability that 2,3,7,8-TCDD is a human carcinogen (see cancer-based criterion derivation).

The carcinogenic potential of 2,3,7,8-TCDD has been established by feeding studies in rodents. The results of the study by Van Miller et al. (1977a,b) are summarized in Table 24, and the findings of the more extensive study by Kociba et al. (1978) are summarized in Table 25. The Van Miller et al. (1977a,b), the Toth et al. (1979) and recent NCI data (U.S. DHHS, 1980a,b) summarized in Tables 28, 29, 30, and 31, reinforce the findings of Kociba et al. (1978) and establish that 2,3,7,8-TCDD is an animal carcinogen and is probably carcinogenic in humans.

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*According to the methods published by EPA (45 FR 79353), an additional uncertainty factor between 1 and 10 must be used because the calculation is based on a LOAEL. An uncertainty factor of 10 was chosen because of the adverse effects seen in rhesus monkeys at 0.0015 µg/kg/day, despite the equivocal nature of the effects in rats seen at the 0.001 µg/kg/day dose level.

Furthermore, the epidemiological findings (Hardell, 1977, 1979; U.S. EPA, 1980c; Hardell et al., 1980; Hardell and Sandstrom, 1979; Ericksson et al., 1979; Theiss and Frentzel-Beyme, 1977; Jirasek et al., 1973, 1974; Pazderova et al., 1974; Axelson et al., 1980; Zack and Suskind, 1980; Zack, 1980; Cook et al., 1980a) are consistent with the conclusion from animal studies that 2,3,7,8-TCDD is a probable human carcinogen. In addition, 2,3,7,8-TCDD has been shown to be a potent liver cancer promoter (Pitot et al., 1980) and a cocarcinogen (Kouri et al., 1978).

Under the Consent Decree in NRDC vs. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." 2,3,7,8-TCDD is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of 2,3,7,8-TCDD in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases, and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of 2,3,7,8-TCDD corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5}, for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the November 1980 Federal Register notice of availability of ambient water quality criteria (45 FR 79318), the U.S. EPA presented a range of concentrations for carcinogens corresponding to cancer risks of 10^{-5}, 10^{-6}

or 10^{-7}, based on the upper 95% confidence level of calculated incremental risk. The criteria for 2,3,7,8-TCDD are shown in the following table:

Exposure Assumptions (per day)	95% Upper-Limit Risk Levels and Corresponding 95% Lower-Limit Criteria (1) for 2,3,7,8-TCDD (μg/ℓ)			
	0	10^{-7}	10^{-6}	10^{-5}
2 ℓ of drinking water and consumption of 6.5 g fish and shellfish. (2)	0	1.3×10^{-9}	1.3×10^{-8}	1.3×10^{-7}
Consumption of fish and shellfish only	0	1.4×10^{-9}	1.4×10^{-8}	1.4×10^{-7}
2 ℓ of drinking water only	0	2.2×10^{-8}	2.2×10^{-7}	2.2×10^{-6}

(1) The animal bioassay data used in these calculations are presented in the Appendix of this document. These levels are calculated by applying a linearized multistage model as discussed in the Human Health Methodology Appendices to the Federal Register notice concerning water quality criteria. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1000 and so forth.

(2) Approximately 94.2% of the 2,3,7,8-TCDD exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 5000-fold. The remaining 5.8% of 2,3,7,8-TCDD exposure results from drinking water. Correspondingly, if no contaminated shellfish or fish are eaten, the water contamination level could be 17 times as high for the same risk level, or 2.2×10^{-7} μg/ℓ for a 10^{-6} upper-limit risk level, vs. 1.3×10^{-8} μg/ℓ when contaminated fish and water are consumed.

Concentration levels were derived assuming a lifetime exposure to various amounts of 2,3,7,8-TCDD, (1) occurring from the consumption of both drinking water and aquatic life taken from waters containing the corresponding 2,3,7,8-TCDD concentrations, (2) occurring solely from consumption of aquatic life grown in waters containing the corresponding 2,3,7,8-TCDD concentrations and (3) occurring from the consumption of drinking water only. Because data indicating other sources of 2,3,7,8-TCDD exposure and their contributions to total body burden are inadequate for quantitative use, the figures reflect the incremental risks associated with the indicated routes only.

