TECHNICAL SUPPORT DOCUMENT
REPORT ON CHLORINATED DIOXINS AND DIBENZOFURANS

PART B - HEALTH EFFECTS OF CHLORINATED DIOXINS AND DIBENZOFURANS

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Table of Contents

1.0	Executive Summary	1-1
2.0	Introduction	2-1
3.0	Pharmacokinetics and Metabolism	3-1
	3.1 Absorption	3-1
	3.2 Distribution	3-2
	3.3 Metabolism	3-3
	3.4 Elimination	3-4
	3.5 Summary and Conclusion	3-5
4.0	Acute and Subacute Effects	4-1
	4.1 Animal Studies	4-1
	4.2 Human Health Effects	4-9
	4.2.1 Effects of Accidental Exposure	4-9
	4.2.2 Effects of Chronic Exposure	4-10
5.0	Reproductive and Teratogenic Effects	5-1
	5.1 Animal Studies	5-1
	5.2 Human Reproductive Effects	5-7
6.0	Genotoxicity	6-1
	6.1 Summary	6-1
	6.2 In Vitro Tests with Bacteria	6-2
	6.3 In Vitro Tests with Eukaryotic Cells	6-6
	6.4 In Vivo Animal Studies	6-11
	6.5 Studies in Human Populations	6-13
	6.6 Direct Interaction with DNA	6-17
	6.7 Conclusion	6-18
7.0	Carcinogenicity	7-1
	7.1 Animal Studies	7-1
	7.1.1 TetraCDD	7-1
	7.1.2 HexaCDD	7-11
	7.2 Human Studies	7-16
	7.2.1 Exposure through Use of Phenoxy Herbicides or Chlorophenols	7-18
	7.2.2 Exposure through Chemical Manufacturing	7-21
	7.2.3 Exposure through Environmental Contamination	7-26
	7.2.4 Discussion of Epidemiologic Studies	7-28
	7.2.5 Statistical Power of Epidemiologic Studies	7-33
8.0	Mechanism of Action	9-1
	8.1 Summary	8-1
	8.2 Cytosolic Binding Mechanism	8-2
	8.3 Carcinogenesis and Mechanism	8-3

9.0	Risk Assessment	9-1
	9.1 Low Dose Extrapolation Models Used	9-4
	9.2 Results of Low Dose Extrapolation	9-6
	9.3 Risk Assessment Estimates for a Mixture of PCDDs and PCDFs	9-20
	9.3.1 Scenario 1	9-21
	9.3.2 Scenarios 2, 3, and 4	9-23
	9.4 Discussion	9-28
Appendix A	Dose Conversion Assumptions and Calculations	A-1
Appendix B	Methods of Inferring Total Potency of a Mixture of PCDD/PCDF	B-1
Appendix C	Consistency of Predicted Risk with Observed Risk in an Epidemiologic Study	C-1
Appendix D	Structure Activity Considerations	D-1

List of Tables

4.1-1	Comparative Single Oral Dose LD_{50} Values for Chlorodiobenzo-p-dioxin Isomers	4-5
4.1-2	Comparative Single Oral Dose LD_{50} Values for Chlorodibenzofurans Compared to TCDD	4-6
4.1-3	Comparative Biologic Activity (In Vitro) of Chlorodibenzo-p-dioxins Relative to TCDD	4-7
4.1-4	Comparative Biologic Activity (In Vitro) of Chlorodibenzofurans Relative to TCDD	4-8
5.2-1	Summary of Studies of Human Reproductive Effects from Exposure to 2,4,5-T, TCDD, and Agent Orange	5-7
7.1-1	Carcinogenicity Bioassays on TCDD	7-2
7.1-2	Tumor Incidences in Male Rats Receiving TCDD in the Diet for Two Years	7-5
7.1-3	Tumor Incidences in Female Rat Receiving TCDD in their Diet for Two Years	7-6
7.1-4	Tumor Incidences in Male and Female Osborne-Mendel Rats Given TCDD by Gavage for Two Years	7-8
7.1-5	Tumor Incidences in Male and Female B6C3F1 Mice Given TCDD by Gavage for Two Years	7-10
7.1-6	Tumor Incidences in Male and Female Osborne-Mendel Rats and Male and Female B6C3F1 Mice Given HexaCDD by Gavage for Two Years	7-12
7.1-7	Incidence of Live Tumors Based on Four Separate Pathological Evaluations of Female Rats Given HexaCDD for Two Years	7-14
7.2-1	Summary of Major Case/Control Studies of Dioxin Exposure	7-30
7.2-2	Summary of Major Cohort Studies of Dioxin Exposure	7-31
9.2-1	TCDD Unit Risk Derived by the Multistage Model for Different Species and Tumor Sites	9-8
9.2-2	TCDD Maximum Likelihood Estimates and 95% Upper Confidence Limits for Excess Lifetime Cancer Risk for Exposure at 10 and 0.1 pg/m^3 Based on Different Low-Dose Extrapolation Models	9-12

9.2-3	HexaCDD Unit Risk Derived by the Multistage Model for Different Species and Tumor Sites	9-17
9.2-4	HexaCDD Maximum Likelihood Estimates and 95% Upper Confidence Limits for Excess Lifetime Cancer Risk for Exposure at 10^{-2} and 10^{-4} ng/m³ Based on Different Low Dose Extrapolation Models	9-18
9.3-1	Summary of Scenarios	9-22
9.3-2	Risk Estimates for Total PCDD/PCDF Under Different Exposure Assumptions	9-24
A-1	Calculated Daily Dose Levels for TCDD Chronic Studies in Rats and Mice	A-7
A-2	Calculated Daily Dose Levels for HexaCDD in the NTP Oncogenicity Bioassay in Rats and Mice	A-8
A-3	Calculated Equivalent Human Exposure to TCDD Based on Daily Animal Dose Levels	A-9
A-4	Calculated Equivalent Human Exposure to HexaCDD Based on Daily Animal Dose Levels from the NTP Oncogenicity Bioassay	A-10
B-1	Estimates of Total Carcenogenic Potency (Relative to 2,3,7,8-TCDD) for a Mixture of PCDDs from Municipal Solid Waste Incinerator Emissions	B-7
B-2	Estimates of Total Carcinogenic Potency (Relative to 2,3,7,8-TCDD) for a Mixture of PCDFs from Municipal Solid Waste Incinerator Emissions	B-8
C-1	Excess Cancers Predicted by Multistage Model from Study by Ott et al. (1980)	C-3

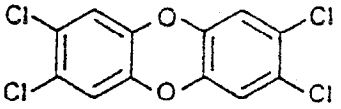
List of Figures

1.0-1	2,3,7,8-Tetrachlorodibenzo-p-dioxin	1-1
9.2-1	MLE and 95% UCL Curves, Mouse Liver Tumors; NCI Study	9-10
9.2-2	Estimated Human Excess Lifetime Cancer Risk Based on Male Mouse Liver Tumors Using the 95% UCL for TCDD	9-13
9.2-3	MLE and 95% UCL Curves, Rat Liver Tumors; NCI Study-Hildebrandt	9-17
9.2-4	Estimated Human Excess Lifetime Cancer Risk Based on Female Rat Liver Tumors Using the 95% UCL for HexaCDD	9-19

1.0 Executive Summary

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are similar classes of compounds. Many of the toxic effects of these compounds are associated with the number and specific placement of the chlorines. 2,3,7,8-TetraCDD (TCDD) (Figure 1.0-1) is probably the most toxic of the isomers. Other more highly chlorinated PCDDs and PCDFs with chlorine placement at the 2,3,7, and 8 positions appear to be significantly more toxic than when one or more of these positions are not chlorinated. Isomers with one, two, three, or eight chlorines are relatively nontoxic compared to those with four, five, six, or seven, and are therefore not considered in this report.

Figure 1.0-1



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

TCDD and other PCDDs and PCDFs are highly toxic in experimental animals when administered acutely, subchronically, or chronically. Toxic effects include severe weight loss, liver necrosis and hypertrophy, skin lesions, immunosuppression, reproductive toxicity, teratogenesis, carcinogenesis and death. The toxicity of 2,3,7,8-TCDD is species-dependent and the relative acute toxicities of other isomers decrease as the degree of chlorination increases. Acute exposure of humans to PCDDs has caused chloracne, a skin lesion which resembles mild to very severe acne and which may last many years. Acute and chronic human exposure has also been associated with liver toxicity. With the

possible exception of carcinogenesis, none of the toxic effects observed in animals or humans is expected to occur at the ambient levels of PCDDs and PCDFs predicted by the staff of the Air Resources Board (See Part A).

Of the numerous PCDD and PCDF isomers, only TCDD and a mixture of hexachlorinated dibenzo-p-dioxins with four of the six chlorines in positions 2,3,7, and 8 (HexaCDDs or 2,3,7,8-HexaCDDs*) have been tested for carcinogenicity. Two independent studies of TCDD resulted in significant increases in the incidence of liver and/or lung tumors in treated rodents (Kociba et al. 1978, NTP 1982). Therefore, the staff of DHS agrees with IARC (1982) that there is sufficient evidence to indicate that 2,3,7,8-TCDD is carcinogenic in animals. A mixture of two 2,3,7,8-HexaCDD isomers was found to produce an increased incidence of liver tumors or neoplastic nodules in treated rats and mice (NTP 1980). DHS staff has concluded that the HexaCDD mixture is also carcinogenic in animals. DHS staff agrees with the approach of IARC that substances found to be carcinogenic in animals should be considered potential human carcinogens. In addition, because of structure-activity considerations and the lack of chronic exposure studies on other PCDD/PCDF isomers containing four, five, six, or seven chlorines, DHS has concluded that these isomers should also be considered potential human carcinogens.

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* In this document 2,3,7,8-HexaCdds refers to hexachlorinated dibenzo-p-dioxins with four of the six chlorines at positions 2,3,7, and 8. Although this abbreviated nomenclature is incomplete, it should not be misleading in this context.

Carcinogenicity in humans cannot be directly inferred from epidemiologic investigations of persons exposed to chemical substances containing minute quantities of PCDD/PCDF. Some epidemiologic studies of workers exposed to PCDD/PCDF-contaminated herbicides have demonstrated increased risks of soft tissue sarcomas: others have not. Small sample sizes, lack of quantitative exposure information, and possible confounding by concomitant exposure to other chemicals make these studies inconclusive as a whole. Therefore, DHS agrees with the approach of EPA to use the results of animal studies for risk assessment.

Health and Safety Code Section 39660(c) calls upon DHS to estimate levels of exposure which may cause or contribute to adverse health effects (where toxicity is governed by a threshold) or to indicate a range of risks for health effects attributable to a nonthreshold process. In conducting a risk assessment for PCDD/PCDF carcinogenicity, the issue of whether the latter phenomenon is governed by a threshold is controversial. Although TCDD and HexaCDD have been shown to be carcinogenic in cancer bioassays, there is also good evidence that TCDD can promote the activity of other genotoxic carcinogens. TCDD's promoter-like action, its lack of effect in several standard assays for genotoxicity, and its binding to a cytosolic receptor that induces metabolic activating enzymes, have led the Ministry of the Environment in Ontario, Canada, to conclude that TCDD (and hence other PCDDs and PCDFs) is not genotoxic and thus can promote, but not initiate, carcinogenesis (1985). One corollary of this judgement is that TCDD's carcinogenicity may be governed by a threshold, which implies the existence of a safe level of exposure. The Canadians calculated this level as an Acceptable Daily Intake (ADI) of 10 pg/kg/day, equivalent to an airborne concentration of about 30 pg/m^3. This

level was calculated on the basis of results from studies of other outcomes (reproductive effects and chronic toxicity) in addition to carcinogenesis.

DHS staff and the U.S. Environmental Protection Agency have interpreted the experimental evidence differently from the Ontario Ministry of the Environment, concluding instead that under current knowledge, TCDD's (and hence other PCDDs' and PCDFs') carcinogenicity should be treated as a non-threshold phenomenon. This judgment is based on issues of both science and public health policy, as follows:

- First, both TCDD and HexaCDDs are positive in standard cancer bioassays at extremely low doses, suggesting that these substances can initiate carcinogenesis. Under the hypothesis accepted by the Canadians, such results could be interpreted as promotion of the effects of ubiquitous background initiators. Acknowledging the existence of evidence that TCDD can act as a promoter, DHS staff members, however, believe that the positive bioassays are compatible with the hypothesis that TCDD has both initiator and promoter activity.

- Second, the experimental evidence regarding genotoxicity is not clear-cut. Although TCDD has been shown to be ineffective in producing mutations in bacterial DNA, there is evidence that it can cause genetic damage in eukaryotic organisms (e.g., in yeast and mammalian cells). Furthermore, short-term tests are generally useful to detect genotoxicity, but are not sensitive enough to rule it out. The staff of DHS, therefore, agrees with IARC (1983) that it is premature to use

such tests as a basis for classification of carcinogens in order to utilize alternate methods of risk assessment.

- Third, in view of TCDD's extraordinary carcinogenic potency, DHS staff members believe that caution is warranted in risk assessment. Practically speaking, this means that a nonthreshold approach is indicated unless both the existence and location of a carcinogenic threshold have been compellingly demonstrated. In this case, the evidence for the existence of such a threshold is arguable, not conclusive. There is no evidence to support a determination of the location of such a hypothetical threshold.

Using the nonthreshold approach, the staff of DHS estimated the carcinogenic risks in human populations from exposure to TCDD and HexaCDD with data from animal bioassays that showed the most sensitive responses. Five low-dose extrapolation models, including the multistage, probit, logit, Weibull, and gamma multihit, were used to estimate the excess lifetime cancer risk. The staff of DHS prefers the multistage model for low-dose extrapolation. The multistage model is consistent with a widely held theory of carcinogenicity and generally gives health-conservative estimates. Therefore, as EPA has done, DHS has used the multistage model to estimate the upper bounds of risk of inducing excess cancers from lifetime exposure to TCDD and HexaCDD.

Using the multistage model for TCDD exposure, the maximum likelihood estimate of excess cancers is 240 per million population for continuous daily exposure for 70 years to an airborne concentration of 10 pg/m^3, with a 95% upper confidence limit of 380 per million. For HexaCDD, the maximum likelihood

estimate of excess cancers is 6 per million population from continuous daily exposure for 70 years to an airborne concentration of 10 pg/m^3, with a 95% upper confidence limit of 10 per million.

DHS recognizes that PCDDs and PCFDs are not uniformly distributed in ambient air. Their main source is thought to be emissions from combustion processes, such as municipal solid waste incinerators. ARB has projected possible levels of Tetra- through OctaCDDs and CDFs which might occur in the air at specific locations in the Los Angeles Basin if several proposed solid waste incinerators were to begin operating. Other sources of PCDDs and PCDFs are not included in these estimates. The resulting range is:

	High Estimate	Low Estimate	Best Estimate
PCDDs	13 pg/m^3	0.7 pg/m^3	4 pg/m^3
PCDFs	27 pg/m^3	1.6 pg/m^3	8.2 pg/m^3

Total PCDD/PCDF in air from sources such as incinerators is composed of a mixture of PCDD and PCDF homologues and isomers, most of which have never been tested for carcinogenicity. Furthermore, the specific chemicals in this mixture are difficult to separate analytically, and the concentrations of each isomer may vary depending upon the emission source.

Therefore, in order to estimate a range of risks that might result from such ambient air mixtures, DHS has used four scenarios. Each scenario uses the

low-dose extrapolation for TCDD and three use the low-dose extrapolation for HexaCDD described above, but the scenarios make different assumptions about: (1) the proportions of the various PCDD and PCDF isomers in the total mixture, and (2) the carcinogenic potencies for the majority of PCDDs and PCDFs that have not been tested. The product of these assumptions is an estimated "TCDD-equivalent concentration," i.e., the amount of the total mixture that is considered to be as carcinogenic as 2,3,7,8-TCDD.

Scenario 1 is the simplest and most conservative approach. Under this scenario, all PCDDs and PCDFs containing more than three chlorines are assumed to be as potent as 2,3,7,8-TCDD. Scenario 2 is similar to the first scenario except that only 2,3,7,8-chlorinated isomers of PCDDs and PCDFs with at least one other position unchlorinated are considered carcinogenic. All of the 2,3,7,8-isomers are considered as potent as 2,3,7,8-TCDD except HexaCDD, for which an actual relative potency has been derived from a bioassay. Under this scenario, the PCDDs and PCDFs were 25% and 20% as potent as under Scenario 1. In Scenario 3, all isomers with four, five, six, or seven chlorines are considered carcinogenic. However, isomers not chlorinated at one or more of positions 2, 3, 7, and 8 are considered less potent than those chlorinated in all of these positions. Each homologue group is assigned a relative potency based upon several factors, including acute toxicity and structure-activity relationships from <u>in vitro</u> tests. This is an approach under consideration at EPA. Under this scenario, the PCDDs and PCDFs were 1% and 0.3% as potent as under Scenario 1. Scenario 4 is similar to Scenario 2 except that a different relative potency is assigned to the higher chlorinated PCDDs and PCDFs because of the structure-acitivity relationship observed in the oncogenicity studies on 2,3,7,8-TCDD and 2,3,7,8-isomers of HexaCDD. Under

this scenario, the PCDDs and PCDFs were found to be 2% and 3% as potent as under Scenario 1.

The staff of DHS favors the use of Scenario 4. Although it is less conservative than Scenarios 1 and 2, it incorporates structure-activity information from cancer studies and therefore more likely reflects actual carcinogenic potencies of the untested isomers. Although Scenario 3 is also based on structure-activity relationships, the use of short-term toxicity tests to estimate carcinogenic potency is a controversial methodology that has not been validated.

Under Scenario 4 the estimated excess risk was extrapolated over four orders of magnitude: from the dose level that induced a significantly increased incidence of tumors in male mice to the above-noted high exposure estimate. The following range of risks was calculated using ARB's best and high exposure estimates:

	TCDD-Equivalent Dose	Upper 95% Confidence Estimate of Excess Lifetime Cancers (per million)
Best Exposure Estimate	0.3 pg/m^3	12
High Exposure Estimate	1 pg/m^3	38

Expressed another way, at a TCDD-equivalent dose of 0.3 pg/m^3, the estimated upper 95% confidence limit (UCL) on an individual's incremental lifetime risk of developing cancer is 1.2/100,000 or about 10^{-5}. Lowering the exposure

level to 0.03 pg TCDD/m^3 would decrease the individual incremental lifetime risk to 10^{-6}.

Under either of ARB's exposure estimates, the level of TCDD-equivalent exposure is less than the ADI calculated by the Ontario Ministry of the Environment (i.e., about 30 pg/m^3). Similarly, it is less than an ADI for TCDD of 1 pg/kg/day or about 3 pg/m^3 calculated by Longstreth and Hushon (1983) from studies of reproductive toxicity in monkeys and immunotoxicity in guinea pigs. The latter ADI incorporates a 1,000-fold safety factor. Thus, as would be expected, utilizing the 95% UCL estimate for a nonthreshold process (carcinogenesis) on the basis of a 10^{-5} or 10^{-6} individual incremental lifetime risk level would protect against threshold-mediated outcomes such as reproductive toxicity and immunotoxicity. In contrast, the use of the above-noted ADIs may not be protective against carcinogenesis and could result in higher individual incremental lifetime cancer risks, as illustrated below:

	TCDD-Equivalent Dose	Approximate Individual Incremental Lifetime Risk
DHS Multistage	0.03 pg/m^3	10^{-6}
"	0.3 pg/m^3	10^{-5}
Longstreth and Hushon ADI	3 pg/m^3	10^{-4}
Canadian ADI	30 pg/m^3	10^{-3}

The staff of DHS has concluded that carcinogenicity is the appropriate basis for risk assessment and that toxic effects of PCDDs and PCDFs other than cancer are not expected to occur at predicted ambient levels.

The estimated lifetime excess risks of cancer reported here must be viewed in the context of the overall probability of developing cancer, which is on the order of 250,000 cases per million population over a lifetime.

2.0 Introduction

This document provides a health assessment for exposure to polychlorinated dibenzodioxins and polychlorinated dibenzofurans in ambient air. In this review, the Department of Health Services (DHS) focuses on one compound, 2,3,7,8-Tetrachlorodibenzo-p-dioxin, on which most research has been conducted, but considers other dioxins and dibenzofurans with four to eight chlorines when data are available.

Several detailed reviews have been published recently and are referred to in this document. These include:

Environmental Protection Agency. Ambient water quality criteria for 2,3,7,8-Tetrachlorodibenzo-p-dioxin. EPA 440/5-84-007. Washington DC: US EPA, Office of Water Regulations and Standards, 1984.

Environmental Protection Agency. Health assessment document for polychlorinated dibenzo-p-dioxins. Review Draft. EPA-600/8-84-014A. Washington DC: US EPA, Office of Health and Environmental Assessment, 1984.

Huff JE, Moore JA, Saracci R, Tomatis L. Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ Health Perspect 1980; 36:221-240.

International Agency for Research on Cancer. Some fumigants, the herbicides 2,4-D and 2,4,5-T, chlorinated dibenzodioxins, and miscellaneous industrial chemicals. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man 1977; 15:41-102.

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

US Veterans Administration. Review of literature on herbicides, including phenoxy herbicides and associated dioxins. Analysis of literature on health effects. Vols I-II. Washington DC. VA Contract Number V101(93) P-823; 1981.

US Veterans Administration. Review of literature on herbicides, including phenoxy herbicides and associated dioxins. Analysis of recent literature on health effects. Vols III-IV. Washington DC. VA Contract Number V101(93) P-953; 1984.

3.0 Pharmacokinetics and Metabolism

There have been a number of reviews published on TCDD pharmacokinetics and metabolism. Therefore, this section will only briefly describe the findings of the large number of varied studies that have been conducted on TCDD and the smaller number of studies that concern other PCDDs and PCDFs.

3.1 Absorption

Absorption of TCDD following oral exposure has been examined in a number of studies. However, no study has been identified that examined TCDD absorption following exposure via inhalation.

The amount of TCDD absorbed from the rat gastrointestinal (GI) tract has been reported to vary from 50% to almost 90% of the administered dose, and appears to depend on the vehicle used for administration. Fries and Marrow (1975) found only 50 to 60% of the administered TCDD was absorbed when given in the diet while Rose et al. (1976) found 86% of the administered TCDD was absorbed when given in an acetone:corn oil (1:25) solution at a similar dose level. Several studies have found that adsorption of TCDD to soil particles reduces the bioavailability of the compound. (Bonaccorsi et al. 1983, McConnell et al. 1984). Poiger and Schlatter (1980) reported that twice as much TCDD was absorbed when given in a 50% ethanol solution compared to a soil/water solution. Van den Berg et al. (1983) fed rats diets containing fly ash or extracts of fly ash that contained Tetra-, Penta-, and HexaCDDs. Following 19 days of administration, the concentration of the CDD isomers in the livers of the animals given fly ash was less than the concentration in

the animals given the extracts. The fly ash appeared to reduce the bioavailability of the higher chlorinated dibenzo-p-dioxins more than that of the TetraCDDs.

As would be expected, dermal absorption of TCDD is also dependent on the vehicle in which it is applied. Poiger and Schlatter (1980) found that less than 1% as much TCDD was absorbed through the skin of rats when applied in a soil/water paste compared to when TCDD was applied in methanol. However, the investigators found that bioavailability increased with an increase in TCDD soil concentration.

3.2 Distribution

The distribution of TCDD in rats, mice and hamsters appears to be similar. In each species the liver has the highest affinity for TCDD, followed by adipose tissue. The guinea pig and nonhuman primates have been found to sequester higher concentrations of TCDD in their adipose tissue than in their livers and were also found to sequester high concentrations in their skin.

Appelgren et al. (1983) used autoradiographic techniques to examine the distribution of ^{14}C-TCDD in mice following administration by intravenous injection. The TCDD was found to localize at the greatest concentration in the liver followed by the nasal mucosa. Using the same technique, the distribution of TCDD in the mouse fetuses at 17 days of gestation was also examined. Although the radioactivity in the fetuses was lower than in the

dams, the highest concentrations were also found in the liver and nasal mucosa.

Nau and Bass (1981) showed that the mouse fetus did not sequester TCDD. They found that the fetal liver had a concentration 10 to 20 times less than that found in the maternal liver, but equal or less than that found in the maternal kidney or lung. Non-hepatic fetal tissue had concentrations 3 to 4 times less than fetal liver. Nau et al. (1982) found that, although fetal tissues do not sequester large amounts of TCDD, neonates that were nursed by treated mothers had tissue levels of TCDD that increased over time, indicating that postnatal exposure via TCDD in mother's milk is a more important source than prenatal transplacental exposure for overall body burden.

3.3 Metabolism

The metabolism of TCDD has been examined in rats, hamsters, guinea pigs and dogs (Neal et al. 1981, Poiger et al. 1982a, Olson 1983). Bile from these animal species treated with radioactively-labeled TCDD was found to contain radioactive compounds that did not chromatograph with TCDD. Treatment of the bile with the enzyme β-glucuronidase changed chromatographic pattern of these newly formed compounds, indicating that at least some of the compounds were glucuronidated phenolic metabolites of TCDD. Although bile from treated dogs contained TCDD metabolites, they did not appear to be conjugated. Urine from treated rats and hamsters was also found to contain some sulfate-conjugated metabolites (Olson and Bittner, 1983). In general, there appeared to be little or no unmetabolized TCDD excreted in either urine or bile.

Vinopal and Casida (1973) failed to detect any water-soluble TCDD metabolites in the livers of treated mice. Rats administered TCDD metabolites isolated from the bile of dogs rapidly excreted the metabolites (Weber et al. 1982). These findings suggest that TCDD metabolites do not accumulate in the body. Poiger et al. (1982a) showed that metabolites obtained from dog bile were at least 100 times less toxic than TCDD to guinea pigs.

Several of the dog bile metabolites of TCDD have been isolated and tentatively identified (Poiger et al. 1982b). These include 2-hydroxy-1,3,7,8-tetrachlorodibenzo-p-dioxin, 2-hydroxy-3,7,8-trichlorodibenzo-p-dioxin and 1,2-dichloro-4,5-dihydroxy-benzene. The structure of some of the metabolites formed suggest that epoxide formation is involved in TCDD metabolism.

3.4 Elimination

In most animal species studied, elimination appears to be a first order process. Elimination in the guinea pig, however, appears to follow zero-order kinetics. The primary route of excretion is through the feces following both oral and intraperitoneal injection, although urinary excretion can also account for significant amounts of the overall elimination. The elimination half-life has been measured in a number of rodent species. The longest half-lives, 30.2 and 31 days, were found in guinea pigs and rats, respectively. (Gasiewicz and Neal 1979, Rose et al. 1976). The shortest half-lives have been found in mice and hamsters. These half-lives were 11.0 and 10.8 days, respectively (Gasiewicz et al. 1983, Olson et al. 1980).

Retention and elimination of TCDD and other PCDDs appear to be related to the degree of chlorination and the position of chlorines on the rings. After feeding fly ash extracts containing Tetra-, Penta- and HexaCDDs to rats for 19 days, Van den Berg et al. (1983) found that the amount of each homologous group retained in the liver as a percentage of total dose was 0.16, 1.18, and 2.99 percent, respectively. 2,3,7,8-TCDD accounted for 3.3% of the tetra-homologue group in the extracts but 77.2% of those found in the liver. Similarly, 1,2,3,7,8-PentaCDD, 1,2,3,6,7,8-HexaCDD, and 1,2,3,7,8,9-HexaCDD made up 4.4%, 10.5% and 10.0% of the isomers in their respective homologue groups in the extract, but 100%, 70.1%, and 29.1% of their homologue groups found in the livers.

3.5 Summary and Conclusion

TCDD and other PCDD are absorbed from the gastrointestinal tract of experimental animals. Absorption is dependent on the vehicle used for administration and how strongly the compounds are adsorbed to particulate matter, such as soil. The liver, adipose tissue, and skin are major tissues that sequester TCDD. The tissues which retain the highest concentration of TCDD differ among species. Once absorbed, most, if not all, TCDD is eliminated in the urine and bile only after biotransformation. The TCDD metabolites appear to be less toxic than TCDD, although some metabolites are likely formed through an epoxide intermediate. A reactive metabolite of this type may account for covalent binding to cellular protein of radioactivity from labeled TCDD. The biological half-life of TCDD has been found to vary in different rodent species from about 11 to 31 days. There is no information on the biological half-life of TCDD in humans. Some evidence

suggests that 2,3,7,8-isomers have longer biological half-lives than other isomers. Unfortunately, there is not sufficient information available to make an interspecies comparison of the pharmacokinetics of TCDD between experimental animals and humans.

References

Applegren LE, Brandt I, Brittebo EB, et al. Autoradiography of 2,3,7,8-tetrachloro-^{14}Cl-dibenzo-p-dioxin TCDD: Accumulation in the nasal mucosa. Chemosphere 1983; 12:545-548.

Bonaccorsi A, diDomenico A, Fanelli R, et al. The influence of soil particle adsorption on TCDD biological uptake in the rabbit. Arch Toxicol 1984; S7:431-434.

Fries GF, Marrow GS. Retention and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rats. J Agric Food Chem 1975; 23:265-269.

Gasiewicz TA, Geiger LE, Rucci G, Neal RA. Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6J, DBA/2J$_1$/J mice. Drug Metabol Disp 1983; 11:397-403.

Gaseiwicz TA, Neal R. 2,3,7,8-Tetrachlorodibenzo-p-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. Toxicol Appl Pharmacol 1979; 51:329-340.

McConnell EE, Lucier GW, Rumbogh RC, et al. Dioxin in Soil: Bioavailability after ingestion by rats and guinea pigs. Science 1984; 223:1077-1079.

Nau H, Bass R. Transfer of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the mouse embryo and fetus. Toxicology 1981; 20:299-308.

Nau H, Bass R, Neubert D. Transfer of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the mouse embryo, fetus and neonate. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related Ccmpounds. impact on the environment. New York: Pergamon Press, 1982; pp.325-337.

Neal RA, Olson JR, Gaseiwicz TA, Gudzinowicz M. The in vivo and in vitro metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the golden syrian hamster. In: Khan MAQ, Stanton RH, eds. Toxicology of halogenated hydrocarbons, health and ecological effects. New York: Pergamon Press, 1981, pp. 259-270.

Olson JR, Gasiewicz TA, Geiger LE, Neal RA. The metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian systems. In: Coulston F, Pocchiari F, eds. Accidental exposure to dioxins: human health aspects. New York:Academic Press, 1983, pp. 81-100.

Olson JR, Bittner WE. Comparative metabolism and elimination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicologist 1983; 3:103.

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

Poiger H, Schlatter C. Influence of solvents and absorbents on dermal and intestinal absorption of TCDD. Food Cosmet Toxicol 1980; 18:477-481.

Poiger H, Weber H, Schlatter C. Special aspects of metabolism and kinetics of TCDD in dogs and rats. Assessment of toxicity of TCDD-metabolite(s) in guinea pigs. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds. Impact on the environment. New York: Pergamon Press, 1982, pp. 317-325.

Poiger H, Buser HR, Weber H, Zweifel U, Schlatter C. Structure elucidation of mammalian TCDD-metabolites. Experientia 1982; 38:484-486.

Rose JQ, Ramsey JC, Wentzler TH, Hummel RA, Gehring PJ. The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. Toxicol Appl Pharmacol 1976; 36:209-226.

Van den Berg M, Olie K, Hutzinger O. Uptake and selective retention in rats of orally administered chlorinated dioxins and dibenzo-p-furans from fly ash and fly ash extract. Chemosphere 1983; 12:537-544.

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

Weber H, Poiger H, Schlatter C. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin metabolites from dogs in rats. Xenobiotica 1982; 12:353-357.

4.0 Acute and Subacute Effects

4.1 Animal Studies

The toxicity of polychlorinated dibenzo-p-dioxins and dibenzofurans has been recently reviewed (EPA 1984, VA 1984). The greatest amount of information concerns the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is believed to be the most potent PCDD congener and more potent than any PDCF congener. Although many of the effects noted for TCDD have also been observed for a number of the other PCDD and PCDF congeners, the primary focus of this discussion will be related to the toxicity of TCDD.

TCDD has been found to be acutely toxic at very low doses in some animal species while it has been found to be up to three orders of magnitude less toxic to other species. The EPA (1984) and VA (1981, 1984) have summarized a large number of acute toxicity studies. Guinea pigs are the most sensitive species tested with the oral LD_{50} ranging from 0.6 to 19 $\mu g/kg$. Rats are less sensitive to TCDD than guinea pigs, followed by mice, then rabbits and hamsters. The range of oral LD_{50} values reported for the hamster, which is the least sensitive species tested, is from 1157 to 5051 $\mu g/kg$. One study on rhesus monkeys indicated that the acute oral LD_{50} for this species is below 70 $\mu g/kg$.

A severe weight loss in animals is observed following acute exposure to TCDD. This "wasting" effect is accompanied by depletion of fat deposits and an inability to utilize ingested nutrients. The reason for the loss of body

weight of up to 50% has not been ascertained but a number of tissues and organs have been found to be affected by TCDD. These include the skin, liver, gastrointestinal tract, and immune system.

TCDD has produced liver changes in most animal species tested. The severity of the lesions at lethal dose levels range from mild to severe and can be a contributor to the cause of death. The most severe lesions include necrosis which may be centrolobular, focal or single cell ,depending on the species, lipid accumulation, and bile duct proliferation. Liver weight relative to body weight has been found to increase in treated mice and rats and a proliferation of rough and smooth endoplasmic reticulum in hepatocytes occurs. A very sensitive indicator of TCDD exposure is the induction of specific hepatic mixed function oxidase activities. This induction can occur in some species at dose levels in the microgram per kilograms body weight range or even lower when TCDD is given subchronically.

Skin lesions have been noted in only a few animal species. The rhesus monkey develops chloracne-like lesions on the lips along with hyperkeratosis of the glands of the eyelids. Acute exposure also produces facial alopecia, blepharitis and loss of fingernails and eyelashes. A similar effect is seen when monkeys are exposed to 2,3,7,8-TCDF. Skin lesions are also seen on the ear of rabbits following dermal application. Suspectibility to this effect appears to be genetically linked in mice.

The immune system appears to be very sensitive to TCDD toxicity. Thymic atrophy is a prominent finding in animals exposed to TCDD and has been observed in all laboratory species examined. Other lymphoid tissues, such

as the spleen, lymph nodes and bone marrow, have also been found to be affected, although, they are less sensitive than the thymus. Studies have indicated that TCDD does suppress both humoral and cell-mediated immunity and alters bone marrow function and host resistance function. Some of these effects have occurred at exposure levels of less than 1 μg/kg. Zinkl et al. (1973) examined the effect of TCDD on humoral and cell-mediated immune responses in the guinea pig at very low doses. The animals were treated with 0.008, 0.04, or 0.2 μg TCDD/kg once a week for eight weeks and then challenged with tetanus toxoid or killed Mycobacterium tuberculosis. Animals in the low-dose group treated with tetanus toxoid, had a significantly reduced lymphocyte count. Although the lymphocyte count was significantly reduced in the other TCDD/tetanus toxoid treated animals, the magnitude of the effect was similar even though the high dose was 25 times greater than the low dose. This effect was not observed in the Mycobacterium tuberculosis treated group at any dose level, although, the leukocyte and neutrophil counts were decreased at the high TCDD dose level.

TCDD has also been found to cause reproductive toxicity, teratogenicity and carcinogenicity in laboratory animals. These toxic effects as well as TCDD's potential genotoxic effect are discussed in more detail in the following sections of this report.

As mentioned earlier, other PCDD and PCDF congeners have qualitatively similar effects, although the magnitude of the response may quantitatively differ from that of TCDD. Kociba and Cabey (1985) have reviewed the literature on the toxicity of a number of PCDD and PCDF isomers and have compiled tables that show quantitative differences between species, test systems, and

isomers. For acute toxicity, Tables 4.1-1 and 4.1-2 list the interspecies differences for TCDD, previously discussed, and list similar differences for other isomers. TCDD is the most toxic isomer listed and increasing or decreasing the degree of chlorination or chlorinating positions other than 2,3,7, and 8 reduces the observed toxicity. Comparable biological activities in in-vitro test systems were also reviewed. Tables 4.1-3 and 4.1-4 show the relative biological activities of PCDDs and PCDFs for a number of effects: induction of the enzyme δ-aminolevulinic acid (ALA) synthetase, induction of mixed function oxidase activity and aryl hydrocarbon hydroxylase (AHH), and induction of cellular keratinization of the cultured epithelial cell line XB/3T3. Again, TCDD is the most potent isomer tested and congeners chlorinated at the 2,3,7, and 8 positions are the most active within each homologue group.

Most of the short-term toxic effects caused by PCDDs and PCDFs are considered to be mediated through the interaction of the compound and a specific cytosolic receptor protein. The structural requirements for binding to this receptor protein are briefly discussed in Appendix D. TCDD structurally has the best fit for the protein's receptor site. PCDD and PCDF congeners chlorinated at the 2,3,7, and 8 positions have higher affinities for the receptor site than the isomers not chlorinated in all these positions. Unfortunately, the mechanisms for the toxic effects, except for enzyme induction, have not been elucidated past receptor binding and are not necessarily correlated with the degree of receptor present and bound.

Table 4.1-1

COMPARATIVE SINGLE ORAL DOSE LD_{50} VALUES FOR CHLORODIBENZO-P-DIOXIN ISOMERS

Chlorodibenzodioxin	Oral LD_{50} Values (µg/kg)						
	Guinea Pig	Mouse	Rat	Monkey	Hamster	Rabbit	Dog
2,3,7,8-Tetra	0.6-2	114-284	22-45	~70	1157-5051	115	>300,<3,000
Unsub		>50,000	>1,000,000				
2,3-Di			>1,000,000				
2,7-Di		>2,000,000	>1,000,000				
2,8-Di	>300,000	847,000,000	>5,000,000				
1,3,7-Tri		>15,000,000	>5,000,000				
2,3,7-Tri	29,444	>3,000	>1,000,000				
1,2,3,4-Tetra			>1,000,000				
1,3,6,8-Tetra	>15,000,000	>2,987,000	>10,000,000				
1,2,3,7,8-Penta	3.1	337.5					
1,2,4,7,8-Penta	1,125	>5,000					
1,2,3,4,7,8-Hexa	72.5	825					
1,2,3,6,7,8-Hexa	70-100	1250					
1,2,3,7,8,9-Hexa	60-100	>1440					
1,2,3,4,6,7,8-Hepta	>600						
Octa		>4,000,000	>1,000,000				

Source: Kociba and Cabey 1985, Table 1

Table 4.1-2

COMPARATIVE SINGLE ORAL DOSE LD_{50} VALUES FOR CHLORODIBENZOFURANS COMPARED TO TCDD

		Oral LD_{50} Values (µg/kg)					
Chlorodioxin/furan	Guinea Pig	Mouse	Rat	Monkey	Hamster	Rabbit	Dog
2,3,7,8-Tetradioxin	0.6-2	114-284	22-45	~70	1157-5051	115	>300,<3000
Chlorodibenzofuran							
2,8-Di		>15,000,000	>15,000,000				
2,4,8-Tri		>15,000,000	>5,000,000				
2,3,7,8-Tet ı	5-10	>6000	>1000	1000			
2,3,4,7,8-Penta	<10						
2,3,4,6,7,8-Hexa	120						

Source: Kociba and Cabey 1985, Table 2

Table 4.1-3

COMPARATIVE BIOLOGIC ACTIVITY (IN VITRO) OF CHLORODIBENZO-P-DIOXINS RELATIVE TO TCDD

Chlorodibenzo-p-dioxin	AHH Activity in Rat Hepatoma Cells	AHH Activity in Chick Embryo Liver	ALA Synthetase in Chick Embryo Liver	Keratinization of XB/3T3 Cells
2,3,7,8-Tetra	1/1	1/1	1/1	1/1
Unsub.	Inactive	Inactive	Inactive	Inactive
1-Chloro		Inactive	Inactive	
1,3-Di	Inactive			
1,6-Di	Inactive	Inactive		Inactive
2,3-Di	Inactive	Inactive	Inactive	Inactive
2,7-Di	Inactive	Inactive	Inactive	Inactive
2,8-Di	Inactive	Inactive	Inactive	
1,2,4-Tri		Inactive	Inactive	
2,3,7-Tri	1/920-1/3060	1/1666	Active	1/100
1,3,7,8-Tetra	1/57-1/242	1/12		1/100
1,2,3,8-Tetra	1/1666-1/5900			
1,2,3,4-Tetra	Inactive	Equiv.	Inactive	
1,3,6,8-Tetra	Inactive	Inactive	Inactive	Inactive
1,2,3,7,8-Penta	1/5-1/53			1/2
1,2,3,4,7-Penta	1/21-1/132	Active	Active	
1,2,4,7,8-Penta	Inactive			
1,2,3,6,7,9-Hexa	Inactive			
1,2,4,6,7,9-Hexa	Inactive	Inact./Equiv.	Inact./Equiv.	
1,2,3,4,7,8-Hexa	1/10-1/20	Active	Active	
1,2,3,7,8,9-Hexa	1/114-1/523	1/5		1/200
1,2,3,6,7,8-Hexa	1/71-1/947			
1,2,3,4,6,7,9-Hepta	1/10,200			
1,2,3,4,6,7,8-Hepta	1/282-1/367			
Octa (99.2%)	1/1666-1/4594			
Octa (>99%)	1/53,000	Inactive	Inactive	

Biologic Activity expressed as fractions relative to TCDD (1/1).

Source: Kociba and Cabey 1985, Table 3

Table 4.1-4

COMPARATIVE BIOLOGIC ACTIVITY (IN VITRO) OF CHLORODIBENZOFURANS RELATIVE TO TCDD

Chlorodioxin/furan	AHH Activity in Rat Hepatoma Cells	AHH Activity in Chick Embryo Liver	Keratinization of XB/3T3 Cells
2,3,7,8-Tetra Dioxin	1/1	1/1	1/1
Chlorodibenzofuran			
Unsub.	Inactive	Inactive	Inactive
2,8-Di	Inactive	Inactive	
2,4-Di		Inactive	
2,4,8-Tri		Inactive	
2,3,8-Tri	1/20,714		
2,4,6-Tri	Inactive		
1,4,6,8-Tetra	Inactive		
1,3,6,7-Tetra		Inactive	
2,3,6,8-Tetra	Inactive		
2,4,6,8-Tetra	Inactive		
2,3,7,8-Tetra	1/92	2/3	1/20
1,2,3,7,8-Penta		1/7	
1,3,4,7,8-Penta	1/1,928		
2,3,4,7,8-Penta		7/10	
1,2,4,7,8-Penta	1/31,428		
1,2,3,4,6,8,9-Hepta	1/24,286		

Biologic Activity expressed as fractions relative to TCDD (1/1).

Source: Kociba and Cabey 1985, Table 4

4.2 Human Health Effects

The literature on human health effects of PCDDs and/or PCDFs has been summarized in the EPA Health Assessment Document for PCDDs (EPA, 1984) and by Huff et al. (1980).

4.2.1 Effects of Accidental Exposure

Accidental exposure to PCDDs has caused chloracne, nausea, headaches, fatigue, and muscular aches and pains. The first report of symptoms from exposure to PCDDs by Butler in 1937 (as cited by Moses et al., 1984) involved 21 cases of chloracne in workers engaged in the production of tetrachlorophenol. This observation has been followed by subsequent reports of other accidental exposures. The most consistent and frequently observed symptom is that of chloracne, which is characterized by cutaneous eruptions of comedones, cysts, and pustules usually on the face and shoulders.

Seventy-nine workers were exposed to PCDDs during an explosion in a British factory in 1968 (May 1973). Eleven of 14 men tested showed abnormal liver function and altered hematological parameters.

Children and adults directly exposed to reactor vapor contaminated with TCDD following an explosion at Seveso, Italy complained of nausea, skin lesions, redness, and swelling. Reduced peripheral nerve conduction velocities were noted in adults and children, with abnormalities being more frequently reported in people residing nearer the chemical plant. Porphyria cutanea

tarda and secondary coproporphyrinuria has also been reported in Seveso victims (Doss et al. 1984).

In Japan in 1968 and in Taiwan in 1978, contamination of rice bran oil (yusho) by polychlorinated biphenyls (PCBs), PCDFs, and polychlorinated quaterphenyls (PCQs) resulted in over 2,000 cases of skin disorders, including chloracne (Reggiani, 1983). Skin and nail pigmentation and numbness of the limbs were reported in the majority of the cases. Mild neurological disorders of sensory and motor nerves were also observed.

4.2.2 Effects of Chronic Exposure

Symptoms of chronic exposure to PCDDs include elevated gamma-glutamyl transpeptidase (GGT) levels, elevated cholesterol levels (Walker and Martin 1979), and abnormal neurological findings (Moses et al. 1984). Moses et al. examined workers with chloracne from a plant in Nitro, West Virginia who had suffered exposure to PCDDs during an explosion 30 years earlier. Suspected exposure continued for 20 years after the accident. Moses found a significantly increased prevalence of abnormal GGT and higher mean GGT in workers with chloracne compared to those without.

Pazderova-Vejlupkova et al. (1981) noted deviations in lipid metabolism, abnormal glucose tolerance, and high urinary excretion of uroporphyrins in 80 workers engaged in the production of 2,4,5-sodium trichlorophenoxyacetate five years after exposure. Polyneuropathy remained evident up to four years following exposure.

References

Doss M, Sauer H, Von Teipermann R, Colombi AM. Development of chronic hepatic porphyria (porphyria cutanea tarda) with inherited uroporphyrinogen decarboxylase deficiency under exposure to dioxin. Int J Biochem 1984; 16:369-373.

EPA (Environmental Protection Agency). Health assessment document for polychlorinated dibenzo-p-dioxins. Review draft. (EPA-600/8-84-014A) Washington DC: US EPA, Office of Health and Environmental Assessment, 1984.

Huff JE, Moore JA, Saracci R, Tomatis L. Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ Health Perspect 1980; 36:221-240.

Kociba R J, Cabey O. Comparative toxicity and biological activity of chlorinated dibenzo-p-dioxins and furans relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Chemosphere 1985; 14:649-660.

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

Moses M, Lilis R, Crow KD, et al. Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. Am J Ind Med 1984; 5:161-182.

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

Reggiani G. An overview on the health effects of halogenated dioxins and related compounds--the Yusho and Taiwan episodes. In: Coulston F, Pocchiari F, eds. Accidental exposure to dioxins: human health aspects. New York: Academic Press, 1983, pp. 39-67.

Veterans Administration. Review of literature on herbicides, including phenoxy herbicides and associated dioxins. Analysis of recent literature on health effects. Vols I-II. Washington DC. VA Contract Number V101(93) P-823; 1981.

Veterans Administration. Review of literature on herbicides, including phenoxy herbicides and associated dioxins. Analysis of recent literature on health effects. Vols III-IV. Washington DC. VA Contract Number V101(93) P-953; 1984.

Walker AE, Martin JV. Lipid profiles in dioxin-exposed workers. Lancet 1979; I:446-447.

Zinkl J, Vos JG, Moore JA, Gupta BN. Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ Health Perspect 1973; 5:111-118.

5.0 Reproductive and Teratogenic Effects

5.1 Animal Studies

Numerous studies with mice have shown TCDD to be both teratogenic and fetotoxic. The primary teratogenic effects observed in mice are cleft palate and kidney abnormalities. At relatively high doses of 200 and 400 μg/kg-day Courtney (1976) found that oral or subcutaneous administration to CD-1 mice on days seven through sixteen of gestation produced maternal toxicity and high rates of abortion. A dose of 100 μg/kg-day decreased fetal weight and survival. All dose levels, including the lowest, caused the malformations noted above as well as hydrocephalus, open eye, edema, and petechiae. The subcutaneous administration route produced a greater effect than oral administration at the lower dose levels.

Teratogenic effects have also been noted in several strains of mice at dose levels of one to ten μg/kg-day given between days six and 15 of gestation by oral or subcutaneous administration. Courtney and Moore (1971) found cleft palate and kidney abnormalities in fetuses of three strains of mice (CD-1, DBA/2J, and C57Bl/6J) given 1 or 3 μg/kg-day TCDD. The C57Bl/6J strain appeared the most sensitive. Work by Moore et al. (1973) suggested that the kidney abnormalities induced in the C57Bl/6J mice might be a toxic and not purely teratogenic effect. Neubert and Dillmann (1972) found TCDD induced cleft palate in NMRI strain mice at a dose level as low as 3 μg/kg-day but did not induce kidney abnormalities at higher dose levels. Poland and Glover (1979) showed that induction of cleft palate in mice was probably related to a genetic trait. Responsive mouse strains, those strains that

are homozygous for the Ah gene locus, which codes for a receptor protein that has a high affinity for TCDD, were much more susceptible to the induction of cleft palate by TCDD than were the non-responsive strains, i.e., strains that are homozygous recessive at that gene locus. A hybrid strain from the cross of dominant and recessive strains had an intermediate response.

Smith et al. (1976) and Neubert and Dillman (1972) examined the teratogenicity of TCDD in mice at dose levels below 1 μg/kg-day. Although Smith et al. (1976) reported that some abnormalities occurred at these lower dose levels, these were not statistically significant. Neubert and Dillman (1972) did not report an effect below 1 μg/kg-day.

Studies in rats have also shown that TCDD is fetotoxic and possibly teratogenic. Fetotoxic effects were observed in a number of studies where TCDD was orally administered over a range of dose levels, from 0.03 to 8 μg/kg on days six to 15 of gestation. Intestinal hemorrhages and subcutaneous edema appeared at dose levels as low as 0.125 μg/kg-day (Courtney and Moore 1971, Sparschu et al. 1971, Khera and Ruddick 1973). A dose level of 0.5 μg/kg-day induced kidney abnormalities (Courtney and Moore 1971); however, exposure to TCDD earlier in the gestational period (1 to 3 days) did not produce these effects. Reduced fetal weight was observed at 2 μg/kg-day and postimplantation losses increased at doses of 0.5 μg/kg-day; no effect was observed in the group treated at 0.125 μg/kg-day. Murray et al. (1979) conducted a three-generation reproduction study in which rats were fed diets that provided dose levels of 0.1, 0.01, and 0.001 μg/kg-day. They concluded that TCDD impaired reproduction in rats at dose levels of 0.1

and 0.01 μg/kg-day, and that 0.001 μg/kg-day could be considered a no-observed-adverse-effect-level (NOAEL). Nisbet and Paxton (1982) reviewed the data and concluded that there were statistically significant incidences of adverse effects at 0.001 μg/kg-day. EPA's Scientific Advisory Panel, however, found that the effects observed at 0.001 μg/kg-day were not consistent enough to be considered significant, and agreed that 0.001 μg/kg-day was a NOAEL (EPA 1979). Kimbrough et al. (1984) suggest that the study is not adequate for human risk assessment because the offspring received a larger dose of TCDD while being nursed than a human child would from a mother receiving a dose comparable to the nursing rat. They also indicate that the study is questionable because of the large variability found in the fertility index of the control and treated groups.

When TCDD was administered to rabbits at dose levels of 0.1 to 1 μg/kg-day on gestational days six through fifteen both maternal toxicity and fetal effects were observed. Maternal toxicity occurred at doses of 0.25 μg/kg-day and above. At these levels there were increases in abortions and resorptions. An increase in the prevalence of extra ribs was seen at dose levels as low as 0.1 μg/kg-day.

Three studies have examined the effects of TCDD on reproduction in nonhuman primates (Schantz et al. 1979, Barsotti et al. 1979, McNulty 1978). These studies had varied protocols and small numbers of animals. In the study reported by Schantz et al. (1979), rhesus monkeys were given a diet containing 50 parts per trillion TCDD for seven months before they were mated with control males. Four monkeys had abortions, one had a stillbirth, two failed

to conceive, and two had normal infants. All eight controls conceived and had normal infants. This report has only been given in abstract form.

These animal studies indicate that TCDD has teratogenic and reproductive effects at very low levels. The no-observed-adverse-effect-level (NOAEL) was 0.5 μg/kg-day for mice, less than 0.1 μg/kg-day and possibly as low as 0.001 μg/kg-day for rats and rabbits, and approximately 0.002 μg/kg-day for monkeys.

5.2 Human Reproductive Effects

Evidence that exposure to dioxins produces adverse reproductive outcomes in humans has not been shown in epidemiological studies (Table 5.2-1). Most investigations of reproductive effects of TCDD exposure following an industrial accident in Seveso, Italy, have not found increases in rates of spontaneous abortions and congenital malformations. Preliminary data reported by Tognoni and Bonoccorsi (1982) suggest that there may be a 67.7% increase in miscarriages in some exposure areas 9-15 months after the accident. However, the Seveso data are not likely to be considered reliable because of a lack of a baseline reference series and incomplete reporting.

Occupational studies of populations involved in the production of TCDD-contaminated materials have shown no alteration in rates of adverse reproductive effects. Townsend et al. (1982) investigated the effects of paternal exposure of dioxins on adverse pregnancy outcomes. Reproductive histories of wives of exposed workers were obtained by interview and compared to histories of wives of non-exposed workers. No statistically

significant association was found between any exposure and pregnancy outcome. However, the power to detect risks of 1.5 or more was only 50% for many of the outcomes studied. Exposure of the workers was also broadly defined, based on job classification, not individual exposure. Furthermore, information on reproductive outcomes was likely to be inaccurate because the events occurred as long as 35 years ago.

May (1982) compared rates of spontaneous abortion and fetal malformations in wives of workers in three exposure groups in a chemical plant where an explosion released TCDD ten years earlier. No malformations and only four miscarriages were reported in all three groups. The number of pregnancies available for analysis in this study was small, limiting the ability of the authors to detect any significant differences in reproductive outcomes between the groups.

Studies investigating the teratogenic effects of Agent Orange and 2,4,5-T, both of which are contaminated with TCDD, have been inconclusive. Tung et al. (1979) found a higher rate of malformations among children of soldiers who served in South Vietnam (Agent Orange exposed) in comparison to those who stayed in North Vietnam. As is the case with all studies of military exposure to Agent Orange, no specific exposure data were available. The North Vietnamese comparison group contained no malformations (compared to an expected rate of 2-3%). Other factors associated with a wartime environment (i.e. explosions, other chemical exposures, stress) may have confounded the analysis. Other studies investigating teratogenic effects of Agent Orange on Vietnam veterans and their offspring have yielded negative results.

Most of the studies of possible reproductive effects of 2,4,5-T exposure are ecological correlation studies using only national or regional defect rates and tonnage of herbicide applied. The best known of these is an Environmental Protection Agency study (1979) which compared spontaneous abortion rates between a study area in Alsea, Oregon, which had been sprayed with 2,4,5-T for forest management, and two control areas. Rates of spontaneous abortions were found to be significantly higher in the study area. This study, however, has been widely criticized for inaccurate data on herbicide application, incomplete case ascertainment, and inaccurate comparisons of the study and control areas. Other epidemiological studies have found no association between potential 2,4,5-T exposure of a population and rates of cleft palate and other congenital malformations (Thomas 1980, Nelson et al. 1979, and Hanify et al. 1981).

In general, all of these studies suffer from a lack of definitive data to show that individuals categorized as exposed have actually been exposed to phenoxy acid herbicides or their contaminants, and from a failure to rule out confounding effects due to multiple exposures to other agents. Furthermore, no quantitative exposure data were available in any of the studies.

Present research has not presented convincing evidence that exposure to dioxin is likely to cause adverse reproductive outcomes in humans. However, because all of the epidemiologic studies are flawed, one cannot rule out a dioxin-related reproductive hazard.

TABLE 5.2-1

SUMMARY OF STUDIES ON HUMAN REPRODUCTIVE EFFECTS FROM EXPOSURE TO 2,4,5-T, TCDD, AND AGENT ORANGE

REFERENCE	STUDY POPULATION	STUDY DESIGN	RESULTS	COMMENTS
Tung (1971)	Vietnamese refugees exposed To Agent Orange	Comparison of rates of chromosomal aberrations in 179 exposed refugees (No. of controls not reported)	Higher rate of chromosomal aberrations found in exposed group	Abnormally low prevalence rate of aberrations found in controls. Information on exposure inadequate and confounding likely
Meselson (1972)	500,000 births in Vietnam from 1960-65	Correlation of birth defect and stillbirth rates and herbicide use	No evidence of correlation between pregnancy outcomes and herbicide use found	No tests of statistical significance performed. Exposure information was inadequate
Meselson (1972)	500,000 births in Vietnam from 1967-70	Correlation of rates of birth defects with likelihood of exposure	Positive correlation between facial clefts and spina bifida and Agent Orange exposure attributed to increased reporting	No controls used. Exposure data insufficient
Reggianni (1978)	Births of women exposed to TCDD in Seveso	Frequencies of spontaneous abortion in pregnant women from contaminated area compared to frequencies prior to contamination	Decreased frequency of spontaneous abortions six months after the accident attributed to high rate of induced abortions	Lack of baseline data and incomplete reporting undermine reliability of study
Rehder (1978)	Aborted fetuses delivered within 6 months of exposure	Morphological examination for malformations	No indication of mutagenic or teratogenic effects found	Fetal tissue was incomplete; exposure status of mothers inaccurate
EPA (1979)	Pregnancies of women in 2,4,5-T sprayed areas	Comparison of spontaneous abortion rates in study and control areas	Significantly higher rate of spontaneous abortions in study area	Study has been widely critized for inadequate exposure data and incomplete case ascertainment
Field (1979)	All births in NSW, Australia, 1965-76	Correlation of annual neural tube defect rate and prior year's usage of 2,4,5-T	Linear correlation found for annual birth rate of neural tube defects and prior years usage of 2,4,5-T	No individual exposure data and high likelihood of confounding
Homberger (1979)	Births in TCDD contaminated regions in Seveso, 1966-77	Frequencies of malformations in Seveso six months after accident compared to frequency 6-18 months after accident	Increase in frequency of malformations attributed to incomplete reporting	Prevalence of defects amond unexposed unknown. No tests of statistical significance performed

TABLE 5.2.1 (cont'd)

REFERENCE	STUDY POPULATION	STUDY DESIGN	RESULTS	COMMENTS
Nelson (1979)	All births in Arkansas from 1943-74	Correlation of annual cleft palate prevalence and level of 2,4,5-T	Significant linear trend with time for facial clefts in high and low exposure groups	Misclassification of exposure groups likely. No individual information on exposure
Tung (1979)	Offspring of soldiers exposed to Agent Orange in South Vietnam	Comparison of malformation rates in children of 658 exposed soldiers vs. 114 unexposed	Higher rates of malformations in offspring of exposed soldiers noted	No tests of statistical significance performed. Controls were inadequte
Thomas (1980)	All births with malformations in Hungary from 1970-76	Correlation of annual incidence of birth defects with prior year's usage of 2,4,5-T	No increase in incidence of any defects was seen with increasing use of 2,4,5-T	No tests of statistical significance performed
Hanify (1981)	All births and stillbirths with malformations in New Zealand from 1960-77	Correlation of malformation incidence with levels of 2,4,5-T spraying	Significant correlation found for levels of 2,4,5-T and talipes only	No information on individual exposure used
May (1982)	Pregnancies of wives of exposed and unexposed workers in TCP factory	Comparison of spontaneous abortion and birth defect rates in wives of 126 workers in three exposure categories	No malformations and only four miscarriages reported in three exposure groups	Number of pregnancies too small for adequate statistical analysis
Smith (1982)	Offspring of New Zealand 2,4,5-T applicators from 1969-80	Retrospective study comparing rates of birth defects in children of 989 exposed and an exposed workers	Relative risks near one obtained for congenital defects and miscarriages	No information collected showing paternal exposure to 2,4,5-T occured during time of pregnancy
Townsend (1982)	Pregnancies of wives of exposed and unexposed workers in TCP factory	Comparison of birth defect and spontaneous abortion rates in 370 exposed and 345 unexposed workers	No statistically significant association was found between any pregnancy outcome and exposure	Power to detect odds ratio of 1.5 or more was 50% for stillbirths, congenital malformations and infant deaths
Balarajan (1983)	Birth malformations in UK from 1974-79	Correlation between malformation rates and father's occupation	Excess of facial clefts, spine bifida, and anencephaly found in infants of agricultural workers	No tests of statistical significance performed. Exposure to other agents besides 2,4,5-T likely
Donovan (1983)	All children with birth defects in Australia	Correlation of 8517 cases of birth defects with service in Vietnam 1966-79	No statistically significant associations were found for birth defects and Vietnam service	No data was collected relating to exposure to Agent Orange

TABLE 5.2.1 (cont'd)

REFERENCE	STUDY POPULATION	STUDY DESIGN	RESULTS	COMMENTS
Golding (1983)	Offspring of agricultural workers in UK from 1965-74	Case-control study of 12 children with birth defects compared with 47 controls	Relative risk to infants of farmers and infants of gardeners for anencephaly was 1.0 and 2.3 respectively. None of the results were significant	Power is generally poor to detect other than large risks. Exposure to other agents besides 2,4,5-T likely
Erickson (1984)	Offspring of Vietnam Veterans born in Georgia, 1968-80	Case-control study of birth defects to determine risk for fathering babies with birth defects	Significant regresson coefficients ($p < .05$) found for fathering babies with spine bifida and cleft lip for Vietnam vets in high exposure groups	Exposure information was based on military records not designed to record health data
Lathrop (1984)	Offspring of Agent Orange applicators in Vietnam	Comparison of malformation rates between 1045 exposed and unexposed veterans	Higher rates for minor birth defects and new born mortality among exposed vets found to be statistically significant	Exposure data was inadequate

References

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

Barsotti DA, Abrahamson LJ, Allen JR. Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. Bull Environ Contam Toxicol 1979; 21:463-469.