The above criteria, which have been calculated on the basis of health effects data, are below the limit of detection for 2,3,7,8-TCDD in water by current analytical methods. The detection limit presently is estimated to be $\sim 3 \times 10^{-5}$ µg/ℓ (Harless et al., 1980). The detection limit should also be considered when issuing guidance based on these criteria.

Estimates by Others of Carcinogenic Potency and Criteria

The U.S. Food and Drug Administration concluded that an advisory level of 25 ppt for Great Lakes fish contaminated with 2,3,7,8-TCDD does not pose an unacceptable risk to public health (FDA, 1981). EPA has reviewed the recent testimony before Congress of Dr. S.A. Miller (FDA, 1983), discussing cancer risk associated with ingestion of these fish. The FDA estimate of the 95% upper-limit carcinogenic potency factor for 2,3,7,8-TCDD is $q_1^* = 1.75 \times 10^4$ $(mg/kg/day)^{-1}$, which is less potent than EPA's estimate of $q_1^* = 1.56 \times 10^5$ $(mg/kg/day)^{-1}$ (see Appendix) by a factor of 9. Even though both Agencies used the same data base (Kociba et al., 1978) and risk extrapolation model, some subtle differences in methodology exist which account for this factor of 9. The major part of this difference is a factor

of 5.38 which EPA uses for rat-to-man extrapolation on the assumption that dose per unit body surface area, rather than dose per unit body weight, is an equivalent dose between species (45 FR 79351). Most of the remaining factor of ~1.7 is due to the FDA's use of the Kociba histopathological diagnosis alone, without including that of Squire, and EPA's adjustment of its calculations to compensate for the high early mortality observed in the Kociba et al. (1978) study (see Appendix).

FDA and EPA also differ in their assessment of human exposure to 2,3,7,8-TCDD in fish, in keeping with their respective regulatory approaches. EPA calculates water quality criteria to protect a body of water as though it were the direct source of 100% of a human population's average daily intake of water and/or freshwater and estuarine fish or shellfish. The concentration of a pollutant in the tissues of all such fish or shellfish is further assumed to be determined by the water concentration and the bioconcentration factor (BCF) of the pollutant. FDA, on the other hand, premised its exposure assessment on the assumption that only limited amounts of fish having 2,3,7,8-TCDD levels at or near the advisory level will actually be consumed. For example, FDA assumed that for this substance, significant contamination problems were limited to bottom feeders such as catfish and carp.* It also assumed that actual average residue levels in the flesh of bottom-feeding species reaching the market would not exceed one-third of the advisory level (i.e., ~8 ppt) and further, that for most individuals, 90% of the fish consumed would be comprised of other species showing no measurable contamination, or would be taken from uncontaminated

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*However, available data indicate that other species, especially trout and salmon, taken from some areas of the Great Lakes may also have tissue residues of 2,3,7,8-TCDD which exceed 25 ppt (see Table 1).

areas. Under these assumptions, and using an upper 90 percentile value for freshwater fish consumption of 15.7 g/day, the FDA potency estimate yields an upper-limit risk estimate of 2.86×10^{-6} for consumers of these fish. If the same exposure assumptions were used with EPA's potency estimate a somewhat higher upper limit risk of 2.92×10^{-5} would result.

The Center for Disease Control (CDC) has also calculated an upper-limit potency value for 2,3,7,8-TCDD (Kimbrough et al., 1983). The CDC estimate is based on the Squire histopathological results, and, like that of FDA, extrapolates from rat to man on a basis of dose equivalence per unit body weight. The CDC difference from both the EPA and FDA approaches is that the curve fit was done, not on administered dose, but on liver concentration at terminal sacrifice. Also, like FDA, CDC did not adjust for high early mortality. The final result is that the CDC 95% upper-limit potency value estimate when converted back to administered dose is $q_1^* = 3.6 \times 10^4$ $(mg/kg/day)^{-1}$ which is more potent by a factor of 2 than that of FDA and less potent by a factor of 4 than that of the EPA.

In January 1984 the three Agencies met to review the differences in carcinogenic potency estimation. The three Agencies agreed that they are using virtually the same methodologies for potency estimation although there are differences in some assumptions used. Further, there was agreement that correction for mortality is appropriate, making the differences less between the EPA estimate and the other estimates. Lastly, the Agencies agreed that the remaining differences are within the range of uncertainty inherent in the risk assessment process.

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*The difference between the EPA and FDA risk estimates results from the difference in potency estimates, described above, and the use by FDA of an average human body weight of 80 kg, versus 70 kg used by EPA.