Constable JD, Hatch MC. Agent Orange and birth defects. New England J Med 1984; 310:653-654.

Courtney KD. Mouse teratology studies with chlorodibenzo-p-dioxins. Bull Environ Contam Toxicol 1976; 16:674-681.

Courtney KD, Moore JA. Teratology studies with 2,4,5-T and 2,3,7,8-TCDD. Toxicol Appl Pharmacol 1971; 20:396-403.

Donovan JW. Case-control study of congenital anomalies and Vietnam service. Report to the Minister for Veterans' Affairs. Commonwealth of Australia 1983. Australia, Sydney.

EPA (Environmental Protection Agency). Spontaneous abortions and exposure during pregnancy to 2,4,5-T. (EPA-560/6-81-006). Washington DC, US EPA, Office of Pesticide Programs, 1981.

EPA (Environmental Protection Agency). Report of the FIFRA Scientific Advisory Panel on Proposed Section 6(b) (2) Notices for 2,4,5-T and Silvex. Memorandum from Dr. H. Wade Fowler, Jr., Executive Secretary FIFRA Scientific Advisory Panel to the Deputy Assistant Administrator for Pesticide Programs. 27 September 1979.

Erickson JD, Milinare J, McClain PW, et al. Vietnam veterans risk for fathering babies with birth defects. JAMA 1984; 252:903-912.

Field B, Kerr C. Herbicide use and incidence of neural tube defects. Lancet 1979; I:1341-1342.

Golding J, Sladden T. Congenital malformations and agricultural workers. Lancet 1983; I:1393.

Hanify JA, Medcalf P, Nobbs CL, Worsley KJ. Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science 1981; 212:349-351.

Homberger E, Reggiani G, Sambeth J, Wiph HK. The Seveso accident; its nature, extent, and consequences. Ann Occup Hyg 1979; 22:327-370.

Khera KS, Ruddick JA. Polychlorodibenzo-p-dioxins: perinatal effects and the dominant lethal test in Wistar rats. Adv Chem Ser 1973; 120:70-84.

Kimbrough RD, Falk H, Stehr P, Fries G. Health implications of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) contamination of residential soil. J Toxicol Environ Heath 1984; 14:47-93.

Lathrop GD, Wolfe WH, Albanese KA. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides: baseline morbidity study results. Project Ranch Hand II 1984; USAF School of Aerospace Medicine, Aerospace Medical Division, Brooks Air Force Base, Texas.

May G. TCDD: a survey of subjects ten years after exposure. Br J Ind Med 1982; 39:129-135.

McNulty WP. Direct testimony before the administrator. US Environmental Protection Agency, FIFRA Docket No. 415, EPA Exhibit No. 106, 1984.

Meselson MS, Westing AH, Constable JD, Cook RE. Background material relevant to presentation at the 1970 AAAS Herbicide Assessment Commission. US Congressional Record, 92nd Congress 2nd Session 1972; 118(32);S3227-3223.

Moore JA, Gupta BN, Zinckl JG, Vos JG. Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ Health Perspect 1973; 5:81-85.

Murray RJ, Smith FA, Nitschke KD,et al. Three-generation reproduction study of rats given 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol Appl Pharmacol 1979; 50:241-251.

Nelson CJ, Holson JF, Green HG, Gaylor DW. Retrospective study of the relationship between agricultural use of 2,4,5-T and cleft palate occurrence in Arkansas. Teratol 1979; 19:377-384.

Neubert D, Dillman I. Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Pharmacol 1972; 272:243-264.

Nisbet ICT, Paxton MB. Statistical aspects of three-generation studies of the reproductive toxicity of TCDD and 2,4,5-T. Am Stat 1982; 36:290-298.

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

Reggiani G. Medical problems raised by the TCDD contamination in Seveso, Italy. Arch Toxicol 1978; 40:161-188.

Rehder H, Sanchioni L, Cefis F, Gropp A. Patholgischembryologische untersuchungen an abortusfallen im zusammenhang mit dem Seveso-Ungluck. Schweiz Med Wschr 1978; 108:1617-1625.

Schantz SL, Barsotti DA, Allen JR. Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Appl Pharmacol 1979; 48:A180.

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

Smith FA, Schwetz BA, Nitschke KD. Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol Appl Pharmacol 1976; 38:518-523.

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

Thomas HF. 2,4,5-T use and congenital malformation rates in Hungary. Lancet 1980; II:214-215.

Tognoni G, Bonoccorsi A. Epidemiological problems with TCDD (a critical review). Drug Metabol Rev 1982; 13:447-469.

Townsend JC, Bodnor KM, Van Peenen PFD, et. al. Survey of reproductive events of wives of employees exposed to chlorinated dioxins. Am J Epidemiol 1982; 115:695-713.

Tung TT, Anh TK, Tuyen BC. Clinical effects of massive and continuous utilization of defoliants on citizens: preliminary survey. Vietnamese Studies 1971; 29:53-81.

6.0 GENOTOXICITY

6.1 Summary

The genotoxicity of TCDD has been examined by several methods. Most short-term *in vitro* tests in bacteria indicate TCDD is not mutagenic, although early tests reported it so; however, in eukaryotic cells (yeast cells and mouse lymphoma cells), TCDD interfered with mitosis and was found to be mutagenic. In tests to examine whether TCDD could induce unscheduled DNA synthesis, results were negative.

In vivo animal tests with TCDD produced conflicting results. Loprieno et al. (1982) reported a significant increase in gaps and chromatid aberrations in cells of animals fed a single dose of TCDD; however, in a second study, these authors did not observe clastogenic effects. Green et al. reported similarly conflicting results in studies examining chromosomal aberrations. (Green and Moreland 1975, and Green et al. 1977).

Evidence that TCDD is clastogenic in humans is also contradictory. A lack of exposure data, long intervals between exposure and time of study, and a small study population were problems in all of the studies of human populations. These factors would tend to obscure the true relationship between TCDD and a clastogenic effect if any existed.

The following chapter presents a brief discussion of the most important studies of genotoxicity of TCDD.

6.2 *In Vitro* Tests with Bacteria

Repeated studies using short-term *in vitro* test systems with bacteria have been conducted with TCDD. These test systems can detect mutations produced by point or frameshift mutagens using several strains of bacteria, primarily specially developed strains of *Salmonella typhimurium* (Ames et al. 1975). In two early studies (Seiler 1973, Hussain et al. 1972), TCDD was found to induce an increase in mutations in *S. typhimurium* TA 1532, a strain sensitive to frameshift mutagens, while strains sensitive to point mutations were not affected.

In the study by Hussain et al. (1972), no increase in mutation frequency was observed until survival was less than 50 percent, which occurred at exposure concentrations greater than 2 to 3 μg TCDD/ml. When survival was between 10 and 50 percent the mutation frequency was 10^3 to 10^4 compared to an apparent background frequency of 10. TCDD was also mutagenic to *Escherichia coli* strain Sd-4 in a similar assay. For the concentration at which the mutation rate increased, survival was 11 and 18 percent in duplicate samples. However, there was almost an order of magnitude difference in mutation rates observed in the latter samples.

Because the solubility of TCDD in water is low, 0.2 μg/ml, there is some question as to whether the solutions used in the study by Hussain et al. (1972) actually contained the concentration of TCDD reported. However, the bacteria were exposed to TCDD in culture medium that also contained 10 percent dimethyl sulfoxide (DMSO), which is a good solvent for TCDD. Therefore, a higher concentration of TCDD could be obtained in this solution than in pure water. In addition, the concentration-related decrease in survival indicates that a gradient of concentrations was studied. A major problem is that the dose-response data were not given for the study using S. typhimurium bacterial strains so the concentrations that induced an increase in mutation rate are not known.

There was little experimental detail given in the report by Seiler (1973). Cited references indicated that the bacteria were exposed to TCDD dissolved in DMSO and placed as a drop in the center of the Petri dish (spot test). Five strains of S. typhimurium were used. Two strains, hisG46 and TA 1530, are sensitive to point mutagens but did not respond. Three strains, TA 1531, 1532, and 1534, are sensitive to frameshift mutagens. Strain TA 1532 showed a strong response: a 10-fold increase in mutation frequency. A response in the other strains was doubtful: a one to twofold increase in mutation frequency. OctaCDD, which was studied at the same time, gave negative results for strains hisG46, TA 1530 and 1531. A doubtful response was reported for strains TA 1531 and 1534. Positive and negative (solvent) controls indicated that the test system was working properly.

Both reports lacked sufficient detail to adequately evaluate the findings. However, both studies suggested that TCDD was mutagenic to strain TA 1532 and not to strain TA 1530. This effect occurred without the addition of a metabolic activating system, which suggests that TCDD was the ultimate mutagen.

More recent studies using the Ames test systems indicate that TCDD is not mutagenic. Geiger and Neal (1981) used S. typhimurium strains TA 100 and 1535, which are sensitive to point mutagens, and TA 98, 1537, and 1538, which are sensitive to frameshift mutagens. These strains are more sensitive to a variety of mutagens than the strains used by Hussain et al. (1972) and Seiler (1973). In addition, Geiger and Neal used the plate incorporation test, which is more quantitative than the spot test used by Seiler (1973). Metabolic activating systems were added in most test series, although a necessary cofactor (NADPH) for the activating system was not included in one test series and no activating system was added in a test using only strain TA 1537. In all cases, TCDD was not mutagenic. This study appears to have been well conducted. The concentrations of TCDD tested, 0.2 to 20 μg/plate, are probably similar to the range tested by Hussain et al. (1972). It is interesting that Geiger and Neal (1981) did not find any evidence of toxicity even at the highest concentration used when Hussain et al. (1972) did report toxicity. This may be due to the fact that Hussain et al. (1972) incubated the bacteria with TCDD for one hour before they were plated while Geiger and Neal (1981) plated the bacteria immediately after adding the TCDD.

Gilbert et al. (1980) also reported that TCDD was not mutagenic to S. typhimurium tester strains TA 98, 100, 1530, 1532, 1535, 1537, 1538, 1950, 1975, 1978, and hisG46 using a plate incorporation method similar to that used by Geiger and Neal (1981). TCDD was reportedly tested in the presence and absence of different metabolic activating systems and under aerobic and anaerobic conditions. TCDD was also reported to be negative in bacterial fluctuation tests using S. typhimurium tester strains. Unfortunately, data were given only for the few compounds that were mutagenic. No actual data are presented in the report on TCDD. This makes it difficult to evaluate the findings except to note that the methodology appeared adequate; however, no positive controls were used.

Mortelmans et al. (1984) have also reported negative findings for TCDD mutagenicity in S. typhimurium tester strains TA 98, 100, 1535, and 1537. Bacteria were preincubated with TCDD for 20 minutes in the presence or absence of a metabolic activating system before they were plated. This is similar to the one hour preincubation used by Hussain et al. (1972), although the latter did not use a metabolic activating system. Mortelmans et al. (1984) used five dose levels: from 10 to 1,000 μg TCDD/plate, none of which was reported to be toxic. The lower dose levels are probably within the upper range used by Hussain et al. (1972) that reportedly induced an increased mutation rate and were toxic. The authors indicate that the lack of TCDD solubility may account for the lack of observed toxicity. These findings most directly contradict the findings reported by Hussain et al. (1972).

Wassom et al. (1978) cited a personal communication with Dr. Joyce McCann about negative findings on the mutagenicity of TCDD. TCDD was reportedly tested by a spot test and the standard plate test using S. typhimurium strains TA 1532, 1535, 1537, and 1538 in the presence and absence of a metabolic activating system. Nebert et al. (1976) also cites unpublished data that showed TCDD was not mutagenic to S. typhimurium tester strains TA 1535 and 1538. These unpublished data cannot be evaluated, but they do support the findings of the more recent studies that indicate TCDD is not mutagenic to the S. typhimurium tester strains.

6.3 In Vitro Tests with Eukaryotic Cells

Several studies have also been conducted using eukaryotic in vitro cell systems instead of prokaryotic bacterial cell systems. Jackson (1972) examined the cytological effects of TCDD, as a contaminant of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), on isolated endosperm cells of the African blood lily. He observed that in cells treated with 0.2 μg/ml TCDD mixed with 2,4,5-T and in those treated with TCDD-contaminated 2,4,5-T TCDD there was a dramatic inhibition in mitosis while cells treated with highly purified 2,4,5-T did not differ significantly from controls. Other effects noted were formation of dicentric bridges, chromatin fusion with formation of multinuclei, or a single large nucleus. This study demonstrates that TCDD interferes with mitosis, which could lead to chromosomal abnormalities; it does not indicate if TCDD has a direct effect on cellular DNA, but it does indicate indirect effects.

Bronzetti et al. (1982) reported that TCDD acted as a mutagen in a yeast test system. The yeast, strain D7 of Saccaromyces cerevisiae, was exposed to concentrations of TCDD that ranged from 0.5 to 10.0 μg/ml and was plated on selective media using standard assay procedures. This strain of yeast permits detection of mitotic gene conversion and point mutations, which were examined in this study, as well as mitotic recombination, which was not examined. TCDD induced a dose-related increase in mitotic gene conversion and point mutation in the presence of a metabolic activating system. No such increase occurred in the absence of a metabolic activating system. Toxicity also occurred in a dose-related fashion, but it was not dependent on the presence of the metabolic activating system. At 2 μg/ml survival was 74 to 85 percent and at 4 μg/ml survival was 43 to 55 percent. The frequency of mitotic gene conversions more than doubled at 4 μg/ml and increased fourfold at the highest concentration. The mutation frequency doubled at 2 μg/ml and also increased fourfold at the highest concentration. These results are the average of four independent experiments and indicate a real effect has occurred.

Bronzetti et al. (1982) reported that TCDD was also mutagenic to S. cerevisiae strain D7 using a host mediated assay. In this study male CD-1 mice were administered a 25 μg/kg dose of TCDD by gavage. Five, 10, 15, or 20 days (or 5, 10, 20, and 30 days -- the report is internally inconsistent) following administration, 1×10^8 yeast cells were injected into the retroorbital sinus of the mice. The mice were sacrificed four hours later and the yeast cells in the liver and kidney of the mice were obtained and plated on selective media plates. Both mitotic gene conversion and mutation

frequency increased approximately twofold in yeast taken from animals treated 20 days earlier with TCDD. Yeasts obtained from liver were more affected than those obtained from kidney. There appeared to be a slight effect on yeast injected 10 days after treatment. At 30 days post-treatment the effect on yeast was less than that at 20 days. The effect observed in this study was small and the temporal pattern of the response was unexpected. Since the highest tissue concentration of TCDD and its metabolites should occur soon after administration, one might expect the highest mutation frequency to occur at the time of the earliest sacrifice, five days after administration; the observations of highest mutation frequencies occurring at 20 days after administration raise concerns that the effect may be a false positive.

Rogers et al. (1982) reported that TCDD was mutagenic in the L5178Y mouse-lymphoma cell assay. In three experiments lymphoma cells were exposed to four different concentrations of TCDD from 0.05 to 0.5 μg/ml for 48 hours, then washed, resuspended, and plated on selective media at 24-hour intervals to obtain the maximum expression time. Five selective marker agents were used: ouabain, excess thymidine, methotrexate, cytosine arabinoside, and thioguanine. In the toxicity studies TCDD had an effect on clone formation and reduced plating efficiency at concentrations as low as 0.001 μg/ml. Dose-related increases in mutation frequency were noted for three of the five selective systems. For the methotrexate, excess thymidine, and thioguanine selective systems, the mutation frequencies at the highest TCDD exposure concentration were an order of magnitude greater than the average

spontaneous mutation frequencies from 10 experiments. (The average spontaneous mutation rates were $1.6 \pm 0.36 \times 10^{-5}$ for methotrexate, $5.55 \pm 0.9 \times 10^{-6}$ for excess thymidine, and $1.93 \pm 0.4 \times 10^{-6}$ for thioguanine).

Two studies, using different methodologies, have been conducted to examine whether TCDD can induce unscheduled DNA synthesis (UDS) in vitro. Loprieno et al. (1982) reported that TCDD did not increase UDS in the heteroploid EUE human cell line following a one-hour exposure to any of eight concentrations of TCDD (0.02 to 5.0 μM). The cells were incubated for three hours with [^{3}H]-thymidine in the presence or absence of hydroxyurea (used to inhibit DNA synthesis) following TCDD exposure. Samples were analyzed by scintillation counting and by autoradiography. An exposure concentration of 2.0 μM and above was found to be toxic. Methylmethane-sulfonate was used as a positive control and did increase UDS as measured by both analysis methods. This study appears to have been adequately conducted, but is not necessarily complete. The addition of a metabolic activating system to the culture system would have been useful, especially in light of the findings by Bronzetti et al. (1982). In addition, TCDD was added to the culture medium in an acetone-corn oil mixture instead of in the more widely used solvent DMSO. The acetone to corn oil ratio was 1:3 at a concentration of 14 μg/ml. This indicates that up to 0.1 ml of corn oil was added to each milliliter of medium. Such a high concentration of oil is unlikely to be miscible in the medium and could sequester TCDD, reducing the actual TCDD concentration. Because toxicity was observed at higher concentrations, however, the TCDD was clearly not completely sequestered from the test cells.

Althaus et al. (1982) also reported that TCDD did not have a significant effect on UDS. This was actually a validation study for a more rapid and sensitive method to measure UDS in isolated rat hepatocytes. Forty-one compounds including TCDD were examined. Isolated hepatocytes were exposed to TCDD concentrations from 0.01 to 0.2 μM for 18 hours in the presence of hydroxyurea and [^{3}H]-thymidine. The number of concentrations used was not reported. The average measured UDS was equal to the control value. One major problem with this report is that it gave no indication whether any of the concentrations of TCDD were cytotoxic. If no cytotoxicity occurred, additional testing at higher concentrations would be needed.

Hay reported that TCDD gave a clear positive response in the baby hamster kidney (BHK) cell transformation assay system (Hay 1983). This author also reported that 2,8-DiCDD and 1,3,7-TriCDD gave weak positive responses while OctaCDD and unchlorinated dibenzo-p-dioxin were not active. TCDD was tested at three concentrations over a range of 0.025 to 0.25 μg/ml. There was a dose-response relationship but toxicity was especially high at the highest TCDD concentration. Significant increases in the transformation rates occurred at concentrations that did not cause more than a 50% decrease in survival. The lack of information on experimental procedure, however, does not allow the results to be fully evaluated.

6.4 In Vivo Animal Studies

In vivo animal studies using different methods have also been conducted to examine TCDD's genotoxicity. Loprieno et al. (1982) reported the results of two such studies. In one study CD-1 male and female mice were given a single dose of 10 μg/kg TCDD by gavage; then bone marrow cell preparations, taken 24 or 96 hours after treatment, were examined for chromosomal aberrations (100 cells/animal). Cells taken from four animals sacrificed 24 hours after treatment were similar to cells from control animals. There was a significant increase in the percentage of cells with gaps and chromatid aberrations for the two animals sacrificed at 96 hours (9.0± 1.41 compared to 2.5± 0.58). This indicates a weak clastogenic effect. Unfortunately, the findings are inconclusive because cells from only two animals were evaluated.

In a second study by Loprieno et al. (1982) the clastogenic effect was not observed. TCDD doses were administered orally to CD-COBS female rats every week for 45 weeks at concentrations of 0.01, 0.1, and 1.0 μg/kg. Twenty-four hours after the final dose the animals were sacrificed and bone marrow cell preparations made from each rat. There was no statistically significant change in the frequency of gaps and chromatid aberrations. Unfortunately, this study was also flawed by the small number of animals and the failure to indicate if the dose levels induced other toxic effects.

Green and Moreland (1975) reported in an abstract, that TCDD, 2,7-DiCDD, and unchlorinated dibenzo-p-dioxin did not produce chromosomal aberrations in

bone marrow cells of treated rats. These animals had been orally treated with the appropriate dioxin for five consecutive days at a dose level of 10 μg/kg and sacrificed six hours after the last administration. In a second study, male rats were given an intraperitoneal injection of 5, 10, or 15 μg TCDD/kg and one other group received an oral dose at 20 μg TCDD/kg. Animals given doses of 15 or 20 μg/kg were sacrificed 24 hours after administration and the other animals were sacrificed 29 days later. No chromosomal aberrations were noted in the TCDD-treated animals while the positive control compound, triethylene melamine, did induce chromosomal aberrations. Unfortunately, the report was not detailed enough for further evaluation.

A positive finding was reported by Green et al. (1977) in Osborne-Mendel male and female rats in a subchronic, 13-week, range-finding study on TCDD. Male rats received oral doses of 0.25, 1, 2, and 4 μg/kg and female rats received oral doses of 0.25, 0.5, 2, and 4 μg/kg twice a week. No control animals were used in this study. Bone marrow cells were examined for mitotic inhibition and chromosomal aberrations. No significant effect on mitosis was observed in either sex. There was a significant increase in chromosomal aberrations in females given 4 μg/kg compared to those given 0.25 μg/kg . Both the 2 and 4 μg/kg dose groups of male rats had a significant increase in cells with chromosomal aberrations compared to the 0.25 μg/kg dose group. However, the authors point out that the male animals given doses of 0.25 or 1.0 μg/kg had unusually low numbers of chromosomal aberrations. If the historic control value were used, only the group given 4 μg/kg would be considered as having a statistically significant elevation

in chromosomal aberrations. The authors concluded that the effect should be considered only weakly positive in both males and females.

6.5 Studies in Human Populations

Several authors have examined the incidence of chromosomal aberrations in human populations exposed to TCDD. Czeizel and Kiraly (1976) reported that workers exposed to the herbicides 2,4,5-trichlorophenoxyethanol and buminol containing less than 0.1 mg/kg TCDD (0.1 ppm) had a significantly increased incidence in chromosomal aberrations in their peripheral lymphocytes. On the other hand, Mulcahy (1980) found that soldiers exposed to "Agent Orange", which was contaminated with TCDD, did not have increased incidences of chromosomal aberrations or sister chromatid exchanges (SCE) in their lymphocytes compared to a matched control group. The 15 exposed soldiers were a self-selected group of men who believed they had symptoms of TCDD poisoning. The amount of actual exposure to the herbicide, which occurred 10 or more years earlier, was unknown, although in two cases investigators believe the soldiers had been sprayed with the herbicide. It is difficult to draw significant conclusions from this study because of the small number of subjects studied, the uncertainty of exposure, and the length of time since exposure.

Reggiani (1980) and Mottura et al. (1981) reported negative findings in cytogenetic examinations of the plant workers and the population living around a plant in Seveso, Italy, where an accidental explosion contaminated the area with TCDD. Reggiani reported that fetal tissue and maternal blood

revealed no abnormalities, but he presented insufficient information to evaluate the results. The report by Mottura et al. was presented as an abstract and also presented few data, preventing an adequate evaluation of the negative findings. The study consisted of an examination of lymphocytes from subjects distributed into three classes of exposure: acute (residences around the plant), chronic (workers in the plant), and none (residences from uncontaminated areas). Microscopic slides made of lymphocytes grown in culture for 48 or 72 hours were examined for aberrations by three different laboratories. It is not clear whether these laboratories examined lymphocytes from the same subjects or different subjects.

DiLernia et al. (1982) examined the same populations and reported positive findings. This group of researchers examined numbers of satellite associations (SAs) per cell, the average number of associated chromosomes per cell, and the SA frequency of the acrocentric chromosomes in lymphocytes taken from control and exposed subjects in 1976 and in 1979. Exposure classifications were the same as those given by Mottura et al. (1981), however, the number of subjects in each class was small: only 8 from each class (acute, chronic, and unexposed) in the 1976 sampling and 8, 2, and 6 from the 1979 sampling, respectively. No significant differences were found in SAs per cell or associated chromosomes per cell for any exposed group compared to the control group; however, there was a significant decrease in SA frequency for large acrocentric chromosomes in the chronic exposure group for both sampling times. There was no difference of this frequency in lymphocytes taken from the acute exposure group in 1976, but it was significantly

decreased in 1979. A similar decrease is seen in lymphocytes exposed to x-rays and the authors suggest that it may be the result of random damage to functional nucleolar organizing regions in the chromosomes; however, x-rays also affected the other parameters: SAs per cell and associated chromosomes per cell. The effect is not well understood and the number of subjects is too small to conclude that this study indicated chromosomal damage produced by TCDD.

In one other study examining a population exposed to TCDD because of the accident in Seveso, Tenchini et al. (1983) did a cytogenetic study on peripheral blood cells and tissue from the placenta, umbilical cord, and fetus from women who were exposed to TCDD during pregnancy or just prior to becoming pregnant and who elected to have an abortion. Tissues from unexposed women were not available at the time tissue samples were taken from the exposed women because of legal restrictions. These restrictions were removed about two years later and control tissues were then obtained under the same procedures used for obtaining tissues from exposed women.

Although cytological analyses of the exposed and unexposed tissues were performed two years apart, they were reportedly done using "as far as possible" the same procedures. Metaphase preparations were prepared with cells from the cultured tissues and scored for the percentage of aberrant cells, including and excluding gaps, the number of aberrations per damaged cell, and the percentage of polyploids.

The authors note that there was marked variability in the frequency of aberrant cells in maternal and fetal tissue from both exposed and unexposed groups. However, no significant differences were observed between exposed and unexposed women. There was significant increase in the percentage of aberrant cells found in fetal tissue from exposed women compared to tissue from unexposed women. The difference in the mean number of aberrations per aberrant cell in fetal tissue was also significantly increased in exposed tissue. It was suggested that the increased aberrations might be artifacts of the laboratory procedure; however, the increased aberrations did not occur in other tissue types. This led the authors to reject the artifact explanation. The results suggest that TCDD was a possible cause of the increase in chromosomal aberrations; however, the sample size was small and exposure data were not available (i.e., tissue concentrations of TCDD).

Cytogenetic analyses of lymphocytes have also been done on other worker populations that were probably exposed to TCDD. Blank et al. (1983) reported finding no significant difference in the frequency of chromosomal aberrations (chromatid/chromosome gaps and breaks) or SCE between groups of workers classified as exposed, possibly exposed, and control (not believed to have been exposed). The numbers of workers in each group examined for chromosomal aberrations were 55, 38, and 40, while the numbers tested for SCE were 8, 20, and 12, respectively. Exposure to TCDD occurred ten years prior to the study, which may have affected the results. However, the authors state that chromosomal aberrations should have been detectable

(i.e., if they had ever been present) since it has been shown that detectable chromosomal damage persists for a long time after irradiation. SCE, on the other hand, may not be detectable long after exposure ends.

6.6 Direct Interaction with DNA

Direct interaction of TCDD with DNA and RNA has not been extensively studied. Kondorosi et al. (1973) found TCDD had no effect on the transfectivity of Qβ phage RNA, a single-stranded RNA. From this the authors suggest that the mutagenic effect seen by Hussain et al. (1972) was through intercalation of the bacterial DNA; however, such an interaction with double-stranded RNA or DNA has not been examined. Poland and Glover (1979) found that radioactivity from tritium-labeled TCDD was strongly bound to liver protein, RNA, and DNA following administration to rats 12 to 168 hours earlier. The amount of binding to RNA and DNA was small, however, and did not vary over time. Therefore, it is likely that the DNA/RNA-bound material is not of biological importance and is an artifact. The protein-bound material, however, is probably a metabolite of TCDD. Neal et al. (1981) demonstrated that in vitro protein binding of radioactivity to hamster microsomes requires NADPH. This is a necessary cofactor for the mixed function oxidase system of the microsomes, which is the principal system for xenobiotic metabolism. The metabolism of TCDD is discussed in Section 3.

6.7 Conclusion

In prokaryotic assays, although early studies indicated TCDD was mutagenic, more recent work suggests it is not; however, in short-term tests in eukaryotic organisms, TCDD interferes with mitosis and is mutagenic. At the present time, our knowledge of these tests and the mechanism by which TCDD acts is not adequate to explain this discrepancy in the findings from the two types of assay.

In vitro tests in bacteria are useful, if the result is positive, for defining a substance as genotoxic. If the result is negative, however, the tests are not sensitive enough to rule out a substance as genotoxic.

Because of the difficulty of ruling out genotoxicity through short-term tests, IARC (1983) indicated that there is insufficient evidence to justify creating separate classes of carcinogens (based on mechanism) for which different risk assessment methods would be used.

In 1982, IARC reviewed the literature on the genotoxicity of TCDD and found it was an inadequate basis from which to form a conclusion. Even with the addition of new data, the staff of the DHS finds the available evidence still inadequate to conclude whether TCDD is genotoxic.

References

Althaus FR, Lawrence SD, Sattler GL, et al. Chemical quantification of unscheduled DNA synthesis in cultured hepatocytes as an assay for the rapid screening of potential chemical carcinogens. Cancer Res 1982; 42:3010-3015.

Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat Res. 1975; 31:347.

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

Bronzetti G, Zeiger E, Lee I, et al. Genetic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in yeast in vitro and in vivo. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds, impact on the environment, Proceedings of a workshop held at the Instituto Superiore di Sanita, Rome, Italy, 22-24 October, 1980. New York: Pergamon Press, 1982; pp. 429-436.

Czeizel E, Kiraly J. Chromosome examinations in workers producing Klorinol and Buminol. In: Banki L, ed. The development of a pesticide as a complex scientific task. Budapest: Medicina 1976; pp 239-256.

DiLernia R, Crimaudo C, Pacchetti G. The study of X-rays and TCDD effects on satellite associations may suggest a simple model for application in environmental mutagenesis. Hum Genet 1982; 61:42-47.

Geiger LE, Neal RA. Mutgenicity testing of 2,3,7,8-tetrachlorodibenzo-p-dioxin in histidine auxotrophs of salmonella typhimurium. Toxicol Appl Pharmacol 1981; 59:125-129.

Gilbert P, Saint-Ruf G, Poncelet F, et al. Genetic effects of chlorinated anilines and azobenzenes on Salmonella typhimurium. Arch Environ Contam Toxicol 1980; 9:533-541.

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

Green S, Moreland FS. Cytogenetic evaluation of several dioxins in the rat. Toxicol Appl Pharmacol 1975; 33:161.

Hay A, Ashby J, Styles JA, et al. The mutagenic properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin. American Chemical Society, Division of Environmental Chemistry 1983; 3:14-22.

Hussain S, Ehrenberg L. Lofroth G, et al. Mutagenic effects of TCDD on bacterial systems. Ambio 1972; 1:32-33.

IARC (International Agency for Research on Cancer). Approaches to classifying chemical carcinogens according to mechanism of activity. Joint IARC/IPCS/CEC Working Group Report, Lyon, France. IARC Internal Technical Reports 1983; 83/001.

IARC (International Agency for Research on Cancer). Chemicals, industrial processes, and industries associated with cancer in humans. IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1982; 1-29:(Suppl 4):238-243.

Jackson WT. Regulation of mitosis. III cytosological effects of 2,4,5-trichlorophenoxyacetic acid and of dioxin contaminants in 2,4,5-T formulations. J Cell Sci 1972; 10:15-25.

Kondorosi A, Fedorcsak I, Solymosy F, et al. Inactivation of QB RNA by electrophiles. Mutat Res 1973; 17:149-161.

Loprieno N, Sbrana I, Rusciano D, et al. In vivo cytogenetic studies on mice and rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds, impact on the environment. New York: Pergamon Press 1982; pp 419-428.

Mortelmanns K, Haworth S, Speck W, et al. Mutagenicity testing of agent orange components and related chemicals. Toxicol Appl Pharmacol 1984; 75:137-146.

Mottura A, Zei G, Nuzzo F, et al. Evaluation of results of chromosome analyses on lymphocytes of TCDD exposed subjects after the Seveso accident. Mutat Res 1981; 85:238-239.

Mulcahy MT. Chromosome aberrations and "Agent Orange". Med J Aust 1980; 2:573-574.

Neal RA, Olson JR, Gasiewicz TA, et al. The in vivo and in vitro metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Golden Syrian hamster. In: Khan MAQ, Staton RH, eds. Toxicology of halogenated hydrocarbons, health and ecological effects. New York: Pergamon Press, 1981, pp 259-270.

Nebert DW, Thorgeirsson SS, Felton JS. Genetic differences in mutagenesis, carcinogenesis, and drug toxicity. In: de Serres FJ, Fouts JR, Bend JR, Philpot RM, eds. In vitro metabolic activation in mutagenesis testing. Elsevier North-Holland Biochemical Press, 1976; 105-122.

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

Reggiani G. Estimation of the TCDD toxic potential in light of the Seveso accident. Arch Toxicol [Suppl 2] 1979; 2: 291-302.

Rogers AM, Anderson ME, Back KC. Mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and perfluoro-n-decanoic acid in L5178Y mouse lymphoma cells. Mutat Res 1982; 105:445-449.

Seiler JP. A survey on the mutagenicity of various pesticides. Experientia 1973; 29:622-623.

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

Wassom JS, Huff JE, Loprieno N. A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutat Res 1978; 47:141-160.

7.0 Carcinogenicity

7.1 Animal Studies

Among the many PCDD and PCDF isomers, only TCDD and a mixture of 2,3,7,8-HexaCDDs have been tested for carcinogenicity in animals. The studies of TCDD were evaluated by IARC (1982) and EPA (1982, 1984) and those of HexaCDD were reviewed by EPA (1984). The reviewers conclude that both TCDD and HexaCDD were carcinogenic in animals. These studies are discussed below and the studies on TCDD are summarized in Table 7.1-1. Additional investigations of TCDD's role as a promoter of carcinogens are discussed in Section 8.

7.1.1 TetraCDD

Van Miller et al. (1977 a,b) reported the results of a study in which rats were fed diets containing from 1 ppt to 1 ppm of TCDD for 78 weeks. Surviving rats were killed after 95 weeks. Laparotomies were performed on all surviving rats at 65 weeks and all tumors were biopsied. Rats in the three highest dose groups, receiving 50 ppb or more, died early. A variety of tumors were found in rats receiving 5 ppt to 5 ppb while no neoplasms were found in the control or low-dose groups. The absence of tumors in these two groups is unusual in this strain of rats. In addition, because of the small number of animals in each group (10) the study is inadequate to determine the carcinogenic potential of TCDD.

Table 7.1-1

Carcinogenicity Bioassays on TCDD

Authors	Species	Strain	Sex	No.[a] Treatment and Other	Experimental Detail	Results	Observations
Van Miller et al. 1977a,b	Rat	Sprague-Dawley	M	10 T 10 C	Diets contained 0.001, 0.005, 0.05, 0.5, 1.0, 5.0, 50, 500, and 1000 ppb of TCDD. Fed for 78 weeks and study ended at 95 weeks.	The 3 high-dose groups died early. In groups receiving 0.005 to 5 ppb, the incidence of total tumors was from 30 to 70%. No tumors found in control group.	The number of animals per group was small and the absence of tumors in the control group is considered unusual for the strain of rat.
Toth et al. 1979	Mouse	Swiss/H/Riop	M	45T 100C	Dosed by gavage once per week at 7.0, 0.7, and 0.007 lg/kg/week for 1 year. Mice followed for life.	High early mortality in the high-dose group. The middle-dose group had an increased incidence of liver tumors.	High early mortality in the high-dose group may have precluded an increased incidence of liver tumors.
Kociba et al.	Rat	Sprague-Dawley	M,F	50T 86C	Given diets containing 2200, 210, and 21 ppt of TCDD for life.	A number of tumors were found to be significantly increased over controls in both males and females.	Adequate study for low-dose extrapolation.
NTP 1982a	Rat	Osborne-Mendel	M,F	50T 75C	Dosed by gavage 2 times per week at 0.01, 0.05, and 0.5 lg/kg/week.	A treatment-related increased incidence of a number of tumors observed in both sexes.	Adequate study for low-dose extrapolation.
NTP 1982a	Mouse	B6C3F1	M,F	50T 75C	Dosed by gavage 2 times per week at 0.01, 0.05, and 0.5 or 0.04, 0.2, lg/kg/week for males and females, respectively.	There was a significance increase in hepatocellular carcinomas in males and an increased incidence in a number of tumors in females.	Adequate for low dose extrapolation.
NTP 1982b	Mouse	Swiss-Webster	M,F	30T 45C	TCDD in an acetone suspension was painted on skin of mice at 0.001 and 0.005 lg per application 3 times per week. One group pretreated with 50lg DMBA.	Female mice treated with TCDD and DMBA/TCDD had significant increases in the incidence of fibrosarcoma of the integumentary system.	Suggest TCDD is carcinogenic and that TCDD does not promote after DMBA initiation, although this study was not adequate to assess TCDD promoting activity.

[a] Number of animals per group. T = Treatment, C = Control.

Toth et al. (1979) administered TCDD to male Swiss/H/Riop strain mice by gavage once a week for a year, then followed them for their lifetime. The weekly doses were 0.007, 0.7, and 7.0 μg/kg. Analysis of the results from this study focused on the incidence of liver tumors. A significant increase in the incidence liver tumors was observed in the intermediate-dose group compared to the four separate control groups. The high-dose group, however, had an incidence of liver tumors that was similar to the control group. This finding may be explained by the early mortality in the high-dose group. The average life span was 424 days for this group, compared to average life spans of between 577 and 651 days for the control groups. Had the treated animals lived longer more tumors may have formed.

Kociba et al. (1978) conducted a two-year feeding study in male and female Sprague-Dawley rats given diets containing 2200, 210, or 22 parts per trillion (w/w) TCDD for two years. Consumption of these diets gave daily doses of 0.1, 0.01, and 0.001 μg/kg-bw, respectively. There were 50 male and 50 female rats in each treatment group and 86 animals of each sex in the control group. There was a statistically significant ($p < 0.05$) increase in cumulative mortality for the high-dose female group in the latter half of the study. Body weights of the male and female high-dose groups were significantly ($p < 0.05$) reduced for the last three quarters of the study; however, food intake was not altered. The combined incidence of hepatocellular carcinomas and hepatocellular neoplastic nodules in the intermediate- and high-dose groups of female rats was increased above the control group. Statistically significant increased incidences of stratified squamous cell carcinomas of the hard palate and/or nasal turbinates were observed in both male and female high-dose groups. The male group also had an increased

incidence of squamous cell carcinoma of the tongue, while the female group had an increased incidence of keratinizing squamous cell carcinoma of the lung.

EPA (1981) reviewed this study and had an independent pathologist, Robert Squire, review the tissue pathology. The incidences of significant tumors reported by Kociba et al. (1978) and by Squire (EPA 1981) are given in Tables 7.1-2 and 7.1-3 for male and female rats, respectively. The results of Squire's review did not differ greatly from those reported by Kociba et al. (1978).

DHS staff members concur with earlier reviewers (IARC 1982, EPA 1984) that the study reported by Kociba et al. (1978) was an adequately conducted chronic carcinogenicity bioassay of TCDD, with significant effects observed at the two higher dose levels.

The National Toxicology Program (NTP 1982a) conducted an oncogenicity bioassay of TCDD in male and female Osborne-Mendel rats. They were administered TCDD in a 9:1 corn oil:acetone vehicle by gavage at dose levels of 0.005, 0.025, or 0.25 μg/kg twice a week for 104 weeks. The treatment groups consisted of 50 rats of each sex and a vehicle control group that was made up of three subgroups of 25 rats of each sex. An untreated control group, also made up of three subgroups of 25 rats of each sex, was included in the study, but not in the statistical analysis of the results by NTP. At the dose levels used, TCDD did not have a significant effect on survival of any treatment group. The high-dose group of male rats did have a statistically significant increased incidence of subcutaneous tissue

Table 7.1-2

Tumor Incidences in Male Rats Receiving TCDD in the Diet for Two Years[a]

Tissue and Diagnosis	Dose level (μg/kg-day) 0 (control)	0.001	0.01	0.1
Tongue				
Stratified squamous cell carcinoma	0/76[b]	1/49	1/49	4/42 p = 0.015[c]
	(0/77)	(1/44)	(1/49)	(3/44) p = 0.046
Nasal turbinates/hard palate				
Squamous cell carcinoma	0/51	1/34	0/27	4/30 p = 0.017
	(0/55)	(1/34)	(0/26)	(6/30) p = 0.002
Total	0/65	2/49	1/49	7/42 p = 0.010
	(0/77)	(2/44)	(1/49)	(9/44) p = < 0.001

a Chi-square test for trend in proportions statistically significant at α= 0.05 level for all tissues and diagnoses.

b Number of animals with tumor over number of animals examined. This is the incidence reported by Kociba et al. (1978). The numbers in parentheses give the incidence reported by Squire.

c P values from Fisher's Exact Test. Only p values less than or equal to 0.05 are reported.

Source: EPA 1984

Table 7.1-3
Tumor Incidences in Female Rats Receiving
TCDD in their Diet for Two Years[a]

Tissue and Diagnosis	Dose Level (μg/kg-day) 0 (control)	0.001	0.01	0.1
Lung				
Keratinizing squamous cell carcinoma	0/86[b]	0/50	0/49	7/49 $p < 0.001$[c]
	(0/86)	(0/50)	(0/49)	(8/47) $p < 0.001$
Nasal turbinates/hard palate				
Squamous cell carcinoma	1/54	0/30	1/27	5/24 $p = 0.009$
	(0/54)	(0/30)	(1/27)	(5/22) $p = 0.001$
Liver				
Hepatocellular hyperplastic nodules/ carcinomas	9/86 [1][d]	3/50	18/50 [2] $p < 0.001$	34/48 [11] $p < 0.001$
	(16/86)	(8/50)	(27/50) $p < 0.001$	(33/47) $p < 0.001$

a Chi-square test for trend statistically significant at $\alpha = 0.05$ level for all tissues and diagnoses.

b Number of animals with tumor over number of animals examined. This is the incidence reported by Kociba et al. (1978). The numbers in parentheses give the incidence reported by Squire.

c P values from Fisher's Exact Test. Only p values less than or equal to 0.05 are reported.

d Number in brackets is the number of animals with hepatocellular carcinomas.

Source: Kociba et al. 1978, EPA 1984

fibromas, but it is not considered biologically significant because of the variability found. All male treatment groups had significantly ($p < 0.05$) increased incidences of thyroid follicular cell adenomas or adenomas and carcinomas, although the low- and intermediate-dose level group incidences were not significant when compared to the untreated control group by DHS staff. The female high-dose group had significantly ($p < 0.05$) increased incidences of several tumor types, including subcutaneous tissue fibrosarcomas, liver neoplastic nodules or hepatocellular carcinomas, and adrenal cortical adenomas. Of these 3 tumors, NTP considered only the liver tumors to be related to TCDD administration. The incidences of these tumors are given in Table 7.1-4. Toxic hepatitis was found in 14 male and 32 female high-dose level rats.

NTP (1982a) also conducted an oncogenicity bioassay with TCDD in male and female B6C3F1 hybrid strain mice. The protocol was similar to that used in the rat study with male mice receiving the same doses of TCDD. Female rats, however, received larger doses of 0.02, 0.1, or 1.0 μg/kg twice a week. These dose levels did not have a statistically significant effect on survival of any treatment group.

Male mice in the highest dose group had a significantly increased incidence of hepatocellular carcinomas. The high-dose female group had significantly increased incidences of subcutaneous tissue fibrosarcomas, hepatocellular adenomas or carcinomas, and thyroid follicular-cell adenomas. NTP considered only liver tumors and thyroid tumors to be related to TCDD administration. NTP also considered histiocytic lymphomas to have been

Table 7.1-4

Tumor Incidences in Male and Female Osborne-Mendel Rats Given TCDD by Gavage for Two years[a]

Tissue and Diagnosis	Dose Level (μg/kg-week) 0 (vehicle control)	0.01	0.05	0.5
Males				
Thyroid				
Follicular cell adenoma	1/69[b]	5/48 p = 0.042[c]	6/50 p = 0.021	10/50 p = 0.001
Follicular cell adenoma/carcinoma	1/69	5/48 p = 0.042	8/50 p = 0.004	11/50 p < 0.001
Females				
Subcutaneous tissue Fibrosarcoma	0/75	2/50	3/50	4/49 p = 0.023
Liver Neoplastic nodules/ hepatocellular carcinoma	5/75	1/49	3/50	14/49 [3][d] p = 0.001
Adrenal Cortical adenoma or adenoma NOS	11/73	8/49	4/49	14/46 p = 0.039

a Chi-square tests for trend in proportions statistically significant at $\alpha = 0.05$ level for all tissues and diagnosis.

b Number of animals with tumors over the number of animals examined.

c P values under group incidences are from the Cochran-Armitage test for dose-related trend. Only p values less than or equal to 0.05 are reported.

d Number in brackets is the number of animals with hepatocellular carcinoma.

Source: NTP 1982a

increased in the high-dose female group, but the staff of DHS do not consider that these lymphomas were increased when the incidences in all control subgroups are considered. The observed tumor incidences in both male and female mice are given in Table 7.1-5. Toxic hepatitis was observed in 44 male and 34 female high-dose group animals. It was also observed in several animals of the other treatment groups.

Both rat and mouse carcinogenicity bioassays conducted by NTP appear to have been done in an adequate manner. The number of treatment groups and the large dose range used in the studies are not typical of NTP bioassays, although it was similar to that used by Kociba et al. (1978). Still it may not have been large enough to include a dose level which produced no effect. Most significantly increased tumor incidences only occurred in the high-dose level groups, but a statistically significant dose-related trend was found in all groups.

NTP (1982b) also conducted a dermal oncogenicity bioassay on TCDD in male and female Swiss-Webster mice. TCDD in an acetone suspension was applied to the skin three days per week for 104 weeks. The male rats received 0.001 μg per application and the females received 0.005 μg per application. Separate groups of male and female mice were treated with one application of 50μg 7,12-dimethylbenzyl(1)anthrene (DMBA) one week prior to the start of TCDD treatments. The only significantly ($p = 0.01$) increased incidences of tumors observed were among female mice. Both the TCDD- and DMBA/TCDD-treated groups had a similar incidences of fibrosarcoma in the integumentary system (8/27 and 8/29, respectively), compared to the vehicle control of 2/41. In NTP's judgement, the results of this experiment indicated that

Table 7.1-5

Tumor Incidences in Male and Female B6C3F1 Mice Given TCDD by Gavage for Two years[a]

Tissue and Diagnosis	Dose Level (μg/kg-week) 0 (vehicle control)	0.01[b] (0.04)	0.05 (0.2)	0.5 (2.0)
		Males		
Liver Hepatocellular carcinoma	8/73[c]	9/49	8/49	17/50 p = 0.002[d]
Liver Hepatocellular adenoma or carcinoma	15/73	12/49	13/49	27/50 p < 0.001
		Females		
Subcutaneous tissue Fibrosarcoma	1/74	1/50	1/48	5/47 p = 0.032
Liver Hepatocellular carcinoma	1/73	2/50	2/48	6/47 p = 0.014
Liver Hepatocellular adenoma or carcinoma	3/73	6/50	6/48	11/47 p = 0.002
Thyroid Follicular cell adenoma	0/69	3/50	1/47	5/46 p = 0.009

a Chi-square test for trend in proportions statistically significant at α= 0.05 level for all tissue and diagnoses.

b Dose administered to male mice. Number in parentheses is dose administered to female mice.

c Number of animals with tumor over number of animals examined.

d P values under treatment group incidences are from Fisher's Exact Test. Only p values less than or equal to 0.05 are reported.

TCDD was carcinogenic. (See Section 8 for additional discussion about this experiment in the context of promotion of carcinogenesis.)

7.1.2 HexaCDD

HexaCDDs have also been tested for carcinogenicity by NTP (1980a) in both Osborne-Mendel rats and B6C3F1 hybrid strain mice. The bioassay tested a mixture of HexaCDDs containing 31 percent 1,2,3,6,7,8-HexaCDD and 67 percent 1,2,3,7,8,9-HexaCDD. Lower chlorinated PCDDs made up the remaining 2% of the mixture, including 0.04 percent TetraCDDs. Male and female rats and male mice received weekly doses of 1.25, 2.5 or 5 μg/kg, administered by gavage twice a week. The female mice were administered doses of 2.5, 5.0, or 10 μg/kg/week.

A dose-related "toxic hepatitis", which was noninflammatory and consisted of degenerative changes in the liver, was observed in treated rats. The treated groups of female rats had significantly increased incidences of liver neoplastic nodules. Four high-dose animals were diagnosed as having hepatocellular carcinoma. The mice also had a dose-related incidence of "toxic hepatitis" and the high-dose male and female mouse groups had statistically significant increased incidences of hepatocellular adenomas and combined incidences of hepatocellular adenomas and carcinomas. The incidences of these tumors are given in Table 7.1-6.

Table 7.1-6

Tumor Incidences in Female Osborne-Mendel Rats and Male and Female B6C3F1 Mice Given HexaCDD by Gavage for Two Years[a]

Tissue and Diagnosis	Dose Level (μg/kg-week)			
	0 (vehicle control)	1.25[b] (2.5)	2.5 (5.0)	5.0 (10)
Female Rat				
Liver				
Neoplastic nodule or hepatocellular carcinoma	5/75[c]	10/50 p = 0.026[d]	12/50 p = 0.007	30/50 p < 0.001
Male Mice				
Liver				
Hepatocellular adenoma	7/73	5/50	9/49	15/48 p = 0.003
Hepatocellular adenoma or carcinoma	15/73	14/50	14/49	24/48 p = 0.001
Female Mice				
Liver				
Hepatocellular adenoma	2/73	4/48	4/47	9/47 p = 0.003
Hepatocellular adenoma or carcinoma	3/73	4/48	6/47	10/47 p = 0.004

a Chi-square test for trend in proportions statistically significant at α = 0.05 level for all tissues and diagnoses.

b Dose level for female rats and male mice. Number in parentheses is the dose level for female mice.

c Number of animals with tumor over number of animals examined.

d P values under treatment group incidences are from Fisher's Exact Test. Only p values less than or equal to 0.05 are reported.

Several pathologists have independently evaluated the slides made from the female rat livers in this bioassay. Each re-evaluation found fewer neoplastic nodules and carcinomas than did the original evaluation.

Although the incidences of neoplastic nodules and carcinomas are probably lower than originally reported, the incidence is still significant in the high-dose group. The results of four separate evaluations of the liver pathology of the female rats are given in Table 7.1-7.

A dermal application carcinogenicity bioassay of the same mixture of HexaCDD in male and female Swiss-Webster mice was also conducted by NTP (1980b). This study was similar to the TCDD dermal oncogenicity bioassay in its protocol. Thirty mice of each sex were treated with 0.005 μg of the dioxin mixture three times per week for the first 16 weeks, which was increased to 0.01 μg thereafter. A similar group was initially treated once with 50 μg DMBA before being treated with the HexaCDD mixture. Thirty untreated and 45 vehicle-treated mice of each sex were used as controls. Although there was a slight increase in fibrosarcomas of the integumentary system, this was not considered by NTP to be a significant carcinogenic response. DMBA pretreatment had no additional affect. (See Section 8 for additional discussion about promotion of carcinogenesis.)

7.1.3 Conclusion

DHS staff members agree with IARC (1982) that there is adequate evidence to support a conclusion that TCDD is carcinogenic to rats and mice and that TCDD should be considered a potential carcinogen to humans. The NTP

Table 7.1-7

Incidence of Liver Tumors Based on
Four Separate Pathological Evaluations of
Female Rats Given HexaCDD by Gavage for Two Years[a]

Pathologist and Diagnosis	Dose Level (μg/kg-week) 0 (control)	1.25	2.5	5.0
NTP (1980a)				
Neoplastic nodules or hepatocellular carcinoma	5/75[b]	10/50 p = 0.026	12/50	30/50(4)[c] p < 0.001
Squire (1983)				
Neoplastic nodules	1/75	4/50	7/50 p = 0.007	7/50 p = 0.007
Haberman and Schueler (Schueler 1983)				
Neoplastic nodules or hepatocellular carcinoma	---	---	---	17/50(3)[d]
Hildebrandt (1983)				
Neoplastic nodules or hepatocellular carcinoma	1/75	5/50 p = 0.037	7/50 p = 0.007	18/50(2) p < 0.001

a Chi-square test for trend in proportions for NTP, Squire, and Hildebrandt studies significant at $\alpha = 0.05$ level.

b Number of animals with tumor over number of animals examined.

c Number of animals diagnosed with hepatocellular carcinoma is shown in parentheses.

d The diagnosis for nine of the animals with neoplastic nodules is considered a matter of judgment by the pathologist.

bioassays (NTP 1980a) of HexaCCDs indicate that the mixture used was tumorigenic. In the latter studies, independent evaluation of the liver pathology in the female rats consistently found lower tumor incidences than those reported by NTP. However, a dose-response effect was observed in each case when all the groups were evaluated and the increases were statistically significant.

7.2 Human Studies

Recent comprehensive reviews of the human studies of dioxin exposure and cancer risk are found in EPA (1984) and Veterans Administration (VA) (1984). The two main types of epidemiologic studies of PCDD/PCDF exposure are:

- Case/control studies--where the exposure histories of persons with cancer are compared to the exposures of comparison subjects, and

- Cohort, or follow-up, studies--where the cancer risk among exposed persons is compared to the risk that would be expected if the exposed group experienced the same risk as (usually) the general population.

Although several studies have been conducted in the US and Europe, they have limitations that prevent their use in quantitative cancer risk assessment. These limitations include:

- Lack of accurate exposure data: Exposure is usually reconstructed historically, sometimes based on job title. Only in rare instances can one estimate the amount of PCDD present. Hence, most studies can speak only of <u>potential</u> exposure.

- Lack of a pure, defined exposure: Dioxins have never been intentional products. In human exposure studies, PCDDs and PCDFs have only been present as contaminants of other toxic chemicals, such as herbicides. Hence all studies of human PCDD/PCDF exposures have been studies of exposure to chemical mixtures that may have contained PCDD and PCDF.

VA (1981, 1984) summarized what is known about the presence of PCDD and PCDF in commercially- used chemicals. In general, PCDDs and PCDFs may be present as contaminants in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Levels of 2,3,7,8-TCDD in 2,4,5-T have been found as high as six parts per million (Rappe et al. 1982). Another widely used herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D) is generally regarded as uncontaminated with TCDD. Cochrane et al. (1982) did detect traces of Di-, Tri-, and TetraCDD as high as one part per billion in technical grade 2,4-D from Canada. However, the TetraCDD isomer found in these samples was the 1,3,6,8-TCDD isomer, not the more toxic 2,3,7,8-TCDD.

Agent Orange, which was a mixture of 2,4,5-T and 2,4-D, has been shown to contain 2,3,7,8-TCDD concentrations as high as 15-47 parts per million with an average of about 2 ppm (VA 1981). PCDDs and/or PCDFs have also been found in the parts per million range in commercially used polychlorinated biphenyls (PCB), trichlorophenol (TCP), tetrachlorophenol, and pentachlorophenol (PCP) (Rappe et al. 1982, Hardell 1983).

The routes of PCDD/PCDF exposure that have been studied in humans are:

- Potential exposure through occupational use of phenoxy herbicides or chlorinated phenols.

- Potential exposure through employment in a chemical manufacturing plant.

- Potential exposure through widespread environmental contamination. The best known examples are dispersal of Agent Orange during the war in Vietnam, and the products of a 1976 chemical explosion at a trichlorophenol-producing plant in Seveso, Italy.

7.2.1 Exposure through Use of Phenoxy Herbicides or Chlorophenols

Several case/control studies have been conducted in Sweden and in New Zealand. In these countries, phenoxyacetic acids and chlorophenols were used extensively for agriculture and forestry. After clinical observations of several patients with soft-tissue sarcomas (STS) and a history of heavy exposure to phenoxyacetic acids, Hardell and Sandstrom (1979) conducted a case/control study of STS and herbicide exposure. Cases were drawn from a university hospital in Northern Sweden, and consisted of 52 adult males with STS diagnosed between 1970 and 1977. Controls were drawn from general population registries, at a 4:1 matching ratio, and matched to cases on sex, age, place of residence, and vital status (whether alive or deceased). The investigators considered only non-malignant deaths for deceased controls. Study subjects (or their next of kin) provided exposure histories by a mailed questionnaire with a telephone follow-up. The odds ratio (or) for exposure to phenoxyacetic acids only (excluding subjects exposed to chlorophenols) was 5.3 (95% confidence interval (CI) 2.4-11.5). For exposure to chlorophenols only (excluding those exposed to phenoxyacetic acids) the OR was 6.6 (95% confidence interval 2.1-20.9).

To confirm these findings, Ericksson et al. (1981) replicated this study in Southern Sweden, using cases from a cancer registry. Similar study methods

were used, including matching controls from a population registry (at a 2:1 ratio), and determining exposure by mail and telephone questionnaires. The investigators calculated separate odds ratios for exposure to phenoxy acids known to be contaminated with PCDD and PCDF (OR=17.0; 95% CI 2.1-140.0) and for exposure to phenoxy acids thought to be free of PCDD and PCDF (OR=4.2; 95% CI 1.2-14.9). When exposure was dichotomized into categories of 30 days or less, or more than 30 days, the ORs were 5.7 and 8.5 respectively, possibly indicating a dose-response trend.

One of the drawbacks of this study is that, exposure histories were provided by the study subjects; therefore, the results may be influenced by recall bias. Cases (or their next of kin) may be more likely to recall an exposure than a healthy person. In order to investigate this possible bias, Hardell (1981) duplicated the study methods using cases of colon cancer. Here there was no significant association with exposure to herbicides. Hence, Hardell concluded that the association with STS was not due to reporting differences between diseased cases and healthy controls.

Smith et al. (1984) reported a similar case/control study in New Zealand. Here, male cases of STS were gathered from a national cancer registry, with controls also being selected from the same registry. This method of control selection was designed to avoid differential recall. Unlike the Swedish studies, however, the New Zealand study showed no significant associations with reported phenoxy herbicide spraying. The authors suggested that if dioxin were the necessary agent, that Swedish herbicides may have been more contaminated than New Zealand herbicides. However, Smith et al. (1984) note that the Swedish investigators also found a significant association between

STS and non-dioxin-contaminated herbicides, indicating that if the association were true, dioxin would not be the sole agent.

Another case/control study reported in brief by Olsen and Jensen (1984) of cases from the Danish Cancer Registry failed to show an association between nasal cancer and chlorophenol exposure, although nasal cancer was associated with occupational exposure to wood dust.

In a letter to Lancet, Milham (1982) reported proportionate mortality data from Washington state indicating that farmers suffered a significantly larger proportion of deaths due to STS. No other group occupationally exposed (foresters, orchardists, tree farmers) showed an excess of STS; however, the exposure assessment was based on occupations taken from death certificates. Furthermore, Milham indicated that 2,4-D was the predominant herbicide used, and 2,4-D is not generally contaminated with 2,3,7,8-TCDD.

A cohort study of phenoxy acid herbicide applicators in Finland was reported by Riihimaki et al. (1983). A historical cohort of 1926 herbicide applicators was assembled from the records of four large employers, including the Finnish Highway Authority and State Railways. These male workers had used chlorinated phenoxyacids for at least two weeks between 1955 and 1971. Their mortality between 1972 and 1980 was studied by comparing their names against population registers. National mortality figures provided expected age-standardized numbers of deaths. Deaths from all causes, and for all cancers, were less than expected. The power of this study to detect an increase in STS was poor, however, as only 0.1 case of STS was expected based on general population rates. Furthermore, as deaths in the cohort

were studied only after 1972, 45 deaths that occurred in this group before 1972 were not tallied. (Even for post-1971 deaths, however, the follow-up period may also have been too short for a sufficient tumor latency period to have elapsed.)

7.2.2 Exposure through Chemical Manufacturing

There have been four potentially exposed occupational cohorts studied in the United States. Zack and Suskind (1980) reported the follow-up of Monsanto employees in Nitro, West Virginia, who were involved in a 1949 accident during the processing of trichlorophenol. A sudden violent reaction released fumes and residues into a building interior. Apparently, the released chemical mixture was not analyzed, but the authors assumed that it contained TCDD, as exposed workers developed chloracne.