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APPENDIX

Summary and Conclusions Regarding the Carcinogenicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)*

2,3,7,8-TCDD is probably carcinogenic for humans on the basis of animal carcinogenicity studies which were positive in multiple species and organs. Epidemiological studies of workers exposed to chemicals contaminated with 2,3,7,8-TCDD such as 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenol are consistent with the position that 2,3,7,8-TCDD is probably carcinogenic for humans; the available evidence indicates an excess incidence of soft tissue sarcomas. Because 2,3,7,8-TCDD is almost always found in association with other materials (e.g., chlorophenols, combustion products, etc.), it may never be possible to evaluate the carcinogenicity of 2,3,7,8-TCDD by itself in humans.

SUMMARY OF HUMAN POTENCY ESTIMATES BASED ON PERTINENT DATA

A summary of 95% upper-limit human carcinogenic potency estimates for 2,3,7,8-TCDD derived from the Kociba et al. (1978) and NCI (U.S. DHHS, 1980b) studies in rats and mice, with two pathologists' findings for the Kociba study, are given in Table 32. These potency estimates have been calculated using the linearized multistage model by a previously described methodology (45 FR 79350-79353). The largest of these potency factors (q_1*) comes from data in an independent pathologist's (Dr. R. Squire) review of the Kociba feeding study of female Sprague-Dawley rats. An adjustment for high early mortality in the high dose groups led to a slightly lower estimate. The mean of the two pathologists' estimates after mortality adjustment is:

$$q_1^* = [(1.51 \times 10^5) \times (1.61 \times 10^5)]^{1/2} = 1.56 \times 10^5 \text{ (mg/kg/day)}^{-1}$$

*This summary was prepared and approved by an expert peer review panel on dioxins convened by the U.S. EPA in Cincinnati on July 17, 1983.

TABLE 32

Summary of Human Potency Estimates for 2,3,7,8-TCDD

Species	Study	Sex	Pathologist	Human Potency Estimate q_1^* in $(mg/kg/day)^{-1}$
Rat	Kociba et al.[a]	male	Kociba	1.47×10^4
			Squire	1.73×10^4
Rat	Kociba et al.[a]	female	Kociba	
			Unadjusted	2.52×10^5
			Adjusted for early mortality	1.51×10^5[b]
			Squire	
			Unadjusted	4.25×10^5
			Adjusted for early mortality	1.61×10^5[b]
Rat	NCI[c]	female	NCI-reviewed	3.28×10^4
Mouse	NCI[c]	male	NCI-reviewed	7.52×10^4
Mouse	NCI[c]	female	NCI-reviewed	4.56×10^4

[a]Source: Kociba et al., 1978

[b]Values used to determine the geometric mean of 1.56×10^5 $(mg/kg/day)^{-1}$

[c]Source: U.S. DHHS, 1980b

These potency estimates were derived from the Kociba feeding study. The responses and parameters of the Kociba feeding study in female rats are given below. The number with tumors refers to the number of animals with at least one of liver, lung, hard palate, and/or nasal turbinate tumors. Adjustment for early mortality refers to eliminating those animals which died during the first year of study. The first tumor appeared in the high dose group during the thirteenth month.

Dose (mg/kg/day)	No. with Tumors/No. Examined Adjusted for Early Mortality	
	Squire	Kociba
0	16/85	9/85
0.001×10^{-3}	8/48	3/48
0.01×10^{-3}	27/48	18/48
0.1×10^{-3}	34/40	34/40
le = 720 days	W_h = 70 kg	
Le = 720 days	W_o = 0.450 kg	
L = 720 days	R = 5000 ℓ/kg	

With these parameters, the mean 95% upper-limit carcinogenic potency factor for humans, q_1^*, is 1.56×10^5 $(mg/kg/day)^{-1}$. For a 70 kg human drinking 2 ℓ water/day and eating 6.5 g of contaminated fish and shellfish, the water concentration should be $<1.3 \times 10^{-8}$ μg/ℓ in order to keep the upper-limit individual lifetime cancer risk below 10^{-6}, for example. If fish and shellfish alone are consumed, the corresponding water concentration for this level of risk should be $<1.4 \times 10^{-8}$ μg/ℓ, and if water alone is consumed, the corresponding water concentration should be $<2.2 \times 10^{-7}$ μg/ℓ.