A historical cohort of 121 white male employees was assembled from company records on the basis of their having exhibited skin disorders "attributed to the 1949 TCP process accident." Their vital status was traced through 1978, providing a maximum of 29 years of follow-up per person. The standardized mortality ratio (SMR) for all causes of death in this cohort (relative to US white males) was significantly decreased (32 observed deaths vs 46.4 expected). One cancer site showed an excess: lung cancer (5 observed vs 2.85 expected), although this SMR of 1.75 was not statistically significant. Interestingly, there occurred one STS, a fibrous histiocytoma. But the authors calculated SMRs (and expected numbers of deaths) only for causes with five or more observed deaths.

Zack and Gaffey (1983) described another cohort from this plant, composed of 884 male workers employed for at least one year between 1955 and 1977. It is not clear whether workers exposed in the 1949 accident were included. The same methods were used to calculate SMRs. Only 25 malignancies occurred, compared to 30.9 expected. However, two specific sites were notably elevated: lung cancer, with 14 observed vs 9.9 expected (SMR 1.4; 95% CI 0.8-2.4), and bladder cancer, with 9 observed vs 0.9 expected (SMR 9.9; 95% CI 4.5-18.8). One STS occurred in a worker judged to have been exposed to TCDD.

One drawback to this study is that exposure histories were only constructed for the 163 decedents--and only 36% of these were judged to have had potential exposure to 2,4,5-T (and therefore TCDD). Hence the true exposed cohort may only have been one-third the size of the entire study group.

Cook et al. (1980) presented a similar historical cohort study of Dow chemical employees. In 1964, chloracne occurred in workers in a trichlorophenol manufacturing area. Industrial hygiene investigations concluded that TCDD was responsible and changes were made in the operations to decrease exposure. Levels of TCDD during this period were unknown because concentrations fell below the existing limit of detection, 0.02 $\mu g/m^3$ of air (Cook 1981a); however, wipe samples were positive for TCDD.

Cook et al. (1980) assembled a cohort of 39 workers thought to have high exposure potential, and 22 workers thought to have lower exposure. Among the high-exposure group, 87% had a history of chloracne, compared to 68% of the low-exposure group. Their vital status was determined through 1978.

There were only four deaths (vs 7.8 expected based on US white males), although three of these deaths were due to neoplasms (vs 1.6 expected). One neoplasm was a fibrosarcoma.

Another Dow cohort was investigated by Ott et al. (1980). This cohort contained 204 white males involved in 2,4,5-T production between 1951 and 1971. The authors determined each worker's vital status through 1976, resulting in a median length of time since first exposure of about 20 years. Only one malignancy (a respiratory cancer) was recorded vs 3.6 expected from US population rates. This cancer death occurred among the employees with 20 or more years of latency; in this group 0.9 deaths were expected.

Besides the small sample size, there are other problems with using this study for risk assessment. The exposure to TCDD may have been minimal. Environmental sampling of the breathing zone in 1969 revealed 2,4,5-T concentrations between 0.2 and 0.8 mg/m^3. Product specifications at that time called for a maximum TCDD concentration of 1 ppm. Assuming the maximum level of both 2,4,5-T in the breathing zone, and TCDD in the 2,4,5-T, the concentration of TCDD in the breathing zone would have been 10^{-6} of the concentration of 2,4,5-T, or 0.8 ng/m^3. Ott et al. also noted that 157 of the 204 workers (77%) were exposed for less than one year. Furthermore, a review of medical records of the cohort uncovered no cases of chloracne.

A further analysis of Dow employees was presented by Bond et al. (1983), who reported a morbidity survey on the combined cohorts previously described by Cook et al. (1980) and Ott et al. (1980). Bond et al. found few differences between the morbidity of these workers and a matched control group of

workers from other locations in the plant. There were, however, more ulcers and diseases of the digestive system (excluding liver) in the 2,4,5-T cohort, at roughly twice the prevalence in the controls. But because the investigators only studied cohort members who participated in company medical programs between 1976 and 1978, only 69% of the original cohort was included. The study did not include workers who had died, retired, or left the company, raising the possibility that the most affected workers might have been missed.

Following the publication of the four US mortality studies, reports began to appear in Lancet of four additional cases of STS among these cohorts, bringing the apparent total to seven (Honchar and Halperin 1981, Cook 1981b, Moses and Selikoff 1981, Johnson et al. 1981). The proportion of deaths in these merged cohorts due to STS appeared to be far greater than would be expected (Fingerhut and Halperin 1983), although there is great difficulty in estimating expected rates of STS using general population statistics (Cook and Cartmill 1984). Fingerhut (cited in VA 1984) has had the diagnoses of the seven cases reviewed by two pathologists. The pathologists could only agree on a diagnosis of STS for three of the seven, another three being reclassified, and the last diagnosis being disputed. Of the three definite cases, only two had frank chloracne to corroborate exposure. The VA review (1984) concluded that the occurrence of even two cases of STS among these relatively small cohorts warranted continued surveillance.

Other cohort studies of occupational exposures have come from Great Britain, West Germany, and the Netherlands. May (1973 and 1982) only briefly described the aftermath of a 1968 accidental release of TCP with a "higher

than normal" concentration of TCDD. A total of 79 cases of chloracne were recorded, but May did not specify how many workers were exposed, so that one cannot calculate an attack rate. A survey of 46 of these workers, who were still with the company 10 years later, revealed that roughly half still had some chloracne (May 1982). Other than this, there were no clinical problems reported, and no cases of cancer (although clearly few if any would be expected in a group this small).

Thiess et al. (1982) published a carefully-reported study of 74 workers exposed to dioxins due to a 1953 reactor accident in a German 2,4,5-T plant. After a 23-year follow-up, this cohort exhibited seven deaths due to malignancies (vs 4.09 expected from West German population rates), including three deaths due to stomach cancer (vs 0.7 expected). The latter was statistically significant at a one-sided 95% level. No cases of STS occurred, although less than 0.1 would have been expected.

A mortality study of workers present at an explosion in an herbicide factory in Amsterdam was summarized by Dalderup and Zellenrath (1983). Between 200 and 500 g of TCDD were thought to have been liberated. The investigation traced 141 of 145 workers potentially exposed, and 69 (49%) had developed chloracne. After 20 years of follow-up, 8 of the workers had died with cancer (vs 6.9 expected), yielding an SMR of 1.2 (95% CI 0.5-2.3). No STS deaths were seen. The authors should have calculated SMRs separately for the group with frank chloracne (an indicator of stronger exposure), as the crude mortality for this chloracne group was 20%, and for the non-chloracne group 15%.

7.2.3 Exposure through Environmental Contamination

Reports are starting to appear in the literature on the effects of Agent Orange herbicide exposure in Vietnam. Unfortunately, most of these reports are as yet anecdotal, or present interim results. Agent Orange was composed of equal parts 2,4-D and 2,4,5-T, and about 90,000 tons of herbicides were sprayed in Vietnam between 1962 and 1971. Hay (1983) mentions evidence from Vietnamese studies that "suggests a link" between herbicide exposure and liver cancer. He gives no details. Sarma and Jacobs (1982) reported three patients with STS who claimed Agent Orange exposure while serving in Vietnam.

The US Air Force's Ranch Hands study (summarized by VA 1984) has released some initial results. This is a cohort study of some 1200 military personnel who worked on Operation Ranch Hand, the herbicide spraying operation. These subjects have been matched (in a 5:1 ratio) with personnel who flew only cargo missions in Vietnam. As of 1983, the total mortality rates were nearly identical between the two groups. Only four cases of cancer have occurred among the exposed, and none were STS. The investigators stressed the preliminary nature of the data, the relatively low power of a study of this size to detect rare tumors such as STS, and the relatively short latency period up to now (12-21 years).

A report by Greenwald et al. (1984) gave the results of a case/control study of STS in New York State. Cases of STS (n=281) diagnosed between 1962 and 1980, who were between the ages of 18 to 29 during the war in Vietnam, were

selected from the state cancer registry. Cases were individually age-matched to living controls drawn from drivers' license files. The investigators gathered exposure information from subjects or next of kin by a telephone questionnaire. The questions focused on Vietnam service (and Agent Orange exposure in particular), but included other exposures such as chemical manufacturing and herbicide spraying in general. Only 3% of the cases and 4% of the controls had a history of Agent Orange, dioxin, or 2,4,5-T exposure. None of the various exposures proved statistically significant.

The power of this study can be criticized, with exposures as rare as they were. Also, one might question the inclusion of cancer cases from the early 1960s. These cases would not have had sufficient latency to have been caused by an exposure in Vietnam.

In 1983, an Australian Royal Commission began investigating the effects of Agent Orange exposure to Australian Vietnam veterans. However, their report, released in 1985, does not supply much information on the effects of PCDDs. The executive summary concluded that "only a very limited number of Australian servicemen were ever directly exposed," and further, that the dose received by the majority of Australian veterans was "so minute that it may, without doubt, be ignored," (e.g., it noted that no Australians developed chloracne). Not surprisingly, the Commission found no evidence of any cancer excess among the "exposed" servicemen (Royal Commission, 1985).

Abate et al. (1982) summarized the series of studies following the 1976 accidental release of TCDD from a TCP-producing plant in Seveso, Italy. The

investigators looked at mortality rates for 11 municipalities for four years after the accident and reported no increase in cancer mortality. These studies serve mainly to provide baseline rates for future studies, because clearly not enough time has elapsed to provide the minimum 10 to 20 years required for an increased cancer risk to become manifest (Bruzzi, 1983).

A summary of the morbidity and mortality of victims ten years after Japan's Yusho incident was reported by Urabe et al. (1979). More than 1600 persons were stricken in 1968 after consuming rice bran cooking oil contaminated with PCB and PCDF. The authors tabulated known causes of death in a group of some 700 victims residing in Fukuoka Prefecture. Of the 51 known to have died, 31 had causes of death identified. Eleven (35%) of these 31 known causes of death were due to neoplasms. This proportion was higher than that observed in the prefecture as a whole. However, the authors did not adjust for sex or age. Furthermore, it is possible that Yusho victims who died of cancer were over-reported among the fraction of deaths of known cases.

7.2.4 Discussion of Epidemiologic Studies

The major epidemiologic studies of PCDDs and cancer are summarized in Tables 7.2-1 and 7.2-2. Some of the limitations of these studies have been alluded to in the above discussions. These include:

- Dubious exposure to PCDD/PCDF: Usually the exposure occurred in the past when there were no sensitive measures of exposure levels. Exposure is often based on job title, self-reported use of substances

which may have had PCDD contamination, or exposure to an event thought to have liberated PCDDs.

- Exposure to other chemicals besides PCDD/PCDF: None of the human exposures described have been solely to PCDDs or PCDFs, but to a mixture of chemicals. PCDDs were but trace contaminants of other toxic chemicals.

- Exposure for a short time: Many of the occupationally exposed subjects were exposed only briefly (e.g., during an accidental release), or worked in a possibly contaminated environment for a short time. For example, more than 75% of the workers studied by Ott et al. (1980) had been exposed for less than one year.

- Studies conducted on males only: No females were included in any of the occupational study groups.

- Small sample sizes: Many studies, including the four US cohorts, have been hampered by small samples. Studies of only a few hundred subjects lack sufficient power to detect small increases in the risk of rare tumors, as discussed below.

Table 7.2-1
Summary of Major Case/Control Studies of Dioxin Exposure

Reference	Nature of Exposure	No. of Cases	Potential Latency	Results	If Negative - Power to Detect a 50% Increase in Risk (one-sided 95% test)
Hardell and Sandstrom (1979)	Self-reported history of using herbicides > 1 day	52 Male cases of soft-tissue sarcoma	≥ 5 yrs	Significant odds ratio for exposure to phenoxy acids and chlorophenols	
Eriksson et al. (1981)	Self-reported history of using herbicides > 1 day	110 Male cases of soft-tissue sarcoma	≥ 5 yrs	Significant odds ratio for exposure to phenoxy acids and chlorophenols	
Hardell et al. (1982)	Self-reported history of using chemicals > 1 day	71 Male cases of nasal and nasopharyngeal cancer	≥ 5 yrs	Significant odds ratio for exposure to chlorophenols (adjusting for wood working)	
Olsen and Jensen (1984)	Occupational data from cancer registry	834 Male cases of nasal, sinus, and naso-pharyngeal cancer	Not given	No association with chlorophenol exposure	0.48
Smith et al. (1984)	Self-reported history of using herbicides ≥ 5 days	82 Male cases of soft-tissue sarcoma	≥ 10 yrs	No significant association with phenoxy herbicides	0.25
Greenwald et al. (1984)	Self-reported history of Vietnam service or using herbicides	281 Male cases of soft-tissue sarcoma	4 to 14 yrs	No association with exposure to Agent Orange, or 2,4,5-T	0.26

Table 7.2-2

Summary of Major Cohort Studies of Dioxin Exposure

Reference	Nature of Exposure	No. of Subjects	Potential Latency	Results	If Negative - Power to Detect a 50% Increase in Risk (one-sided 95% test)
Zack and Suskind (1980)	Trichlorophenol process accident	121 Males	29 years	No excess total cancer SMR for lung cancer = 1.8 (not significant)	For total cancer = 0.38 For lung cancer = 0.14
Cook et al. (1980)	Employment in a trichlorophenol process	39 Males with "high exposure potential" 22 Males with "low exposure potential"	15 years	SMR for total cancer = 1.9 (not significant)	For total cancer = 0.10
Ott et al. (1980)	Employment in a 2,4,5-T plant	204 Males, but only 47 exposed > 1 yr	20 years	No excess total cancer	For total cancer = 0.18
Theiss et al. (1982)	2,4,5-T process accident	74 Males	Mean = 23 years	SMR for total cancer = 1.7 (not significant) SMR for stomach cancer = 4.3 (significant at 95% one-sided level)	For total cancer = 0.17
Dalderup and Zellenrath (1983)	Herbicide process accident	141 Males	20 years	SMR for total cancers = 1.2 (not significant)	For total cancer = 0.34
Riihimaki et al. (1983)	Herbicide applicators	1926 Males	$\geq$ 10 years	No excess total cancer	For total cancer = 0.68
Zack and Gaffey (1983)	Employment in a 2,4,5-T plant	884 Males, but number actually exposed to 2,4,5-T much less	median approx. 30 years	SMR for lung cancer = 1.4 SMR for bladder cancer = 9.9 (significant at 95% level)	For total cancer = 0.80 For lung cancer = 0.42

Therefore, DHS staff members have concluded that the epidemiologic data provide insufficient information to conclude whether or not PCDDs or PCDFs are human carcinogens.

Much has been made in the literature of the Swedish case/control studies by Hardell et al., which found odds ratios for exposure to PCDD-containing herbicides to be dramatically high. An attempt to replicate these findings in New Zealand failed, however. Smith et al. (1984) suggested that past levels of TCDD 2,4,5-T in New Zealand (which had never been measured) may have been less than the 1 ppm found in Swedish 2,4,5-T. There exists the possibility that the association found in the Swedish studies resulted from an over-reporting bias among cases or their next of kin. Hardell (1981) examined this possibility by repeating their methodology in a case/control study of colon cancer, here he found no association. This suggests that differences in herbicide exposure between STS cases and controls can probably not be explained solely by reporting differences.

The majority of cohort studies have not demonstrated an association between PCDD/PCDF exposure and cancer in humans. Some SMRs for total cancer mortality have been reported in the 1.5 to 1.9 range, but have not been statistically significant. Of course, these cohort studies suffer from the limitations of uncertain exposures and small sample sizes. There have been too few cases of STS among each individual cohort to adequately test the association seen in the case/control studies. A plan to combine exposed cohorts from many industrial sites may prove fruitful in the future (Fingerhut and Halperin 1983).

7.2.5 Statistical Power of Epidemiologic Studies

As noted earlier, the lack of association between PCDD/PCDF exposure and cancer may be due in part to small sample sizes. To evaluate this possibility, we refer to a 1980 Occupational Safety and Health Administration (OSHA) policy, which states that a "non-positive" human study (a study which failed to show an exposure effect) should only be considered evidence of "no effect" if the study had sufficient statistical power to detect a 50% increase in risk (Haines and Shannon 1983). Statistical power is the probability that the null hypothesis of no effect (e.g., SMR=1.0) would be rejected if the true SMR were, in this case, 1.5. Usually, a power of 0.80 or more is considered adequate. Power is greater in studies of larger sample sizes or longer follow-up. Studies with low power do not have the sensitivity to detect a small increase in risk, nor to declare a small increase in risk statistically significant if one were to be observed.

Tables 7.2-1 and 7.2-2 present for each non-positive study the power to detect a 50% increase in risk (i.e., SMR=1.5 or OR=1.5). The power for each case/control study was calculated from the investigators' data on sample size and prevalence of exposure among the control subjects, using the formula given in Rothman and Boice (1979). The power for each negative cohort study was calculated from the investigators' data on sample size and the expected number of deaths, using the Poisson power formula in Ginevan (1982) and the tables in Pearson and Hartley (1966). As shown in Tables 7.2-1 and 7.2-2, few studies realized adequate power to detect a 50% increase in total

cancers. And none had sufficient power to detect a 50% increase in cancer at any single site.

The most statistically powerful study might appear to be that by Zack and Gaffey (1983), because of their relatively large cohort and long follow-up time. But while the cohort contained a total of 884 men drawn from a plant's employment rolls, a specific history of 2,4,5-T exposure was searched for only among the decedents. After the examination of work histories, only 36% of the decedents were judged to have been exposed. If the same proportion held for the entire group of workers, the size of the exposed cohort might have been as small as 320, lessening the power considerably.

Therefore, using the OSHA criterion, none of the non-positive PCDD/PCDF studies would be accepted as evidence of no effect.

The staff of DHS favors the use of a less arbitrary, but more stringent criterion for proof of "no effect" in human studies. This is to determine if the study had the power to detect the number of additional cases that would be predicted, using the reported exposure measurements and the risk estimates derived from the animal studies. The study by Ott et al. (1980) of Dow chemical workers is the only cancer risk study to include exposure measurements. As described in Appendix C, the predicted number of cancers extrapolated to this cohort from the most sensitive animal bioassay is less than 0.1 excess case. A study of the size of Ott's, with 204 subjects, would not have the power to detect an increase this small.

References

Abate L, Basso P, Belloni A, et al. Mortality and birth defects from 1976 to 1979 in the population living in the TCDD polluted area of Seveso. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds: impact on the environment. Oxford: Pergamon Press, 1982; 571-587.

Bellin JS, Barnes DG. Health hazard assessment for chlorinated dioxins and dibenzofurans other than 2,3,7,8-TCDD. Presented at the Symposium on Advances in Health Risk Assessment for Systemic Toxicants and Chemical Mixtures. Cincinnati OH. 25 October 1984.

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

Bruzzi P. Health impact of the accidental release of TCDD at Seveso. In: Coulston F, Pocchiari F, eds. Accidental exposure to dioxins; human health aspects. New York: Academic Press, 1983:215-225.

Cochrane WP, Singh J, Miles W, et al. Analysis of technical and formulated products of 2,4-dichlorophenoxy acetic acid for the presence of chlorinated dibenzo-p-dioxins In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds: impact on the environment. Oxford; Pergamon Press,1982: pp. 209-213.

Cook RR, Cartmill JB. Dioxin: comparing apples to oranges. Chemotech. 1984; 14:534-537.

Cook RR. Author's response. J Occup Med 1981a; 23(1):8.

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

Cook RR, Townsend JC, Ott MG, et al. Mortality experience of employees exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J Occup Med 1980; 22:530-532.

Dalderup LM, Zellenrath D. Dioxin exposure: 20 year follow-up. Lancet 1983; II:1134-1135.

Environmental Protection Agency (EPA). Health assessment document for poly chlorinated dibenzo-p-dioxins. Review Draft. Part 2. Washington DC, US EPA Office of Health and Environmental Assessment, EPA-600/8-84-014A. 1984.

Environmental Protection Agency (EPA). Risk assessment on 2,4,5-trichlorophenoxy propionic acid, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Washington DC: US EPA Carcinogen Assessment Group, EPA 600/6-81-003. 1981.

Environmental Protection Agency (EPA). Health assessment document for poly-chlorinated dibenzo-p-dioxins. Review Draft. Washington DC: US EPA Office of Health and Environmental Assessment, EPA-600/8-84-014A. 1984.

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

Fingerhut MA, Halperin WE. Dioxin exposure and sarcoma. JAMA 1983; 249:3176-3177.

Greenwald P, Kovasznay B, Collins DN, et al. Sarcomas of soft-tissues after Vietnam service. JNCI 1984; 73(5):1107-1109.

Ginevan ME. A Poisson trials approach to interpopulation comparisons of cause of death data. Environ Res 1981; 25:147-159.

Haines T, Shannon H. Sample size in occupational mortality studies. J Occup Med 1983; 25:603-608.

Hardell L. Epidemiological studies on soft-tissue sarcoma, malignant lymphoma, nasal and nasopharyngeal cancer, and their relation to phenoxy acid or chlorophenol exposure. In: Choudhary G, Keith LH, Rappe C, eds. Chlorinated dioxins and dibenzofurans in the total environment. Boston: Butterworth Publishers, 1983; pp 367-374.

Hardell L, Johansson B, Axelson O. Epidemiological study of nasal and nasopharyngeal cancer and their relationship to phenoxy acid or chlorophenol exposure. Am J Ind Med 1982; 3:247-257.

Hardell L, Relation of soft-tissue sarcoma, malignant lymphoma, and colon cancer to phenoxy acids, chlorphenols, and other agents. Scand J Work Environ Health 1981; 7:119-130.

Hardell L, Sandstrom A. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 1979; 39:711-717.

Hay A. Defoliants in Vietnam: the long term effects. Nature 1983; 302:208-209.

Hildebrandt PK. Letter to EE McConnell, NIEHS/NTP Research Triangle Park, NC. 31 October 1983.

Honchar PA, Halperin WE. 2,4,5-T, trichlorophenol, and soft tissue sarcoma. Lancet 1981; I:268-269.

IARC (International Agency for Research on Cancer). Chemicals, industrial processes, and industries associated with cancer in humans. IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1982; 1-29: Suppl 4.

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

Kociba RJ, Keyes DG, Beyer JE, et al. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol Appl Pharmacol 1978; 46(2):279-303.

May G. Chloracne from the accidental production of tetrachlorodibenzo-dioxin. Br J Ind Med 1973; 30(3):276-283.

May G. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. Br J Ind Med 1982; 39(2):128-135.

Milham S. Herbicides, occupation, and cancer. Lancet 1982; I:1464-1465.

Moses M, Selikoff IJ. Soft tissue sarcomas, phenoxy herbicides, and chlorinated phenols. Lancet 1981; I:1370.

National Toxicology Program. Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (gavage study). DHHS Publ No. (NIH) 82-1765. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, RTP, Box 12233, NC. 1982a.

National Toxicology Program. Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (Dermal study). DHHS Publ No. (NIH) 80-1757. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, RTP, Box 12233, NC. 1982b.

National Toxicology Program. Bioassay of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin for possible carcinogenicity (dermal study). DHHS Publ. No. (NIH) 80-1758. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, NTP, Box 12233, NC. 1980a.

National Toxicology Program. Bioassay of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (gavage) for possible carcinogenicity. DHHS Publ. No. (NIH) 80-1754. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, RTP, Box 12233, NC. 1980b.

Olsen JH, Jensen OL. Nasal cancer and chlorophenols. Lancet 1984; II:47-48.

Ott MG, Holder BB, Olson RD. A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. J Occup Med 1980; 22:47-52.

Pearson ES, Hartley HO. Biometrika tables for statisticians. Vol 1. Cambridge; University Press, 1966:227.

Rappe C, Nygren M, Buser HR, et al. Occupational exposure to polychlorinated dioxins and dibenzofurans. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds: impact on the environment. Oxford: Pergamon Press, 1982, pp 495-515.

Riihimaki V, Asp S, Pukkala E, et al. Mortality and cancer morbidity among chlorinated phenoxyacid applicators in Finland. Chemosphere 1983; 12:779-784.

Rothman KJ, Boice JD. Epidemiologic analysis with a programmable calculator. Washington DC: US National Institutes of Health, NIH publication no. 79-1649; 1979:33-34.

Royal Commisission on the Use and Effect of Chemical Agents on Austrailian Personnel in Vietnam. Final Report. Canberra: Australian Government Publishing Service 1985 ; 8:XV12-13, XV23.

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

Schuler RL. Report: Review of selected neoplastic and non-neoplastic-Liver lesions in rats given hexachlorodibenzo-p-dioxins (C03703). Submitted to B Haberman, USEPA, Washington DC, 21 September 1983.

Smith AH, Pearce NE, Fisher DO, et al. Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. JNCI 1984; 73(5):1111-1117.

Squire RA. Report: An assessment of the experimental evidence for potential carcinogenicity of hexachlorodibenzo-p-dioxins. Submitted to: TA Robinson, Vulcan Chemicals Birmingham, AL. 29 June 1983.

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

Toth K, Somfai-Relle S, Sugar J, et al. Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. Nature 1979; 278(5704):548-549.

Urabe H, Koda H, Asahi M. Present state of Yusho patients. Ann NY Acad Sci 1979; 320:273-276.

Van Miller JP, Lalich JJ, Allen JR. Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere 1977a; 6:625-632.

Van Miller JP, Lalich JJ, Allen JR. Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere 1977b; 6:537-544.

Veterans Administration. Review of literature on herbicides, including phenoxy herbicides and associated dioxins. Vol I: Analysis of literature. Washington DC: Veterans Administration Department of Medicine and Surgery. VA Contract No. V101(93) P-823. 1981.

Veterans Administration. Review of literature on herbicides, including phenoxy herbicides, and associated dioxins. Vol. III: Analysis of recent literature and health effects. Washington DC; Veterans Administration Department of Medicine and Surgery. VA Contract No. V101(93)P-953. 1984.

Zack JA, Gaffey WR. A mortality study of workers employed at the Monsanto Company plant in Nitro, West Virginia. In: Tucker RE, Young AL, Gray AP, eds. Human and environmental risks of chlorinated dioxins and related compounds. New York: Plenum Press, 1983; pp 575-591.

Zack JA, Suskind RR. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J Occup Med 1980; 22:11-14.

8.0 Mechanism

8.1 Summary

In animal bioassays TCDD acts as a promoter, a weak initiator, and as a cocarcinogen. When administered alone it induces changes in liver cells considered pre-cancerous.

There is substantial evidence that one mechanism by which TCDD acts to cause toxic effects and possibly carcinogenesis is binding to a specific receptor protein in the cytosol. The TCDD-cytosolic receptor complex moves to the nucleus where it interacts with DNA to increase mixed function oxidase synthesis.

Poland and his colleagues conducted a series of experiments with TCDD examining toxicity, carcinogenicity, and cytosolic receptor binding. They found that the toxic potency of a series of halogenated aromatic hydrocarbons, including TCDD, was correlated with their affinity to bind to the cytosolic receptor; that promoter activity was correlated with the ability to bind with the cytosolic receptor; and that carcinogenesis through the mechanism of cytosolic binding depends on the expression of an unspecified genetic component.

The following section contains a brief discussion of the evidence concerning mechanism and a description of each of the studies mentioned therein.

8.2 Cytosolic Binding Mechanism

The mechanisms for TCDD's toxicity are not well understood with the excpetion of the induction of mixed-function oxidase enzyme activities.

A cytosolic receptor protein, found in many mammalian cell types, has a high affinity for TCDD and other aromatic hydrocarbons. This TCDD-receptor complex can move from the cytosol to the nucleus, where it interacts with the chromatin to induce the synthesis of cytochrome P-450 proteins and to increase measurable enzyme activities. Investigators reported a stochiometric relationship between the synthesis of cytochrome P-450 messenger RNA and the amount of TCDD-receptor complex in the nucleus. (Tukey et al. 1981, 1982, Gonzalez et al. 1984, and Israel and Whitlock 1983).

Furthermore, the toxic potency of halogenated hydrocarbons, including TCDD, is correlated with their binding affinity for the cytosolic receptor (Poland et al. 1982a). Toxic effects linked to the presence of cytosolic receptor binding include liver damage (necrosis and hypertrophy), gastrointestinal damage, increased mixed-function oxidase activity, and, possibly, carcinogenicity.

Investigators have shown that the cytosolic receptor protein is the product of one specific gene locus, the Ah locus (Poland and Knutson 1982a). However, not all cell types or tissues which undergo TCDD-enzyme induction display other toxic effects; therefore, Poland suggests that expression of other genes may be necessary for certain toxic responses to occur.

8.3 Carcinogenesis and mechanism

TCDD may act as a cocarcinogen through the induction of the mixed function oxidase system. Studies demonstrate that TCDD can both increase and reduce the carcinogenic potency of polynuclear aromatics (Norman et al. 1982, Berry et al. 1979, Cohen et al. 1979, DiGiovanni et al. 1979, 1980, Kouri et al. 1978). In a short-term in vitro test with bacteria, TCDD increased the formation of mutagenic metabolites of 2-aminoanthracene (Norman et al. 1982); however, in tests of TCDD with 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP), TCDD inhibited the carcinogenic response, apparently by altering the metabolism of BaP and DMBA to less carcinogenic metabolites. Evidence for this effect was found in Cohen's experiments: the level of normal DNA-BaP adducts in TCDD-treated animals was lower that controls, despite an increase in other DNA-BaP adducts (Cohen et al. 1979).

TCDD also appears to act as a promoter. Two groups of investigators have reported promotion activity (Poland et al. 1982b, Pitot 1980). Two others obtained negative results (Berry et al. 1978, NTP 1982); however, the findings of Poland and his colleagues (that a specific genetic component may have to be present) may explain the negative results.

In a series of experiments Poland et al. (1982b) investigated the correlation between cytosolic receptor binding, promotion activity, and specific strains of mice. He observed that (1) promotion activity occurred only in the compounds able to bind with the cytosolic receptor; (2) the affinity of the cytosolic receptor to bind with TCDD varied according to the strain of mouse; and (3) TCDD acted as a promoter in a strain of mouse homozygous at

the hr gene locus, but it did not promote carcinogenesis in a congeneic strain heterozygous at the hr gene locus. The Poland work indicates that an additional genetic component must be expressed for TCDD to promote carcinogenesis.

Poland also found that the toxic potency, including carcinogenicity, of a series of halogenated hydrocarbons was correlated with their affinity to bind with the cytosolic receptor; TCDD was the most potent and showed the greatest affinity for the cytosolic receptor.

Pitot et al. (1980) also observed that TCDD acted as a promoter; TCDD was administered after the initiator diethylnitrosamine (DEN). The investigators observed increased tumor incidence of liver carcinomas when both agents were present; TCDD also induced liver cell changes considered precancerous when administered alone. Because TCDD had not been demonstrated to be mutagenic in short-term assays in bacteria, the authors concluded that TCDD was not an initiator; therefore, they interpreted the liver cell changes as evidence that TCDD promotes cells initiated by other agents such as background radiation or diet.

Kociba (1984) proposed that the mechanism for TCDD-induced carcinogenesis is through tissue response to chronic toxicity.

Finally, there is evidence that TCDD acts as an initiator. DiGiovanni et al. (1977) reported a small positive response when mice were treated with an

initiating dose of TCDD, then with the promoter 12-0-tetra-decanoylphorbal-13-acetate (TPA). When the mice were treated with the initiator DMBA and TCDD, the authors observed an additive effect.

Effects On Other Carcinogens

TCDD can both increase and reduce the carcinogenic potency of polynuclear aromatics. In several studies using the 2-stage mouse skin tumorigenesis bioassay, TCDD reduced the carcinogenic potency of polynuclear aromatics such as benzo(a)pyrene (BaP) and 7, 12-dimethylbenz(a)anthracene (DMBA) (Berry et al. 1979, Cohen et al. 1979, DeGiovanni et al. 1979, 1980). TCDD (0.1 to 1 μg) was administered topically or by intraperitoneal injection from three to ten days prior to the administration of the procarcinogen. The final incidence of skin tumors decreased following promotion by 12-0-tetradecanoylphorbol-13-acetate (TPA). Cohen et al. examined the covalent binding of radioactively labeled BaP and DMBA to DNA of mouse skin applied three days after the area was treated topically with 1 μg of TCDD. In the case of DMBA, the amount of DNA-bound material was much less in mice pretreated with TCDD. This correlated well with a the decrease in the tumor incidence observed for DMBA in mice preteated with TCDD. With BaP, on the other hand, there was an increase in DNA-bound material in pretreated mice; but the level of normal BaP DNA adducts was lower than in control animals. This suggests that the increased DNA binding was of adducts that are noncarcinogenic or much less potent than the normal BaP adducts. Thus, TCDD likely inhibits the carcinogenic response of polynuclear aromatics in the 2-stage mouse skin tumorigenesis assay system by altering or increasing biotransformation to less carcinogenic metabolites.

However, in an in vitro mutagenicity test, TCDD increased the formation of mutagenic metabolities of 2-aminoanthracene. The test used a metabolic activating system from TCDD-pretreated rabbits (Norman et al. 1982).

Kouri et al. (1978) found TCDD acted as a cocarcinogen. A dose of 100 μg TCDD was administered by intraperitoneal injection to DBA/2 Cam (D2) strain mice at the same time as 3-methylcholanthrene (3-MC) was administered by subcutaneous injection; the incidence of fibrosarcomas was significantly increased over animals treated with 3-MC alone. This effect may have been due to an increase in the formation of carcinogenic 3-MC metabolites. However, two factors raise questions about whether the mechanism is by induction of the mixed-function oxidase system: 1) the strain of mice that showed a positive effect is an unresponsive strain (induction of the mixed-function oxidases requires a large dose of TCDD, and induction is still not as great as in responsive strains) while a responsive strain did not show a positive effect; 2) TCDD administered two days prior to 3-MC administration had no effect, although, this would be more advantageous for optimum induction of the mixed function oxidases.

Promotion Activity

In two studies (NTP 1982, Berry et al. 1978) TCDD failed to promote the induction of skin tumors in the 2-stage mouse skin tumorigenesis assay system using the initiator DMBA. The NTP (1982) study has already been discussed in Section 7. No response was observed in male mice treated with 0.001 μg of TCDD three times per week for 104 weeks. In female mice treated with 0.005 μg TCDD a similar increase in fibrosarcomas was observed in both

the group treated with DMBA and TCDD and the group treated only with TCDD. Thus, there was no indication of promoting activity. In the study by Berry et al. (1978) DMBA-treated and control mice received 0.1 μg TCDD topically twice a week for 30 weeks. No tumors occurred in either group, although TPA did promote skin tumors in another group of DMBA-treated mice. A mixture of 31% 1,2,3,6,7,8- and 67% 1,2,3,7,8,9-hexaCDD also failed to promote skin tumors in DMBA-initiated mice (NTP 1980).

TCDD has been observed to promote initiated cells. Poland et al. (1982b) reported the results of a study in which TCDD did act as a promoter in the mouse skin two-stage tumorigenesis bioassay, using a strain of mouse homozygous for the recessive hairless trait at the hr gene locus. Both the classical promoter TPA and TCDD gave positive results, althought TCDD was effective at a dose level three orders of magnitude less than that of TPA. The TCDD dose was 3.75 to 30 ng/mouse/2 times weekly; the TPA dose was 1 to 3 μg/mouse/2 times weekly. Histologically, the skin of TPA-treated mice showed typical changes of acute inflammation and hyperplasia while TCDD-treated mouse skin showed hyperplasia and hyperkeratosis with no acute inflammation. In the congeneic mouse strain TCDD did not act as a promoter, nor did it produce skin changes.

Poland et al. (1982b) also used the hairless mouse strain to test the promoting activity of other halogenated hydrocarbons. They found that 2,3,7,8-TetraCDF acted as a promoter when applied at a dose of 1 μg per mouse. A similar response was observed for TCDD at 15 μg per mouse. However, only one dose level of 2,3,7,8-TetraCDF was used, so it is not known whether lower levels would give the same results. The brominated

biphenyl 3,4,5,3',4',5'-Hexabromobiphenyl (HBB) also acted as a promoter while the dioxin and biphenyl 2,7-DiCDD and 2,4,5,2',4',5'-HBB did not. Poland et al. (1982b) note that the compounds that acted as promoters bind to the cytosolic receptor while the non-promoters did not. They suggest that cytosolic binding is necessary for the promoting activity of the compounds; however, the cytosolic receptor is found in both strains of mice, but the promoting effect is seen in only one. Therefore, the authors suggest that an additional battery of genes must be expressed and that cytosolic receptor binding is necessary for expression of these genes in genetically susceptible mice.

TCDD was also found to be a promoter by Pitot et al. (1980) in the two-stage model of hepatocarcinogenesis. TCDD was administered by subcutanious injection at 14 bi-weekly treatments. The rats were partially hepatectomized and were given one initiating dose of diethylnitrosamine (DEN). TCDD doses were 0.14 and 1.4 μg/kg.

TCDD treatments significantly increased the number of enzyme-altered foci found in the liver. These foci are thought to be prescursors of hepatocellular carcinomas. At the high dose level there was a significantly increased incidence of hepatocellular carcinomas (five out of seven had tumors). Neoplastic nodules were found in three out of five rats given the lower TCDD dose level and in one rat given the high dose level. When phenobarbital was given as the promoter instead of TCDD, the number of foci increased and eight out of ten animals had hepatocellular carcinomas. Thus, TCDD and phenobarbital acted in a similar manner. When TCDD was given to partially hepatectomized rats that were not treated with DEN, no tumors were

found; however, the rats did have 25 and 34 enzyme-altered foci per cubic centimeter of liver. No foci were found in rats given a partial hepatectomy.

The authors conclude that because TCDD has not been shown to be a mutagen and, therefore, is not an initiator, it must be acting as a promoter of DEN-initiated hepatic carcinoma. The fact that TCDD induced hepatic foci when DEN was not administered is considered to show that TCDD will also promote cells initiated by ambient environmental conditions such as diet or background radiation. They propose that TCDD only appears to be complete carcinogen in chronic toxicity studies because of its promoting activity.

Matsumura (1983) has suggested that the promoting activity of TCDD, particularly as shown by Pitot et al. (1980), is due to cytosolic receptor binding through its effect on the plasma membrane of the cells. TCDD causes changes on the surface of the plasma membrane that resemble precancerous and transformed cells. One type of change, a loss of gap-junction, is linked to tumor promotion because of a loss of cell-to-cell communication. But because these changes are not observed in cultured cell lines treated with TCDD, Matsumura argues that the effect is not directly on the plasma membrane; but rather through another process such as the cytosolic receptor.

Carcinogenisis as response to injury

Kociba (1984) proposes that the mechanism of TCDD-induced carcinogenesis is through tissue response to chronic toxicity, e.g., the increase in fibrosarcomas in mice topically treated with TCDD in the study conducted by NTP (1982). (See discussion in Section 7). Inflammation, necrosis, and ulceration of the underlying subcutaneous tissue preceded or accompanied the fibrosarcomas. In a second example, liver tumors in rats only occurred in dose groups that also had liver toxicity. (Kociba et al. 1978). (See discussion in Section 7).

Initiator activity

A study by Di Giovanni et al. (1977) suggests TCDD may act as an initiator. In this study, mice were treated with a single 2 μg initiating dose of TCDD on their skin and then treated with promoter TPA over a 32-week period. Three out of 21 surviving mice had a skin tumor. When animals were treated with the initiator DMBA and TCDD an additive effect was observed; however, the effect observed for TCDD alone was weak. However, the DiGiovanni study with TPA did not include an adequate control group treated only with TPA.

Findings in chronic bioassays also suggest TCDD could act as an initiator. (Kociba et al. 1978, NTP 1982b). (See discussion in Section 7).

Further studies of mechanism

More recently, Poland et al. (1984) examined histological changes in the skin (e.g., keratinization, hyperplasia, and keratinized cyst formation). They tested eight mouse strains that are homozygous recessive at the hr locus. In all cases histological changes were noted. Seven strains were treated with 0.3 μg of TCDD topically once a week for 4 weeks. One strain studied did not have measurable amounts of the cytosolic receptor; although it did have a receptor with a much lower affinity for TCDD. This strain of mouse was treated with 1.0 μg of TCDD, instead of 0.3 μg, and a histological response was observed.

These findings do not confirm the authors' hypothesis that cytosolic receptor binding is the mechanism for the toxic skin response. There was no correlation between the concentration of cytosolic receptor in the liver and the TCDD skin response, and even mice with deficient cytosolic receptor protein responded to TCDD treatment; however, it is possible that the dose levels were sufficiently high that enough receptor binding occurred, even in mice with deficient cytosolic receptors, for the effects to appear.

References

Berry DL, DiGiovanni J, Juchau MR, Bracken WM, Gleason GL, Slaga TJ. Lack of tumor-promoting ability of certain environmental chemicals in a two-stage mouse skin tumorigenesis assay. Res Comm Chem Pathol Pharmacol 1978; 20:101-108.

Berry DL, Slaga TJ, DiGiovanni J, Juchau MR. Studies with chlorinated dibenzo-p-dioxins, polybrominated biphenyls, and polychlorinated biphenyls in a two-stage system of mouse skin tumorigenesis: Potent anticarcinogenic effects. Ann NY Acad Sci 1979; 320:405-414.

Cohen GM, Bracken WM, Iyer RP et al. Anti-carcinogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene tumor initiation and its relationship to DNA binding. Cancer Res 1979; 39:4027-4033.

DiGiovanni J, Berry DL, Gleason GL, Kishore GS, Slaga TJ. Time-dependent inhibition by 2,3,7,8-tetrachlorodibenzo-p-dioxin of skin tumorigenesis with polycyclic hydrocarbons. Cancer Res 1980; 40:1580-1587.

DiGiovanni J, Berry DL, Juchau MR, Slaga TJ. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Potent anticarcinogenic activity in CD-1 mice. Biochem Biophys Res Commun 1979; 86:577-584.

DiGiovanni J, Viaje A, Berry DL, Slaga TJ, Juchau MR. Tumor-initiating ability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and Arochlor 1254 in the two-stage system of mouse skin carcinogenesis. Bull Environ Contam Toxicol 1977; 18:552-557.

Gonzalez FJ, Tukey RH, Nebert Dw. Structural regulation of cytochrome P_1-450 and P_3-450 mRNA levels by 3-methylcholanthrene. Mol Pharmacol 1984; 26:117-121.

Israel DI, Whitlock JP Jr. Induction of mRNA specific for cytochrome P_1-450 in wild type and variant mouse hepatoma cells. J Biol Chem 1983; 258:10390-10394.

Kociba, RJ. Summary and critique of rodent carcinogenicity studies of chlorinated dibenzo-p-dioxins. In: Lowrance, WE, ed. Public Health Risks of the Dioxins, proceedings of a symposium held on October 19-20, 1983 at the Rockefeller University, New York City. Los Altos, California. William Kaufmann, 1984; 77-98.

Kouri RE, Rude TH, Joglekar R, et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin as cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at Ah Locus. Cancer Res 1978; 38:2777-2783.

Matsumura, F. Biochemical aspects of action mechanisms of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related chemicals in animals. Pharmac Ther 1983; 19:195-209.

National Toxicology Program. Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (dermal study). Carcinogenesis Test Prog., NCI, NIH, Bethesda, MD and NTP, Research Triangle Park, NC. Pub. No. 80-1757, 1982.

Norman RL, Muller-Eberhard U, Johnson EF. Effect of microsomal cytochrome P-450 isozyme induction on the mutagenic activation of 2-aminoanthrene. Cancer Res. 1982; 42:1722-1726.

Pitot HC, Goldsworthy T, Poland A. Promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res 1980; 40:3616-3620.

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

Poland A, Palen D, Glover E. Tumor promotion by TCDD in skin of HRS/J mice. Nature 1982b; 300(5889):271-273.

Poland A, Knutson J, Glover E. A consideration of the mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons. In: Tucker RE, Young AL, Gray AP, eds. Human and environmental risks of chlorinated dioxins and related compounds. New York: Plenum Press, 1983; 539-559.

Poland A, Knutson JC, Glover E. Histological changes produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the skin of mice carrying mutations that affect the integument. J Invest Dermatol. 1984; 83:454-459.

Tukey RH, Nebert DW, Negishi M. Structural gene products of the [Ah] Complex. Evidence for transcriptional control of cytochrome P_1-450 induction by use of a cloned DNA sequence. J Biol Chem. 1981; 256:6969-6974.

Tukey RH, Hannah RR, Negishi M, Nebert DW, Eisen HJ. The Ah locus: Correlation of intranuclear appearance of inducer-receptor complex with induction of cytochrome P_1-450 RNA. Cell 1982; 1:275-284.

9.0 Risk Assessment

TCDD is one of the most acutely toxic compounds known, although its potency is species-dependent. This is also true of TCDD's other adverse effects. Animal studies have shown TCDD to be teratogenic, immunotoxic, and hepatotoxic (i.e., inducing enzyme activities) at dose levels in the microgram and submicrogram per kilogram body weight range.

Longstreth and Hushon (1983) developed an acceptable daily intake (ADI) level for TCDD of one picogram per kilogram per day. Their ADI is based on the lowest observed effect level (LOEL) from two separate studies: (1) a study by Schantz et al. (1979) that reported reproductive toxicity in monkeys at a daily dose level of approximately 1.5 ng/kg body weight per day (see Section 5.1), and (2) a study by Zinkl et al. (1973) that reported an immunotoxic effect in guinea pigs at a daily dose level of approximately 1.1 ng/kg body weight per day (see Section 4.1). Therefore, there is a safety factor of over 1000 incorporated in Longstreth and Hushon's ADI. The airborne concentration necessary to give an exposure equivalent to the ADI is approximately 3 picograms of TCDD per cubic meter.

Calculation of an ADI assumes that there is a threshold dose level below which no adverse effect will occur. TCDD and HexaCDD, however, have been found to produce carcinogenic responses in rats and mice in cancer bioassays and carcinogenicity is generally considered as a nonthreshold phenomenon. Specifically, it is assumed that for a carcinogen which produces direct DNA damage, measured in short-term assays, there is no threshold for carcinogenicity. In such cases, an ADI based on a threshold assumption

would be inappropriate. However, if a compound is found to induce cancer by an indirect means, not by causing DNA damage, then the process may have a threshold and its risk could possibly be assessed in a manner similar to that done by Longstreth and Hushon (1983). As discussed in the previous sections, there is inconclusive evidence that TCDD is genotoxic and thus able to cause direct DNA damage. There is sufficient evidence, however, that it does induce cancer in laboratory animals and it acts as a potent promoter of cancer induced by initiators.

TCDD induced cancer in animals in several standard two-year oncogenicity bioassays. This finding suggests either that TCDD is an initiator or that TCDD may be a powerful promoter and has promoted cells initiated by environmental carcinogens. Studies have shown that TCDD is a potent promoter of initiated cells. These studies, however, do not differentiate whether or not TCDD is acting solely as a promoter or also has the ability to initiate cells. Thus, the staff of DHS has made a health-conservative assumption that TCDD is both a promoter and initiator.

The Ontario (Canada) Ministry of the Environment (1985) has reviewed the same information and concluded that TCDD acts solely as a promoter and, as such, possesses a threshold level below which tumors would not be promoted. The threshold level was estimated from the NOEL of two animal studies, a three-generation reproduction study in rats reported by Murray et al. (1979, see Section 5) and the two-year chronic toxicity study in rats reported by Kociba et al. (1978, see Section 7). A NOEL of 1.0 ng/kg/day for TCDD was found for both studies. The ADI was calculated using a safety factor of 100, which includes a factor of 10 for the extrapolation from animals to

humans and a second factor of 10 for differential sensitivities within the human population. Thus, the ADI for TCDD was set at 10 pg/kg/day or an equivalent airborne concentration (calculated by DHS) of 30 pg/m^3. This ADI is ten times higher than that developed by Longstreth and Hushon (1983), but it is still somewhat below the "best" and "high" airborne concentrations of total PCDDs and PCDFs estimated by ARB.

The staff of DHS disagrees with the approach taken by the Ontario Ministry of the Environment and used the non-threshold approach for the following reasons:

- First, TCDD is positive in standard cancer bioassays at extremely low doses, suggesting that this substance can initiate carcinogenesis. Under the hypothesis accepted by the Canadians, such results could be interpreted as promotion of the effects of ubiquitous background initiators. Acknowledging the existence of evidence that TCDD can act as a promoter, DHS staff members, however, believe that the positive bioassays are compatible with the hypothesis that TCDD has both initiator and promoter activity.

- Second, the experimental evidence regarding genotoxicity is not clear-cut. Although TCDD has been shown to be ineffective in producing mutations in bacterial DNA, there is evidence that it can cause genetic damage in eukaryotic organisms (e.g., in yeast and mammalian cells). Furthermore, short-term tests are generally useful to detect genotoxicity, but are not sensitive enough to rule it out. The staff of DHS, therefore, agrees with IARC (1983) that it is premature to use

such tests as a basis for classification of carcinogens in order to utilize alternate methods of risk assessment.

- Third, in view of TCDD's extraordinary carcinogenic potency, DHS staff members believe that caution is warranted in risk assessment. Practically speaking, this means that a nonthreshold approach is indicated unless both he existence and location of a carcinogenic threshold have been compellingly demonstrated. In this case, the evidence for the existence of such a threshold is arguable, not conclusive.

There are no animal studies that demonstrate a NOEL for the promoting effect. Furthermore, because the dose-response relationship for promotion is unknown a study that does show a NOEL may not actually be statistically powerful enough to make such a claim. This view has been supported in the literature (Perera 1984, Weinstein 1983). The nonthreshold approach for quantitative risk assessment of TCDD has previously been taken by EPA (1984a, 1984b) and by Kimbrough (1984). Since the mechanism of action of HexaCDD is likely to be similar to that of TCDD, the same nonthreshold approach to the risk assessment for HexaCDD was used.

9.1 Low-Dose Extrapolation Models Used

The mathematical models used to extrapolate potential human risks from dose response relationships seen in animal studies have been used by DHS before (DHS 1985). Below is a brief summary of the various models:

a. Multistage model: This stochastic model assumes that a single cell can generate a malignant tumor only after it has undergone a certain number of heritable changes. The cellular changes are independent and can be thought of as representing stages in the carcinogenic process that are characterized as being of infrequent and improbable occurrence. The model can be derived in a dichotomous or temporal format. A limiting form of this model is the one-hit model.

b. Probit model: This is a statistical or tolerance distribution model. The tolerance concept assumes that each animal in the population has its own tolerance to the test compound and that a response occurs when this tolerance is exceeded. The probit model assumes that the Gaussian normal distribution function describes the tolerance distribution of the carcinogen. The probit function predicts that the log of the dose is linearly dependent on the cumulative normal probability function. At low doses, below the experimental range, the slope of this curve is shallow and provides for a practical threshold. This model is applicable in a dichotomous or temporal format.

c. Logit model: This is a statistical or tolerance distribution model. The model is used in classical toxicology to describe the cumulative probability distribution (sigmoidal curve) for adverse effects with a threshold. Alternatively, this model can be directly associated with a tolerance distribution of the function: $1/[1 + \exp(-x)]$. This model is only derived in a dichotomous format.

d. Weibull model: This is a statistical or tolerance distribution model, classically known as the extreme function model, x to the x power. The usual form of this model is as the dose to the beta power. The model can be derived in both temporal and dichotomous formats. If the power of the dose is less than one but greater than zero, the dose response curve can be supralinear (concave downward or convex).

e. Gamma Multihit model: This stochastic model assumes that a response occurs when a tissue gets sufficient "hits" by a biologically effective unit of dose within a specific time period. As originally derived this model assumes independence between spontaneous background tumors and dose-related tumors.

It is assumed that the number of hits ("k") is a Poisson process. In the mathematical formulation, k need not be an integer; thus, the dose response curve can be supralinear (concave upward or convex). The model can be derived in both dichotomous and temporal formats. A limiting case of this model, when k = 1, is the one-hit model.

The computer program used in applying the multistage model was GLOBAL79, by KS Crump and WW Watson. The probit, logit, Weibull, and gamma models were run using RISK81, a computer program by J Kovar and D Krewski.

9.2 Results of Low-Dose Extrapolation

A primary assumption listed in Appendix A is that the multistage theory appropriately describes the process of carcinogenesis, and that the low-dose

extrapolation model based on this theory, as described in 9.1, is an appropriate method for low-dose risk extrapolation. With this extrapolation model, the estimated excess human cancer risks from exposure to TCDD were calculated based on a number of different tumor types. These tumors were found to have significantly increased incidence rates in rats and mice from the two-year exposure studies by Kociba et al. (1978) and NTP (1982)(see Tables 7.1-2 and 7.1-3). A list of the tumor incidences examined is shown in Table 9.2-1. Because the tissue pathology from the Kociba study was evaluated twice, the estimated excess cancer risks were calculated based on information from each evaluation.

GLOBAL79 uses maximum likelihood theory to fit the multistage model to the experimental dose-response data. The model provides point estimates of the extra risk for both the maximum likelihood estimate (MLE) and the linearized 95% upper confidence value (UCL). The UCL is calculated by maximizing the linear term of the model, or forcing a best fitting linear term if one is not present. This method of calculating the UCL is consistent both with the expected low-dose linearity and the linear nonthreshold theory of carcinogenesis. The slope of the 95% UCL, q*, is taken as a plausible upper bound of potency of the chemical inducing cancer at low doses.

Because the animal dose levels for TCDD were converted to human equivalent exposure from inhalation (see Appendix A), the 95% UCL, q*, is a measure of the greatest potential excess cancer risk for humans. If the lifetime daily exposure is expressed in ng/m^3, then q* is the excess risk associated with this exposure. Since q* for humans is a unit measure of excess lifetime

Table 9.2-1
TCDD

Unit Risk Derived by the Multistage
Model for Different Species and Tumor Sites

Study (Pathologist)	Species	Sex	Tissue: Diagnosis	Unit Risk $[(ng/m^3)^{-1} \times 10^3]$
Kociba et al. 1978 (Kociba)	Rat	Male	Nasal turbinates/tongue Squamous cell carcinoma	4.2
Kociba et al. 1978 (Squire)	Rat	Male	Nasal turbinates/tongue Squamous cell carcinoma	4.9
Kociba et al. 1978 (Kociba)	Rat	Female	Liver: carcinomas and neoplastic nodules	27
Kociba et al. 1978 (Squire)	Rat	Female	Liver: carcinomas and neoplastic nodules	25
NTP 1982 (NTP)	Rat	Female	Liver: carcinomas and neoplastic nodules	9.4
NTP 1982 (NTP)	Mouse	Male	Liver: carcinomas and adenomas	38
NTP 1982 (NTP)	Mouse	Female	Subcutaneous tissue: fibrosarcoma	2.4

cancer risk associated with exposure to TCDD, it will be termed the unit risk. With the unit risk, the 95% UCL of excess risk may be calculated for any low-level exposure to TCDD by the equation:

$$R = \text{unit risk} \times \text{dose}$$

where R is the 95% UCL of excess lifetime cancer risk.

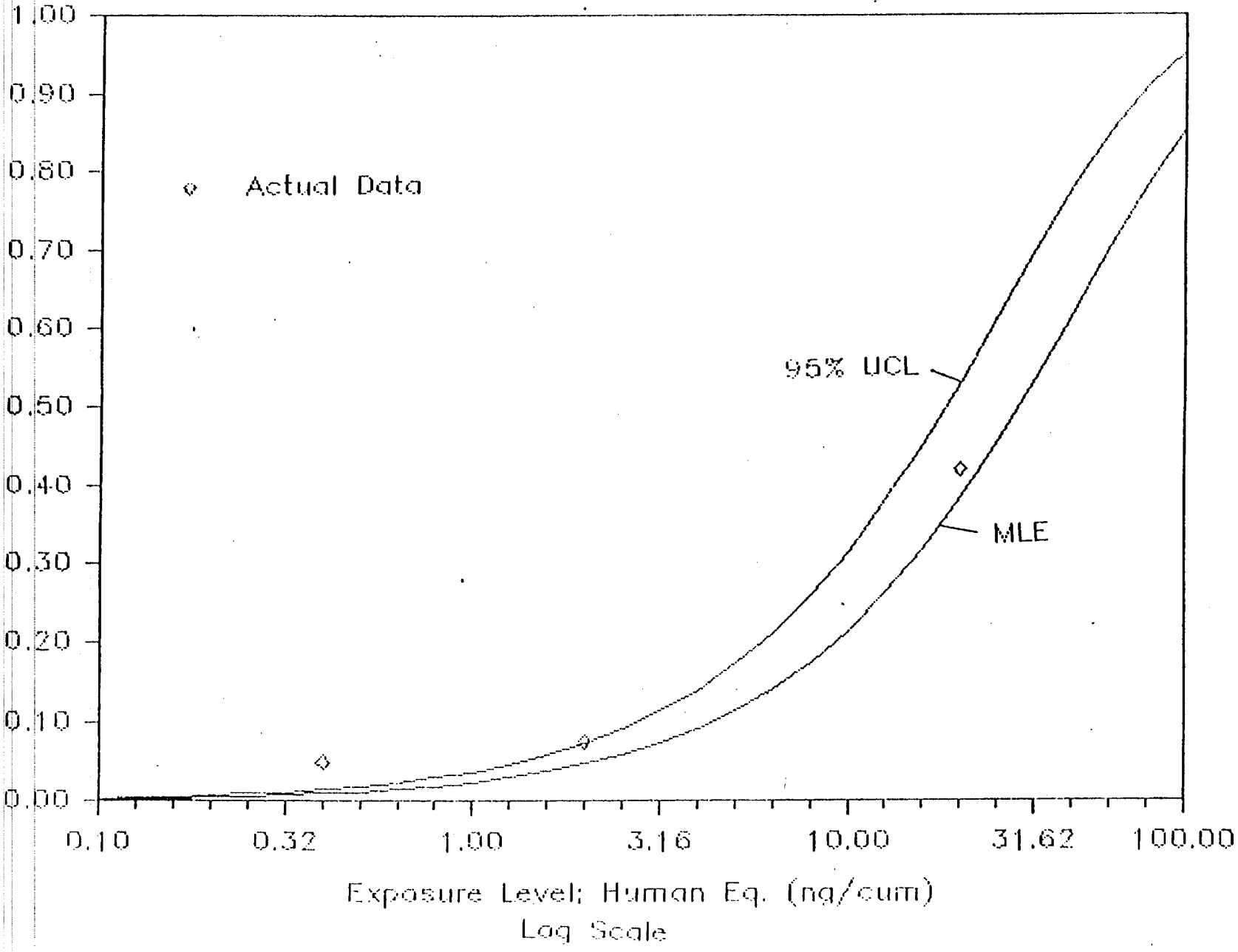
The unit risk is given in Table 9.2-1 for each tumor site studied. Because the unit risk is the upper bound of the potency of a chemical inducing cancer at low doses, it can be used as a measure of sensitivity of the animal carcinogenic response. In this case, the most sensitive species, sex, and site for the induction of cancer by TCDD is the male mouse with hepatocellular adenomas or carcinomas. This response is an order of magnitude greater than the least sensitive species, sex, and site examined, the female mouse subcutaneous fibromas. It is interesting to note that there is less than a four-fold difference in the unit risk between animal species for liver tumors.

A graph of the MLE and 95% UCL was fitted to incidence data of the male mouse liver tumors (See Figure 9.2-1). These plots provide a visual sense of how the curves fit the data. As seen in the graph and in Table 7.1-5, the low- and mid-dose level groups did not have significantly increased incidences of tumors. It is interesting to note that although there is an order of magnitude difference in dose between the high-dose level group that had a significant increase in tumors and the mid-dose level group that did not have an increased tumor incidence, if the incidence remained the same

Figure 9.2-1

MLE and 95% UCL CURVES

Mouse Liver Tumors; NCI Study



but the dose levels were closer together (e.g., 2.2, 11.0, and 22.0 ng/m^3) then the unit risk derived from the multistage model would be about 40 percent less. Had an intermediate response also been observed at the new mid-dose level, the unit risk would be about 10 percent less. Therefore, the large dose intervals used in this study do not appear to substantially affect the calculated unit risk, although it is possible that it could lead to some overestimation of the risk.

Although the DHS staff believe that the multistage model is the appropriate method to estimate low-dose risk, other models were used to show the range of risk estimates that can be obtained. For comparison, the maximum likelihood estimate and 95% UCL of excess human lifetime cancer risk for humans (based on liver tumor incidence in male mice) are presented in Table 9.2-2 for each model at two possible environmental exposure levels. These environmental exposure levels are believed to be within the range of ambient levels expected in California. The 95% UCL dose-response curve for each model can be seen in Figure 9.2-2. Usually the multistage model predicts the highest risk at low dose levels. The probit generally predicts the least, followed in order of increasing risk by the gamma multihit, logit, and Weibull models, but in this case, the multistage model appears to predict lower risks than the gamma multihit, logit, and Weibull models.

These three models predict higher risks than the multistage model (at low doses) becauce they use a supralinear curve. This requires (at least for the gamma multihit and Weibull models) that the exponential factor be less

Table 9.2-2

TCDD

Maximum Likelihood Estimates and 95% Upper Confidence Limits for Excess Lifetime Cancer Risk for Exposure at 10 and 0.1 pg/m^3 Based on Different Low-Dose Extrapolation Models[a]

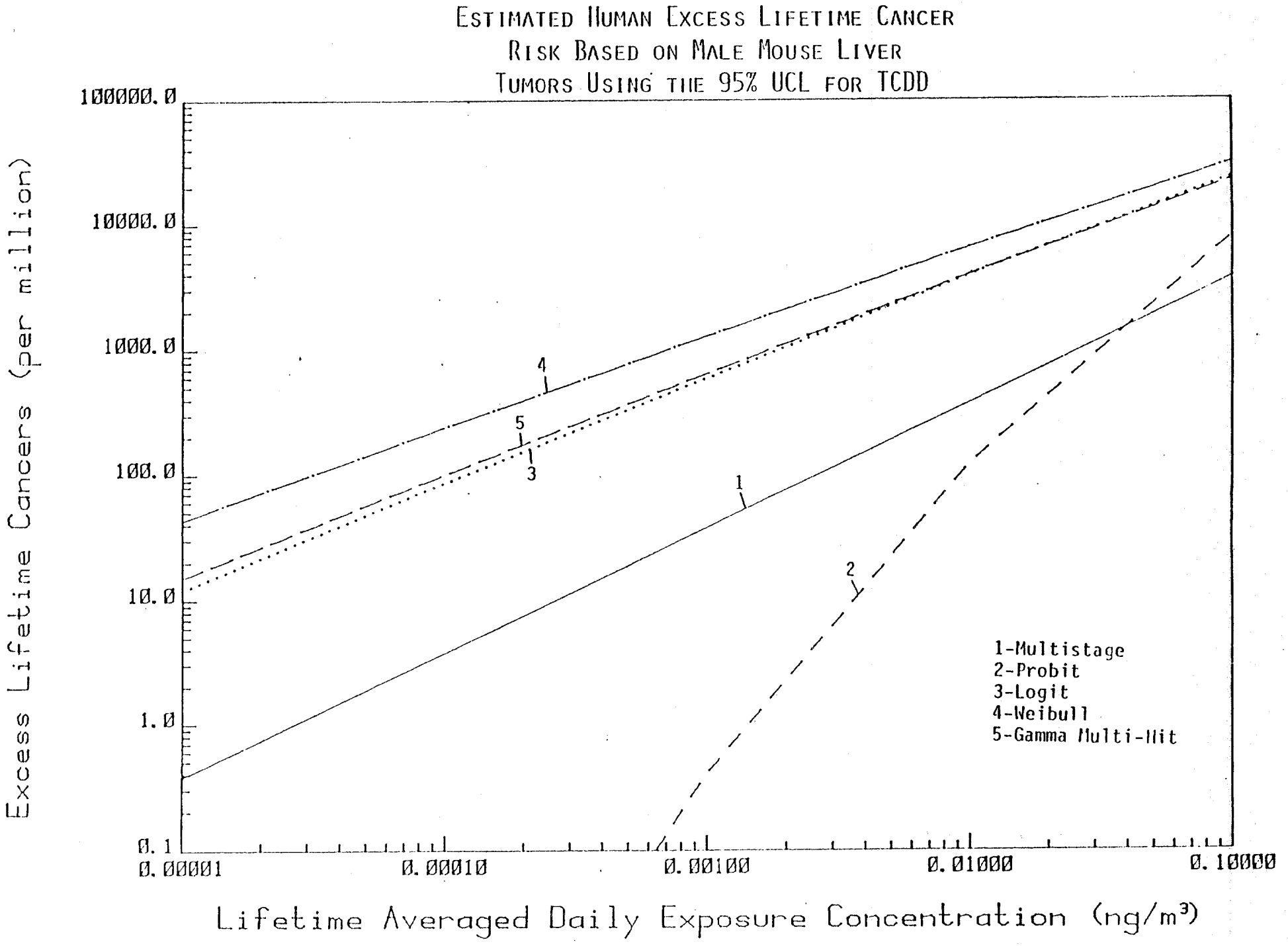
	Ambient Air Concentration (pg/m^3)			
	10		0.1	
Model	MLE[b]	UCL[c]	MLE	UCL
Multistage	$240/10^6$	$380/10^6$	$2/10^6$	$4/10^6$
Probit	$7/10^6$	$120/10^6$	$< 1/10^6$	$< 1/10^6$
Logit	$470/10^6$	$3,900/10^6$	$7/10^6$	$85/10^6$
Weibull	$840/10^6$	$6,500/10^6$	$20/10^6$	$240/10^6$
Gamma-Multihit	$420/10^6$	$4,000/10^6$	$7/10^6$	$100/10^6$

a Based on liver tumor incidence (adenoma and hepatocellular carcinoma) in male mice given TCDD by gavage, as reported by NTP (1982)

b Maximum likelihood estimate, expressed as excess lifetime cancer cases per million population

c 95% upper confidence limits, expressed as excess lifetime cancer cases per million population

Figure 9.2-2


ESTIMATED HUMAN EXCESS LIFETIME CANCER
RISK BASED ON MALE MOUSE LIVER
TUMORS USING THE 95% UCL FOR TCDD
Excess Lifetime Cancers (per million)
100000.0
10000.0
1000.0
100.0
10.0
1.0
0.1
0.00001
0.00010
0.00100
0.01000
0.10000
Lifetime Averaged Daily Exposure Concentration (ng/m³)
1-Multistage
2-Probit
3-Logit
4-Weibull
5-Gamma Multi-Hit

than one, but greater than zero. Such a number would not be consistent with the biological basis of the models (Crump 1985). Therefore, the staff of DHS considers the multistage model the most appropriate to use in this case.

To compare the risks estimated by Longstreth and Hushon (1983), the Ontario Ministry of the Environment (1985), and DHS, we examined the number of excess cancers predicted using the DHS risk assessment at an exposure equivalent to the ADIs. For the Longstreth and Hushon ADI of 3 pg/m^3 the maximum excess cancer incidence is estimated to be 114 cases per million exposed population. For the Ontario Ministry of the Environment ADI of 30 pg/m^3, the maximum excess cancer incidence is estimated to be 1140 cases per million exposed population. The airborne concentration necessary to increase the excess cancer incidence by one per one million exposed population, based on the DHS risk assessment, is 2.6×10^{-2} pg/m^3.

The estimated excess human cancer risk from exposure to HexaCDD was calculated as for TCDD except that only the tumor incidence data on liver tumors in female rats and mice were used (see Tables 7.1-6 and 7.1-7). Liver tumors in female rats are the most sensitive indicator of the carcinogenic potential of HexaCDD (based on unit risk estimates). The initial pathological evaluation of this tissue by NTP was challenged, resulting in independent reviews by three other pathologists. The tumor incidence data from two of these new evaluations were sufficient to use in low-dose risk extrapolation. As can be seen in Table 9.2-3, the unit risks, based on the evaluations by Hildebrandt (1983) and Squire (1983), are less

Table 9.2-3

HexaCDD

Unit Risk Derived by the Multistage
Model for Different Species and Tumor Sites

Study (Pathologist)	Species	Sex	Tissue: Diagnosis	Unit Risk $[(ng/m^3)^{-1} \times 10^3]$
NTP 1980 (NTP)	Mouse	Female	Liver: carcinomas and adenomas	0.83
NTP 1980 (NTP)	Mouse	Female	Liver: carcinomas and adenomas	0.62
NTP 1980 (NTP)	Rat	Female	Liver: carcinomas and neoplastic nodules	1.5
NTP 1980 (Hildebrandt 1983)	Rat	Female	Liver: carcinomas and neoplastic nodules	1.0
NTP 1980 (Squire 1983)	Rat	Female	Liver: neoplastic nodules	0.63
NTP 1980[a] (Squire 1983)	Rat	Female	Liver: neoplastic nodules	1.0

a High-dose group censored in analysis

than the unit risk calculated from the original NTP data. The incidence data obtained from Squire's evaluation gave the lowest unit risk; however, as can be noted in Table 7.1-7, the mid- and high-dose groups had the same tumor incidence level. This suggests that the tumor incidence observed at these dose levels is the maximum effect that could occur because of some limiting factor such as liver toxicity or metabolic saturation of some type. Therefore, the high-dose group was censored from the evaluation to prevent the incidence observed in this dose group from affecting the dose-response portion of the curve. The unit risk obtained in this manner was similar to the unit risk obtained when Hildebrandt's data were used.

The Hildebrandt (1983) evaluation was used as the basis for the DHS risk assessment because the results were similar to the tumor incidence in the high-dose level group found in the evaluation by Schueler (1983). The results were also similar to the Squire (1983) evaluation for the lower dose level groups. Thus, there was some agreement in these findings between pathological evaluations.

A graph of the MLE and 95% UCL is given in Figure 9.2-3. Unlike the incidence data used for TCDD, all dose levels for HexaCDD had a significantly increased incidence of tumors.

Using the incidence data from the Hildebrandt evaluation, the probit, multihit, logit, and Weibull models all predict a lower risk than the multistage model when the 95% UCL is considered (see Table 9.2-4 and Figure 9.2-4).

Figure 9.2-3

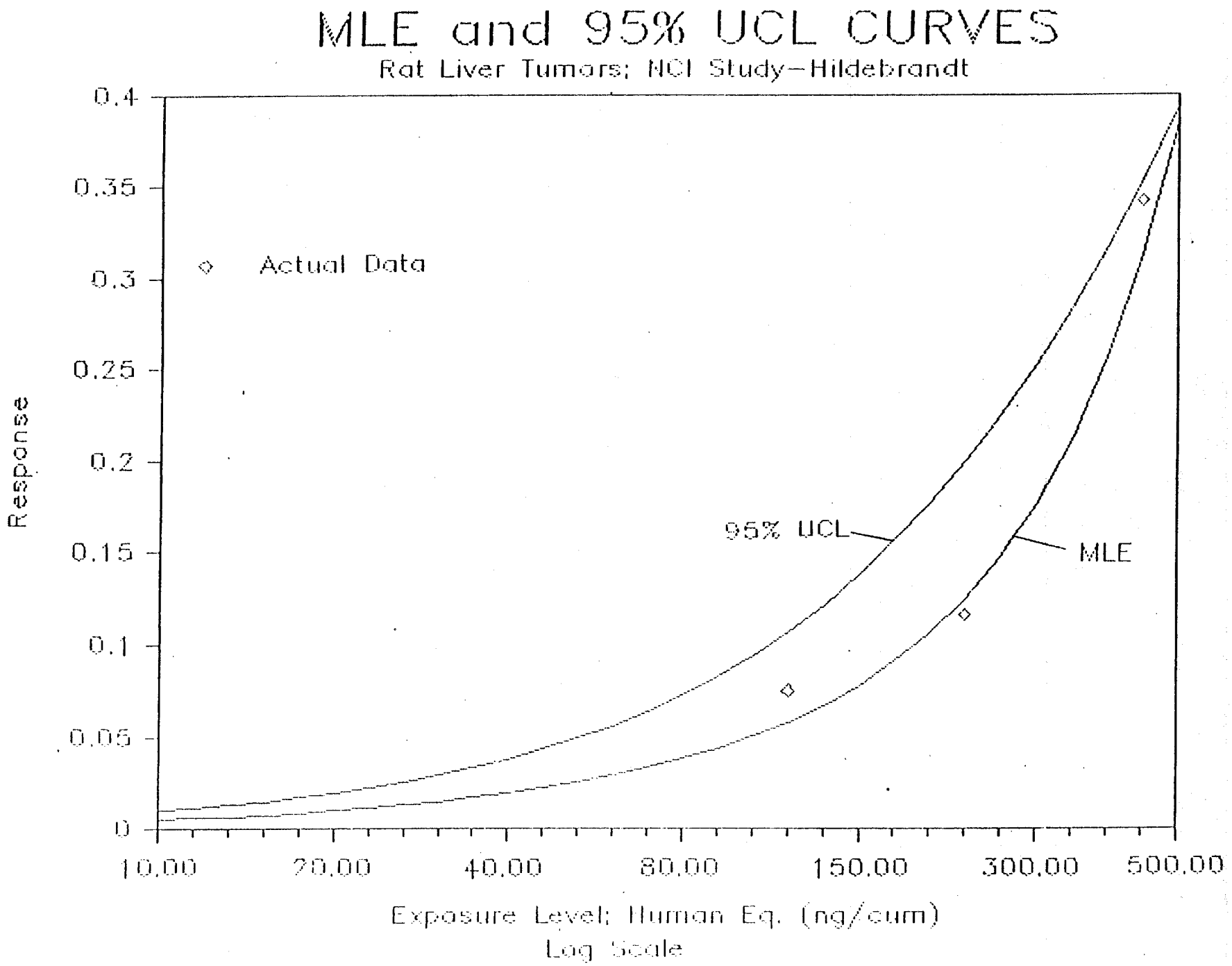

MLE and 95% UCL CURVES
Rat Liver Tumors; NCI Study—Hildebrandt
Response
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Actual Data
95% UCL
MLE
10.00
20.00
40.00
80.00
150.00
300.00
500.00
Exposure Level; Human Eq. (ng/cum)
Log Scale

Table 9.2-4
HexaCDD

Maximum Likelihood Estimates and 95% Upper Confidence Limits for Excess Lifetime Cancer Risk for Exposure at 10 and 0.1 pg/m^3 Based on Different Low-Dose Extrapolation Models[a]

	Ambient Air Concentration (pg/m^3)			
	10		0.1	
Model	MLE[b]	UCL[c]	MLE	UCL
Multistage	$5.7/10^6$	$10/10^6$	$< 1.0/10^6$	$< 1.0/10^6$
Probit	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$
Logit	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$
Weibull	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$
Gamma Multihit	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$	$< 1.0/10^6$

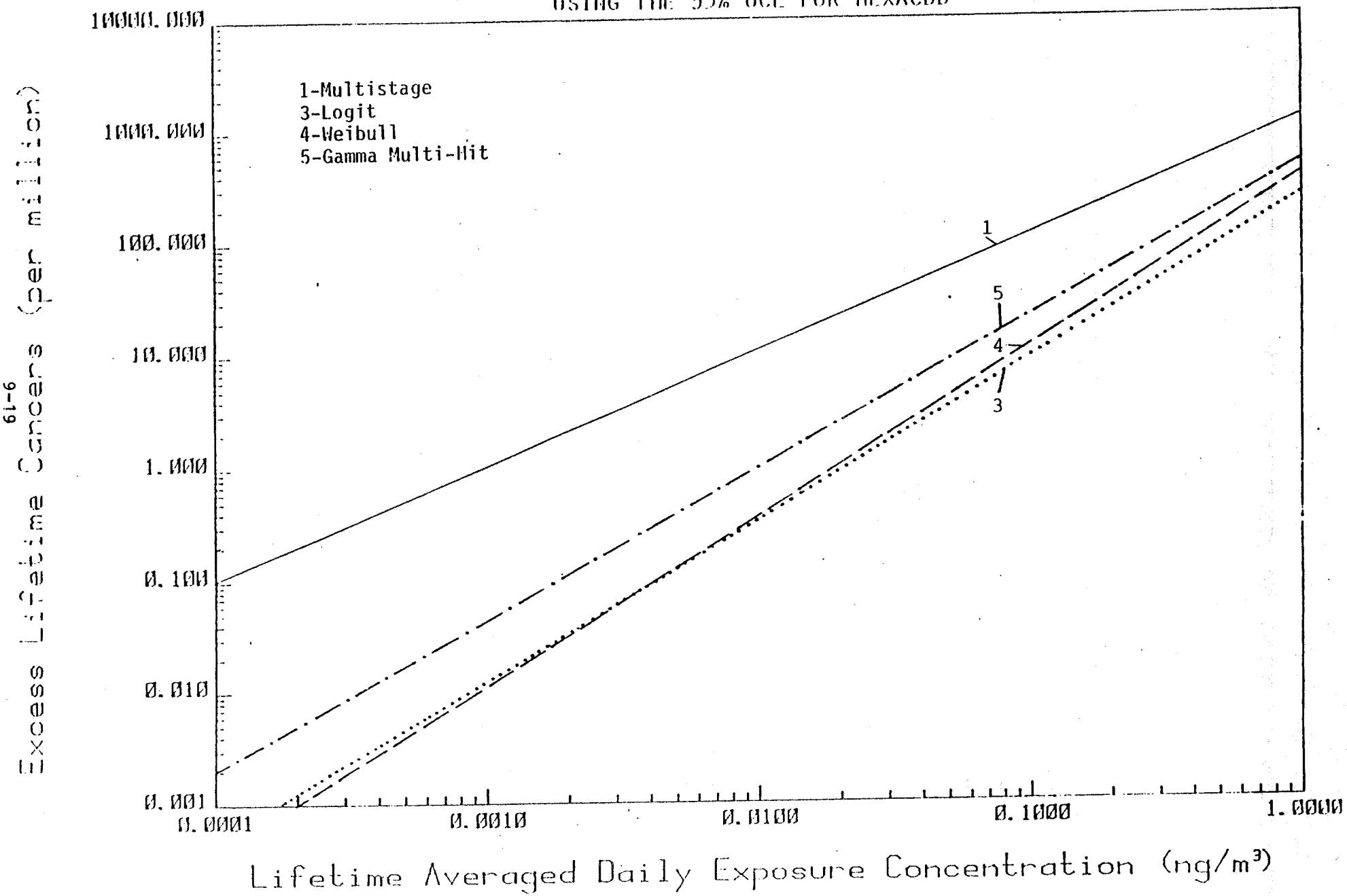
a Based on liver tumor incidence (neoplastic nodules) in female rats dosed by gavage with HexaCDD as reported by NTP (1980) and pathology evaluated by Squire (1983). The high-dose group was not included in these analyses.

b Maximum likelihood estimate, expressed as excess lifetime cancer cases per million population

c 95% upper confidence limit, expressed as excess lifetime cancer cases per million population

Figure 9.2-4

ESTIMATED HUMAN EXCESS LIFETIME CANCER
RISK BASED ON FEMALE RAT LIVER TUMORS
USING THE 95% UCL FOR HEXACDD[a]



9.3 Risk Assessment Estimates for a Mixture of PCDDs and PCDFs

The ARB has provided estimates for ambient concentrations of PCDDs and PCDFs with four through eight chlorines which might occur in the Los Angeles Basin if several proposed solid waste incinerators were operating. The contribution of other sources of PCDDs and PCDFs are not included in the estimates. The estimated ranges are:

	High Estimate	Low Estimate	Best Estimate
PCDDs	13 pg/m^3	0.7 pg/m^3	4 pg/m^3
PCDFs	27 pg/m^3	1.6 pg/m^3	8.2 pg/m^3

Tables 9.2-2 and 9.2-4 give lifetime excess cancer risk estimates for air concentrations of 2,3,7,8-TCDD and HexaCDD in this range. However, DHS recognizes that total PCDD/PCDF in the air is composed of dozens of PCDD and PCDF homologues and isomers. The chemicals in this mixture are difficult to quantitate analytically. As a result, the only measurements feasible to make are total PCDD and total PCDF, as the ARB has done.

To estimate cancer risks from such mixtures requires information about: (1) the proportion of each PCDD and PCDF in the mixture, and (2) the carcinogenic potency of each. However, these data are not available. the proportion of isomers has been measured a few times and differs depending on the emission source. For carcinogenic potency only three isomers have been tested (2,3,7,8-TCDD and a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HexaCDD).

In the following sections, we present four "scenarios", or ways of estimating the risk. These scenarios differ in the assumptions made about relative concentrations and relative carcinogenic potency of PCDDs and PCDFs. A summary is presented in Table 9.3-1.

Each scenario estimates a "TCDD-equivalent proportion," or the proportion of the total mixture that is assumed to be as carcinogenic as 2,3,7,8-TCDD. This TCDD-equivalent proportion is multiplied by the total PCDD/PCDF concentration to derive a TCDD-equivalent concentration. The TCDD-equivalent concentration can be multiplied by the carcinogenic potency of TCDD (Section 9.2) to derive the estimated excess cancer risk for the total PCDD/PCDF mixture.

9.3.1 Scenario 1

Scenario 1 assumes that all airborne PCDD/PCDF containing more than three chlorines is present as 2,3,7,8-TCDD (or equivalently, all PCDDs and PCDFs are as potent as 2,3,7,8-TCDD). The TCDD equivalent proportion under this scenario is therefore 1.0 (i.e., the mixture is as potent as 2,3,7,8-TCDD). The total TCDD-equivalent concentration is obtained by multiplying the total PCDD and PCDF concentrations by the TCDD-equivalent proportion (1.0) and summing.

Table 9.3-1
Summary of Scenarios

	1	2	3	4
Mixture Assumption Theoretical Ratios	No	Yes	Yes	Yes
Potency Assumption	All measured PCDDs and PCDFs with 4-8 chlorines are considered carcinogenic. All are considered equipotent to TCDD.	Only 2,3,7,8-chlorinated PCDD and PCDF isomers from the homologue groups of tetra, penta, hexa, and hepta are considered equipotent to TCDD, except HexaCDD which has a relative potency to TCDD based on its own risk assessment.	All tetra, penta, hexa, and hepta-chlorinated carcinogenic. 2,3,7,8-chlorinated isomers. are 100 times more potent than the non 2,3,7,8-chlorinated isomers. Relative potencies are based on short-term and *in vitro* studies.	Only 2,3,7,8-chlorinated PCDD and PCDF isomers from the homologue groups of tetra, penta, hexa, and hepta are considered carcinogenic. The Tetra- and PentaCDD/CDF isomers are considered equipotent to TCDD. The Hexa- and HeptaCDD/CDF isomers are considered equipotent to HexaCDD.

As an example, for the ARB's high ambient estimate (See Section 9.3), the TCDD-equivalent concentration is:

$$(1.3 \times 10^{-2} \text{ ng PCDD/m}^3)\ (1.0) + (2.7 \times 10^{-2} \text{ ng PCDF/m}^3)\ (1.0)$$

$$= 4.0 \times 10^{-2} \text{ng TCDD-equivalent/m}^3.$$

The estimated cancer risk is obtained by multiplying this dose by the carcinogenic potency (unit risk) of TCDD (38×10^{-3} $[\text{ng/m}^3]^{-1}$). The result is a lifetime excess risk of 1500 cancers per million population, expressed as the estimated 95% upper confidence limit (Table 9.3-2).

The advantage of Scenario 1 is that it requires no assumptions about the proportions of various PCDDs and PCDFs in the total mixture. As 2,3,7,8-TCDD is believed to be the most toxic of the PCDDs and PCDFs, one can be reasonably sure that this scenario will not underestimate the true risk. However, Scenario 1 does not take into account the observation that HexaCDDs are substantially less potent than TCDD and thus, to the extent that such other isomers are present, will consistently overestimate risks.

9.3.2 Scenarios 2, 3, and 4

Scenarios 2,3 and 4 refine the estimates from Scenario 1 by making assumptions about the relative concentrations of individual PCDD and PCDF isomers in the total concentration and about the carcinogenic potencies of the various PCDDs and PCDFs.

Table 9.3-2

Risk Estimates for Total PCDD/PCDF
Under Different Exposure Assumptions

Potency Scenarios for a Mixture of PCDD/PCDF	High Exposure Estimates: Equivalent TCDD Dose ($X\ 10^{-2}$ ng/m^3)	High Exposure Estimates: Upper 95% Estimated Excess Lifetime Cancers (per million)	Low Exposure Estimates: Equivalent TCDD Dose ($X\ 10^{-2}$ ng/m^3)	Low Exposure Estimates: Upper 95% Estimated Excess Lifetime Cancers (per million)	Best Exposure Estimates: Equivalent TCDD Dose ($X\ 10^{-2}$ ng/m^3)	Best Exposure Estimates: Upper 95% Estimated Excess Lifetime Cancers (per million)
Scenario 1*	4.0	1500	0.23	87	1.22	460
Scenario 2**	0.87	330	0.05	19	0.26	100
Scenario 3***	0.02	8	0.001	0.4	0.006	2
Scenario 4****	0.10	38	0.006	2	0.03	12

* Assumes all PCDD is 2,3,7,8-TCDD, or that all PCDD/PCDF is equipotent to 2,3,7,8-TCDD.

** Uses potencies derived from bioassays for 2,3,7,8 chlorinated TCDD and HexaCDD; assumes all other isomers chlorinated on 2,3,7,8 positions are equipotent to 2,3,7,8-TCDD; assumes that isomers not chlorinated on the 2,3,7,8 positions are non-carcinogenic; assumes that OctaCDD/CDF are noncarcinogenic.

*** Uses potencies derived from bioassays for 2,3,7,8 chlorinated TCDD and HexaCDD; for other isomers, uses potency assumptions from Bellin and Barnes (1984), and Barnes (personal communication, 1985).

**** Uses potencies derived from bioassays for 2,3,7,8 chlorinated TCDD and HexaCDD; assumes that 2,3,7,8 chlorinated isomers of TetraCDF, PentaCDD, and PentaCDF are equipotent to 2,3,7,8-TCDD; assumes that 2,3,7,8 chlorinated isomers of HexaCDF, HeptaCDD, and HeptaCDF are equipotent to 2,3,7,8 isomer HexaCDD; assumes that isomers not chlorinated on the 2,3,7,8 positions are non-carcinogenic; assumes that OctaCDD/CDF are noncarcinogenic.

DHS used the method recommended by the ARB to estimate the concentration of each 2,3,7,8-isomer (Tetra- through OctaCDD/CDF). The percentage of 2,3,7,8-isomers in the emissions is assumed proportional to the number of 2,3,7,8-isomers possible in each homologue group. The homologue groups assumed present are taken from published data of homologue distribution for municipal solid waste incinerators. For a detailed discussion of this method see Appendix B.1.

In scenarios 2, 3, and 4, assumptions about the relative concentrations of PCDD and PCDF are the same, but the cancer potency assumptions differ. Scenarios 2, 3, and 4 use the potencies of TCDD and HexaCDD derived from animal bioassays, as described in Section 9.3. For the other isomers Bellin and Barnes (1984) tabulated eight different approaches to estimating the relative potencies. Scenarios 2,3, and 4 present three such approaches.

9.3.2.1 Scenario 2

Scenario 2 uses the potencies of TCDD and HexaCDD derived from their bioassays. For untested PCDDs and PCDFs, isomers chlorinated on the 2,3,7, and 8 positions with at least one ring position unchlorinated are assumed to be as potent as 2,3,7,8-TCDD. Isomers not chlorinated on the 2,3,7, and 8 positions or without at least one unoccupied ring position (i.e., OctaCDD and OctaCDF) are assumed to be noncarcinogenic. DHS used this approach previously to estimate the cancer risk of exposure to products of a PCB fire (DHS 1983, Milby et al. 1985).

Using these assumptions the TCDD-equivalent proportion for the mixture of Tetra- through OctaCDD is 0.25, and for Tetra- through OctaCDF, 0.20. See Appendix B, Tables B-1 and B-2 for calculations. In other words, under Scenario 2, the carcinogenic potency of a mixture of PCDD would be 25% that of an equivalent amount of 2,3,7,8-TCDD. Similarly, the potency of a mixture of PCDF would be 20% that of TCDD.

For the ARB's high exposure estimate, the TCDD-equivalent dose is 8.7 pg/m^3*. The total estimated cancer risk is obtained by multiplying this dose by the unit risk for TCDD. The estimated 95% upper confidence limit on the excess lifetime cancer risk is 330 per million (Table 9.3-2).

9.3.2.2 Scenario 3

Scenario 3 uses the same assumptions as Scenario 2 about the proportions of the various PCDD and PCDF isomers found in total air, but it relies on different assumptions about the carcinogenic potencies of each PCDD and PCDF.

Scenario 3 uses the carcinogenic potencies of TCDD and HexaCDD obtained from their bioassays, as above. It also assumes, as before, a zero potency for OctaCDD, OctaCDF, and other PCDDs and PCDFs with fewer than four chlorines. For other PCDDs and PCDFs that have not been bioassayed, Scenario 3 assigns

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*Calculating the relative potency on a molar to molar basis instead of a weight to weight basis reduces the TCDD-equivalent dose (see Appendix B).

relative potencies based upon several factors, including their acute toxicity and structural/activity relationships from in vitro tests. These relative potencies were derived by staff of EPA: Tetra- through PentaCDD and Tetra- through HexaCDF from Bellin and Barnes (1984), and HeptaCDD and HeptaCDF from Barnes (personal communication, 1985).

Using this approach, the potency of a given concentration of PCDDs would be 1% of the potency of TCDD. The potency of a mixture of PCDFs would be 0.3% of the potency of TCDD. Calculations are shown in Appendix B.

9.3.2.3 Scenario 4

Scenario 4 also recognizes that not all 2,3,7,8-isomer PCDDs and PCDFs are equally carcinogenic. The results of the bioassays on TCDD and HexaCDD suggest that carcinogenic potency may decline in homologues more chlorinated than TCDD. Unlike Scenario 3, however, Scenario 4 does not scale carcinogenic potencies to acute toxicity.

Scenario 4 assumes, as before, that PCDDs and PCDFs that are not chlorinated on the 2,3,7,8 positions or do not have at least one ring position open are noncarcinogenic. Scenario 4 uses the established potencies for 2,3,7,8-isomer Tetra and HexaCDD. It also considers that the 2,3,7,8-isomer PentaCDD has a carcinogenic potency equivalent to TCDD, and that 2,3,7,8-isomer HeptaCDD is equivalent to 2,3,7,8 isomer HexaCDD. The potencies for the homologous PCDDs are also used for the PCDFs.

Using this approach, the potency of a given concentration of PCDDs would be 2% of the potency of TCDD. The potency of a mixture of PCDFs would be 3% of the potency of TCDD (Appendix B).

9.4 Discussion

DHS staff have used established methodologies for estimating unit risks for the two PCDDs for which data are available; but estimating the risk for a mixture of Tetra- through OctaCDDs and CDFs requires making assumptions about two unknowns: the relative amounts of each PCDD and PCDF homologue in emissions, and the relative potency of each PCDD and PCDF.

The staff calculated the total excess cancer risk under four sets of assumptions or scenarios (Table 9.3-2). Each scenario has its advantages and disadvantages. Scenario 1 is appropriate as a "worst case" estimate when one does not know the concentrations of the individual PCDDs and PCDFs, or is unwilling to guess what they might be. Scenario 1 avoids this uncertainty by assuming that all PCDD/PCDF is present as 2,3,7,8-TCDD. Scenario 1 leads to the largest estimated cancer risk, and hence this approach is the most health-conservative. If other less potent PCDDs and PCDFs are actually present, however, this approach will overestimate the true risk.

Scenario 3 yields the lowest total cancer risk and is the least health-conservative approach. Furthermore. it requires the greatest number of assumptions. Its basic assumption that relative carcinogenic potencies can be calculated from acute toxicities is tenuous because the majority of

PCDDs and PCDFs have never been subjected to long-term chronic exposure studies.

DHS does not favor using short-term toxicity tests to estimate carcinogenic potency, although such a method has recently been suggested by Zeise et al. (1984). These authors found linear correlations between the logarithm of carcinogenic potency and the logarithm of acute toxicity. Bernstein et al. (1985) also found a correlation between carcinogenic potency and the maximum dose tested for compounds studied in the NTP carcinogenicity bioassay series. They concluded, however, that a major component of this correlation results from the way the bioassays are conducted, although there may be a biological component as well. In both reports there were compounds whose carcinogenic potency were orders of magnitude different from those predicted from the calculated correlations. In general, there is not much evidence for a common biological mechanism between acute toxicity and carcinogenicity, although in particular cases a similar mechanism may exist. For example, the mechanisms of aflatoxin's acute liver toxicity and carcinogenicity both occur via a reactive metabolite. Despite this, Zeise et al. (1984) found that the carcinogenic potency of aflatoxin was not well predicted by its acute toxicity; thus mechanistic insights do not necessarily predict toxicity/carcinogenicity correlations.

In the case of PCDD/PCDFS, although cytosolic receptor binding may be involved as a common mechanism for TCDD's acute toxicity and carcinogenicity, DHS staff members believe that it would be premature to utilize acute toxicity data as a surrogate for determining carcinogenic potency.

DHS favors the use of Scenario 4. This scenario recognizes that total PCDD/PCDF is composed of a mixture of PCDDs and PCDFs. It assumes that only the PCDDs and PCDFs chlorinated on the 2,3,7, and 8 positions and which have at least one ring position open are carcinogenic. It assigns potencies derived from the multistage model for the 2,3,7,8-isomers that have been bioassayed (TCDD and HexaCDD). For 2,3,7,8-isomers that have not been bioassayed, this scenario assumes that the PentaCDD is more similar to TCDD than to HexaCDD, and that HeptaCDD is more similar to HexaCDD than to OctaCDD. The potencies of the PCDFs are taken to be equal to their PCDD homologues.

The excess risk estimates derived under Scenarios 3 and 4 are not markedly different, and in fact overlap (Table 9.3-2). For Scenario 3 the upper 95% confidence level estimates range from 0.4 to 8 excess cancers per million. For Scenario 4, these estimates are 2 to 38 excess cases per million.

Both the present risk assessment, based on a nonthreshold approach, and the threshold approach of the Ontario Ministry of the Environment (1985) predict a risk from lifetime exposure to level of PCDDs and PCDFs. (The non-threshold approach in this case applies Scenario 1 using the high exposure estimates). The Canadian assessment estimates an ADI of 30 pg TCDD/m^3 (calculated by DHS from the reported ADI of 10 pg/kg/day) which is below the 4 ng/m^3 "TCDD-equivalent dose" from Scenario 1. The DHS assessment estimates an excess lifetime cancer incidence of 1500 per one million exposed population for the same exposure level. However, using the best exposure estimates, the Canadian assessment indicates that there is no risk from exposure while the DHS assessment still estimates an excess lifetime cancer

rate of 460 per one million. When Scenarios 3 and 4 are used to estimate TCDD-equivalent exposure, the exposures are from one to three orders of magnitude below the Canadian ADI. According to the Canadian approach these exposures would not result in any cancers. However, the DHS approach, which assumes that every dose has some probability of producing cancer, estimates the lifetime excess cancer rate at these exposures to be from 2 to 38 per million. Thus, the DHS risk assessment is more health-conservative than the Canadian assessment.

References

Bellin JS, Barnes DG. Health hazard assessment for chlorinated dioxins and dibenzofurans other than 2,3,7,8-TCDD. Presented at the Symposium on Advances in Health Risk Assessment for Systemic Toxicants and Chemical Mixtures, Cincinnati OH. 25 October 1984. (unpublished)

Barnes DG. Personal Communiation. EPA. 1985.

Bernstein L, Gold LS, Ames BN, et al. Some tautologous aspects of the comparison of carcinogenic potency in rats and mice. Fund Appl Toxicol 1985; 5:79-86.

Crump KS. Mechanism leading to dose-response models. In: Ricci P, ed. Principles of health risk assessment. Prentice-Hall, Inc. Englewood Cliffs, NJ: 1985, pp 235-277.

DHS (Department of Health Services). Interim guidelines for acceptable exposure levels in office settings contaminated with PCB and PCB combustion products. Epidemiological Studies Section, California Department of Health Services. Berkeley, California. November 1983.

DHS (Department of Health Services). Guidelines for chemical carcinogen risk assessment and their scientific rationale. State of California, Health and Welfare Agency, Department of Health Services. Sacramento, California. November 1985.

EPA (Environmental Protection Agency). Ambient water quality criteria for 2,3,7,8-tetrachlorodibenzo-p-dioxin. EPA 440/5-84-007. Washington DC: US EPA Office of Water Regulations and Standards, 1984a.

EPA (Environmental Protection Agency). Health assessment document for polychlorinated dibenzo-p-dioxins. Review Draft. Part 2. EPA-600/8-84-014A. Washington DC: US EPA, Office of Health and Environmental Assessment, 1984b.

Hildebrandt PK. Letter to EE McConnell, NIEHS/NTP Research Triangle Park, NC. 31 October 1983.

IARC (International Agency for Research on Cancer). Approaches to classifying chemical carcinogens according to mechanism of activity. Joint IARC/IPCS/CEC Working Group Report, Lyon, France. IARC Internal Technical Reports 1983; 83/001.

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

Kociba RJ, Keyes DG, Beyer JE, et al. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol Appl Pharmacol. 1978; 46:279-303.

Longstreth JD, Hushon JM. Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In: Tucker RE, Young AL, Gray AP, eds. Human and environmental risks of chlorinated dioxins and related compounds. New York: Plenum Press 1983, pp 639-664.

Milby TH, Miller TL, Forrester TL. PCB-containing transformer fires: decontamination guidelines based on health considerations. J Occup Med 1985; 24(5):351-356.

Murray RJ, Smith FA, Nitschke KD, et al. Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol Appl Pharmacol 1979; 50:241-251.

NTP (National Toxicology Program). Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (gavage study). DHHS Publ No. (NIH) 82-1765. Carcinogenesis Testing Program, NCI, Bethesda, MD, NIH, and Research Triangle Park, NC, NTP. 1982.

NTP (National Toxicology Program). Bioassay of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin for possible carcinogenicity (gavage study). DHHS Publ No. (NIH) 80-1758. Carcinogenesis Testing Program, NCI, Bethesda, MD, NIH, and Research Triangle Park, NC, NTP. 1980.

Ontario Ministry of the Environment. Scientific Criteria Document for Standard Development No. 4-84. Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). (Preprint Copy.) Ontario, Canada: prepared for: Hazardous Contaminants and Standards Branch, Ontario Ministry of the Environment. September 1985.

Perera FP. The genotoxic/epigenetic distinction: relevance to cancer policy. Environ Res 1984; 34:175-191.

Schantz SL, Barsott DA, Allen JR. Toxicologic effects produced in non-human primates exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Applied Pharmacol 1979; 48:A180.

Schuler RL. Report: Review of selected neoplastic and non-neoplastic liver lesions in rats given hexachlorodibenzo-p-dioxins (C03703). Submitted to B. Haberman, USEPA, Washington DC, 21 September 1983.

Squire RA. Report: An assessment of the experimental evidence for potential carcinogenicity of hexachlorodibenzo-p-dioxins. Submitted to: TA Robinson, Vulcan Chemicals, Birmingham, AL. 29 June 1983.

Tiernan TO, Taylor ML, Garrett JH, et al. Chlorodibenzodioxins, chlorodibenzofurans, and related compounds in the effluents from combustion processes. Chemosphere 1983; 12:595-606.

Weinstein IB. Letter to the editor. Science. 1983; 219:794-796.

Zeise L, Wilson R, Crouch E. Use of acute toxicity to estimate carcinogenic risk. Risk Anal 1984; 4:163-176.

Zinkl J, Vos JG, Moore JA, et al. Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ Health Perspect 1973; 5:111-118.

Appendix A

Summary of Assumptions

Several assumptions underlie a quantitative risk assessment involving extrapolation of observed dose-response data to low-dose levels and the use of animal data to estimate human risk. These assumptions have been used previously by DHS (DHS 1985), and are summarized below:

- Animal data are applicable to humans.

- There is not necessarily an exact correspondence between the histological distribution of animal and human cancers.

- The use of most sensitive animal species, sex, and tumor site to predict human effects is justified.

- High-dose bioassays are appropriate for determining low-dose responses.

- Average lifetime daily dose is the appropriate dose to use for dose-response assessments.*

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*Lifetime daily dose is the cumulative dose divided by the number of days in the animal's life.

- The route of exposure need not be identical in animals and humans if the tumors of interest appear distal to the point of exposure.

- A threshold for the observed carcinogenic effect does not exist.

- Benign and malignant tumors may be combined for dose-response assessment, if observed to occur in the same tissues or organs.

- Dose per surface area is equivalent between species.

- The multistage theory most appropriately describes the phenomenon of carcinogenesis and is linear at low doses. The extrapolation procedure developed by Crump, based on the multistage theory, is an appropriate method for low-dose-response extrapolation.

These assumptions are, for the most part, general and can be applied to most carcinogenic compounds. More specific assumptions are used for the particular cases of TCDD and HexaCDD in this risk assessment. The specific assumptions are made to reduce the uncertainty involved in animal-to-human extrapolation and deal with differences in routes of exposure, absorption, metabolism, and elimination.

Dose Conversion Assumptions and Calculations

To estimate the risk to humans from TCDD exposure we need to convert data from animal studies into equivalent human doses by applying scaling factors. Scaling factors, in concept, account for the differences between humans and animals in body weight, surface area, metabolic rate, pharmacokinetics, and life expectancy.

The staff of the DHS previously reviewed the literature to determine the most appropriate scaling factor (DHS 1985) and chose the parameter of surface area as a scaling factor because it produces a result, or dose, which is intermediate to the dose-estimate produced by the other methods.

Scaling factors provide a technique for converting animal dose to human dose but it is impossible to account for many factors which affect the amount of delivered dose. Differences in route of exposure, vehicle of exposure, metabolism, and pharmacokinetics will all affect the dose. For instance, animal exposure in these studies is by oral ingestion; in humans it will be by inhalation. The vehicle of exposure will also affect the quantity of carcinogen absorbed. For example, TCDD in an acetone: corn oil mixture is absorbed more readily than TCDD on a soil particle or food particle. Unfortunately, there is not sufficient information at this time to allow us to measure and account for these factors; therefore, we must make the following assumptions:

- Oral and inhalation routes are equivalent;

- The concentration of TCDD in the air is assumed to be the daily oral dose;

- The route of exposure does not affect absorption;

- There is no difference in metabolism and pharmacokinetics between animals and humans.

Based on these assumptions, the animal experimental dose levels were converted to human exposure levels. The first step was to change the reported animal dose levels to daily dose levels. For the Kociba et al. (1978) study, the daily dose levels were reported and those levels were used. For the NTP (1980, 1982) studies, the total weekly dose levels were averaged over the entire week to get the daily dose level. This procedure assumes that daily dosing of the animals in the NTP studies would have given the same results as did the actual twice weekly dosing schedule. Since the half-life of TCDD is relatively long, both dosing schedules should produce similar concentrations of TCDD in the animal tissues, and therefore would be expected to give similar results. The calculated daily doses are given in Tables A-1 and A-2.

Conversion of the animal daily dose to a human daily dose based on surface area was accomplished by the same methods used by EPA (1984) with the following formula:

$$D_H = D_A(W_A/W_H)^{1/3}$$

where D_H is the human dose in mg/kg/day, D_A is the animal daily dose in mg/kg/day given in Tables A-3 and A-4, W_A is the animal body weight also given in Tables A-3 and A-4, and W_H is the average human body weight which is assumed to be 60 kg for men and women. These dose levels were then converted to airborne concentrations (ng/m^3), using the conversion factor such that:

$$D_{Hatm} = D_H(W_H/V_H) \text{ X } 10^{-3}$$

where D_{Hatm} is the human equivalent dose as an airborne concentration in ng/m^3 and V_H is 18 m^3, which is the assumed average daily ventilation volume for humans. This conversion assumes, as previously discussed that all TCDD or HexaCDD inhaled is retained and is available for absorption. As an example, the airborne concentration of TCDD that gives a human the equivalent daily dose given to female rats in the low dose group of the Kociba et al. (1978) study was calculated as follows:

$$D_H = 0.001 \ \mu g/kg/day(0.45 \text{ kg}/60 \text{ kg})^{1/3} = 0.0002 \ \mu g/kg/day$$

$$D_{Hatm} = 0.0002 \ \mu g/kg/day \ (60 \text{ kg}/18 \text{ m}^3) \text{ X } 10^3 = 0.65 \text{ ng/m}^3$$

where D_{Hatm} is the human equivalent dose as an airborne concentration. The calculated airborne concentrations are listed in Tables A-3 and A-4. These were used in the low dose extrapolation models to estimate excess lifetime cancer risk at low ambient air levels.

Table A-1

Calculated Daily Dose Levels for
TCDD Chronic Studies in Rats and Mice

Study	Animal	Reported Dose Level	Calculated Dose Level
Kociba et al. (1978)	Male and Mice	22 ppt[a]	0.001 μg/kg-day[b]
	Rats	210 ppt	0.01 μg/kg-day
		220 ppt	0.1 μg/kg-day
NTP (1982a)	Male and Female Rats, Male Mice	0.01 μg/kg-week	0.0014 μg/kg-day
		0.05 μg/kg-week	0.0071 μg/kg-day
		0.5 μg/kg-week	0.071 μg/kg-day
	Female Mice	0.04 μg/kg-week	0.0057 μg/kg-day
		0.2 μg/kg-week	0.029 μg/kg-day
		2.0 μg/kg-week	0.29 μg/kg-day

a Concentration in the diet, parts per trillion (w/w)

b Reported by the authors

Table A-2

Calculated Daily Dose Levels for HexaCDD
in the NTP Oncogenicity Bioassay in Rats and Mice

Animal	Reported Dose Level	Calculated Dose Level
Female Rats	1.25 μg/kg-week	0.18 μg/kg-day
	2.5 μg/kg-week	0.36 μg/kg-day
	5.0 μg/kg-week	0.71 μg/kg-day
Female Mice	2.5 μg/kg-week	0.36 μg/kg-day
	5.0 μg/kg-week	0.71 μg/kg-day
	10 μg/kg-week	1.40 μg/kg-day

Table A-3

Calculated Equivalent Human Exposure to TCDD
Based on Daily Animal Dose Levels

Study	Animal	Daily Dose Level (μg/kg-day)	Airborne Concentration for Equivalent Human Exposure (ng/m^3)
Kociba et al. (1978)	Male Rat (0.60)[a]	0.001	0.72
		0.01	7.2
		0.1	72
	Female Rat (0.45)	0.001	0.65
		0.01	6.5
		0.1	65
NTP (1982a)	Female Rat (0.45)	0.0014	0.93
		0.0071	4.6
		0.071	46
	Male Mice (0.048)	0.0014	0.44
		0.0071	2.2
		0.071	22
	Female Mice (0.04)	0.0057	1.7
		0.029	8.4
		0.29	84

a Number in parentheses is animal body weight in kilograms

Table A-4

Calculated Equivalent Human Exposure to HexaCDD Based on Daily Animal Dose Levels from the NTP Oncogenicity Bioassay

Animal	Daily Dose Level (μg/kg-day)	Airborne Concentration for Equivalent Human Exposure (ng/m^3)
Female Rats (0.45)[a]	0.18	120
	0.36	230
	0.71	460
Female Mice (0.04)	0.36	100
	0.71	210
	1.43	420

a Number in parentheses is animal body weight in kilograms

References

DHS (Department of Health Services). Guidelines for chemical carcinogen risk assessment and their scientific rationale. State of California, Health and Welfare Agency, Department of Health Services. November 1985.

EPA (Environmental Protection Agency). Health assessment document for polychlorinated dibenzo-p-dioxins. Review Draft. Part 2. Washington DC:USEPA Office of Health and Environmental Assessment, EPA-600/8-84-014A. 1984.

Kociba RJ, Keyes DG, Beyer JE, et al. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol Appl Pharmacol. 1978; 46:279-393.

NTP (National Toxicology Program). Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (gavage study). DHHS Publ No. (NIH) 82-1765. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and NTP, RTP, Box 12233, NC. 1982.

National Toxicology Program (NTP). Bioassay of 1,2,3,6,7,8- and 1,2,3,7,8,9-herachlorodibenzo-p-dioxin for possible carcinogenicity (gavage study). DHHS Publ No. (NIH) 80-1758. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and NTP, RTP, Box 12233 NC. 1980.

Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol. 1982;22:517-554.

Appendix B

Methods of Inferring Total Potency of a Mixture of PCDD/PCDF

The total amount of PCDD/PCDF in ambient air, from sources such as solid waste incinerators, is typically composed of a mixture of PCDD and PCDF homologues and isomers. In order to produce the best estimate of the total cancer risk from such a mixture, one would need to know:

(1) The proportion of each PCDD and PCDF isomer in the total mixture--This has been measured in some instances, but various sources give somewhat different results.

(2) The carcinogenic potency of each PCDD and PCDF isomer in the total mixture--In fact, of the Tetra through Octa CDD/CDFs only 2,3,7,8,-TCDD, and a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HexaCDD have been tested for carcinogenicity in bioassays.

This presents the problem of inferring the cancer risk resulting from an uncertain mixture of chemicals, most of which have never been tested for carcinogenicity. In light of these uncertainties, we have made different assumptions about (1) and (2) above in order to produce four potency scenarios:

Scenario 1:

--Assume that all PCDD/PCDF in air is present as 2,3,7,8-TCDD (or equivalently that all PCDDs and PCDFs are as potent as 2,3,7,8-TCDD). The total cancer risk is obtained simply by applying the carcinogenic potency (unit risk) for 2,3,7,8-TCDD to the total PCDD/PCDF concentration.

Scenario 2:

--Use the proportion of each PCDD and PCDF homologue measured from combustion sources. Various attempts to measure these compounds have resulted in different distributions, depending on the source. We have followed the recommendations of ARB, and used the results of Tiernan et al. (1983) because these measures were obtained from mass burn municipal solid waste incinerators of the type currently proposed in California. This is also one of the few studies which provides data on all PCDDs and PCDFs with four through eight chlorines.

--Estimate the proportion of each isomer chlorinated on the 2,3,7,8 positions in a given homologue group, by assuming an even distribution of possible isomers (Olie et al. 1982). For example, there are 22 isomers of TCDD, and only one of these configurations has the four chlorines on the 2,3,7, and 8 positions (EPA, 1980). Therefore, under this assumption, 1/22, or 4.5% of total TCDD, is assumed to be 2,3,7,8-TCDD. This method produces results which agree reasonably well with a few studies that have measured isomer distribution in particular situations (R. Barham, California Air Resources Board, personal communication, 1985). Although there are other ways of

deriving a hypothetical distribution (K. Crump, personal communication, 1985), we stress that all methods are speculative, and until the actual distribution can be established, we will follow the ARB recommendations to use the method outlined above.

--Assume the carcinogenic potencies of 2,3,7,8-TCDD and 1,2,3,6,7,8- and 1,2,3,7,8,9-HexaCDD obtained from their bioassays. For other PCDDs and PCDFs that have not been bioassayed, assume that PCDDs and PCDFs that are chlorinated on the 2,3,7,8 positions, and that have at least one ring position unchlorinated, are as toxic as 2,3,7,8-TCDD. Isomers that are not chlorinated on the 2,3,7,8 positions, or do not have at least one ring position open (i.e. OctaCDD and OctaCDF), are assumed to be non-carcinogenic. DHS used this approach previously to estimate the cancer risk of exposure to products of a PCB fire (DHS 1983).

Tables B-1 and B-2 express the various potencies of PCDDs and PCDFs, respectively, relative to 2,3,7,8-TCDD under Scenario 2. The relative potencies are scored as 1 for 2,3,7,8-TCDD and other 2,3,7,8 isomers, and 0 for the non-2,3,7,8 isomers. The relative potency of 2,3,7,8 isomer HexaCDD (0.03) is calculated by taking the ratio of its carcinogenic potency (1.0×10^{-3} $[ng/m^3]^{-1}$, as derived from Squire's review of the NTP bioassay, omitting the highest dose group) to the potency of 2,3,7,8-TCDD (38×10^{-3} $[ng/m^3]^{-1}$, from the NTP bioassay). These unit risks were presented in Section 10.3.

Scenario 3:

--Assume that the proportion of each PCDD and PCDF homologue is the same as measured from combustion sources, given above.

--Assume that the proportion of isomers chlorinated on the 2,3,7,8 positions isthe same as given above.

--Use the carcinogenic potencies of TCDD and HexaCDD obtained from their bioassays, as above. Assume a potency of zero for OctaCDD and OctaCDF, because of the lack of an open ring position. For other PCDDs and PCDFs that have not been bioassayed, assign relative potencies (i.e., potencies relative to 2,3,7,8-TCDD) based upon several factors, including their acute toxicity and structural/activity relationships in in vitro tests. The relative potencies used in Tables B-1 and B-2 are those used by EPA: Tetra- through PentaCDD and Tetra through HexaCDF from Bellin and Barnes (1984), and HeptaCDD and HeptaCDF from Barnes (personal communication, 1985).

Scenario 4:

--Assume that the proportion of each PCDD and PCDF homologue is the same as measured from combustion sources, given above.

--Assume that the proportion of isomers chlorinated on the 2,3,7,8 positions is the same as above.

--Use the carcinogenic potencies of TCDD and HexaCDD obtained from their bioassays, as above. Assume a potency of zero for OctaCDD and OctaCDF because of the lack of an open ring position. Assume a potency of zero for isomers that are not chlorinated on the 2,3,7,8 positions. For 2,3,7,8 isomer PCDDs, assume that PCDDs with six or more chlorines generally decrease in carcinogenic potency compared to TCDD, based on the bioassay results of HexaCDD. Hence, assign the potency of 2,3,7,8-TCDD to 2,3,7,8 isomer PentaCDD, and assign the potency of 2,3,7,8 isomer HexaCDD to 2,3,7,8 isomer HeptaCDD. Assign the potencies for the PCDFs equal to their homologous PCDDs.

Tables B-1 and B-2 illustrate Scenarios 2, 3, and 4 for PCDDs and PCDFs, respectively. Under Scenario 2, the carcinogenic potency of a mixture of PCDDs at a given concentration would be 25% of the potency that would obtain if the entire concentration were pure 2,3,7,8-TCDD. Similarly, the potency for a mixture of PCDFs would be 20% of the potency if the entire concentration were 2,3,7,8-TCDD. The assumptions embodied in Scenario 2, therefore, reduce the cancer risk estimate compared to pure TCDD by about 1/5, but less than by an order of magnitude.

Under Scenario 3, the potency of a given concentration of PCDDs would be 1% of the potency of pure TCDD. The potency of a mixture of PCDFs would be 0.3% of the potency of TCDD. This is because the assumptions of Bellin and Barnes (1984) rank each PCDF somewhat less potent than its homologuous PCDD counterpart, due to PCDF's lower acute toxicity. Hence, the assumptions of Scenario 3 reduce the cancer risk estimate (relative to TCDD) by about two orders of magnitude.

Under Scenario 4, the potency of a given concentration of PCDDs would be about 2% of the potency of pure TCDD, and the potency of a mixture of PCDFs would be about 3% that of TCDD.

Under Scenarios 2 and 4 the relative potencies for the untested homologue groups are developed on a weight to weight basis with TCDD or Hexa-CDD. A more appropriate method may have been to develop the relative potencies on a molar to molar basis. When this is done for Scenario 2, using the distribution of isomers given in Tables B-1 and B-2, the total TCDD equivalent exposure was 20 percent less. For four, the total TCDD equivalent exposure remained the same.

Table B-1

Estimates of Total Carcinogenic Potency (Relative to 2,3,7,8-TCDD)
for a Mixture of PCDDs from Municipal Solid Waste Incinerator Emissions

Homologue	Proportion of Homologue in Emissions*	Proportion of Isomer in Homologue Group**	Proportion of Isomer in Emissions	Scenario 2+ Potency Score	Scenario 2+ Equivalent TCDD Proportion	Scenario 3++ Potency Score	Scenario 3++ Equivalent TCDD Proportion	Scenario 4+++ Potency Score	Scenario 4+++ Equivalent TCDD Proportion
TetraCDD	0.09	2,3,7,8 isomer = 0.045	0.004	1.00	0.004	1.00000	0.0041	1.00	0.004
		Non 2,3,7,8 = 0.955	0.086	0.00	0.000	0.01000	0.0009	0.00	0.000
PentaCDD	0.12	2,3,7,8 isomer = 0.071	0.009	1.00	0.009	0.20000	0.0017	1.00	0.009
		Non 2,3,7,8 = 0.929	0.112	0.00	0.000	0.00200	0.0002	0.00	0.000
HexaCDD	0.20	2,3,7,8 isomer = 0.300	0.060	0.03	0.002	0.03000	0.0016	0.03	0.002
		Non 2,3,7,9 = 0.700	0.140	0.00	0.000	0.00040	0.0001	0.00	0.000
HeptaCDD	0.47	2,3,7,8 isomer = 0.500	0.235	1.00	0.235	0.00100	0.0002	0.03	0.007
		Non 2,3,7,8 = 0.500	0.235	0.00	0.000	0.00001	0.0000	0.00	0.000
OctaCDD	0.11	2,3,7,8 isomer = 1.000	0.110	0.00	0.000	0.00000	0.0000	0.00	0.000
	1.0		1.0	Total =	0.25	Total =	0.01	Total =	0.022

* From Tiernan et al. (1983)

** Assumes equal distribution of isomers in homologue group (Olie et al., 1982)

\+ Scenario 2: Relative potency is one for isomers chlorinated on 2,3,7,8 position, 0 otherwise, 0 for OctaCDD, and 0.03 for HexaCDD (from multistage model)

++ Scenario 3: Relative potencies from Bellin and Barnes (1984), and Barnes (personal communication, 1985)

+++ Scenario 4: Relative potency for 2,3,7,8-chlorinated isomers of PentaCDD is equal to 2,3,7,8-TCDD, relative potency for 2,3,7,8-chlorinated isomers of HeptaCDD is equal to 2,3,7,8-isomer HexaCDD.

Table B-2

Estimates of Total Carcinogenic Potency (Relative to 2,3,7,8-TCDD)
for a Mixture of PCDFs from Municipal Solid Waste Incinerator Emissions

Homologue	Proportion of Homologue in Emissions*	Proportion of Isomer in Homologue Group**	Proportion of Isomer in Emissions	Scenario 2+ Potency Score	Scenario 2+ Equivalent TCDD Potency	Scenario 3++ Potency Score	Scenario 3++ Equivalent TCDD Potency	Scenario 4+++ Potency Score	Scenario 4+++ Equivalent TCDD Potency
TetraCDF	0.31	2,3,7,8 isomer = 0.026	0.008	1.00	0.008	0.10000	0.000800	1.00	0.008
		Non 2,3,7,8 = 0.974	0.302	0.00	0.000	0.00100	0.000302	0.00	0.000
PentaCDF	0.19	2,3,7,8 isomer = 0.072	0.0137	1.00	0.0137	0.10000	0.001370	1.00	0.0137
		Non 2,3,7,8 = 0.928	0.176	0.00	0.000	0.00100	0.000176	0.00	0.000
HexaCDF	0.21	2,3,7,8 isomer = 0.252	0.053	1.00	0.053	0.01000	0.000530	0.03	0.0016
		Non 2,3,7,8 = 0.748	0.157	0.00	0.000	0.00010	0.000016	0.00	0.000
HeptaCDF	0.26	2,3,7,8 isomer = 0.500	0.130	1.00	0.130	0.00100	0.000130	0.03	0.0039
		Non 2,3,7,8 = 0.500	0.130	0.00	0.000	0.00001	0.000001	0.00	0.000
OctaCDF	0.02	2,3,7,8 isomer = 1.000	0.020	0.00	0.000	0.00000	0.000000	0.00	0.000
	1.0		1.0	Total =	0.20	Total =	0.003	Total =	0.027

* From Tiernan et al. (1983)

** Assumes equal distribution of isomers in homologue group (Olie et al., 1982)

\+ Scenario 2: Relative potency is 1 for isomers chlorinated on 2,3,7,8 position, 0 otherwise, and 0 for OctaCDF

++ Scenario 3: Relative potencies from Bellin and Barnes (1984), and Barnes (personal communication, 1985)

+++ Scenario 4: Relative potencies for 2,3,7,8-chlorinated isomers of Tetra- and PentaCDF are equal to 2,3,7,8-TCDD, relative potencies for 2,3,7,8-chlorinated isomers of Hexa- and HeptaCDF are equal to 2,3,7,8-isomer HexaCDD.

References for Appendix B

Bellin JS, Barnes DG. Health hazard assessment for chlorinated dioxins and dibenzofurans other than 2,3,7,8-TCDD. Presented at the Symposium on Advances in Health Risk Assessment for Systemic Toxicants and Chemical Mixtures, Cincinnati, OH. 25 October 1984.

Department of Health Services (DHS). Interim guidelines for acceptable exposure levels in office settings contaminated with PCB and PCB combustion products. Epidemiological Studies Section, California Department of Health Services. Berkeley, California. November 1983.

Olie K, Lustenhouwer JWA, Hutzinger O. Polychlorinated dibenzo-p-dioxins and related compounds in incinerator effluents. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds: Impact on the environment. Oxford: Pergamon Press, 1982; 227-224.

Tiernan TO, Taylor ML, Garrett JH, et al. Chlorodibenzodioxins, chlorodibenzofurans, and related compounds in the effluents from combustion processes. Chemosphere 1983; 12(4/5):595-606.

Appendix C

Consistency of Predicted Risk with Observed Risk in an Epidemiologic Study

Although for the most part, epidemiologic studies of PCDD/PCDF exposure can not be used for risk assessment (as discussed in Section 7.2), the results from a human study can be used to check the predicted risks extrapolated from animal data.

The limiting factor that prevents most human studies of PCDD/PCDF exposure from being used for risk assessment is the lack of an estimate of exposure. The only epidemiologic study of cancer risk that provided an estimate of exposure is the one by Ott et al. (1980) of 204 Dow Chemical workers. This study has been described in Section 7.2.2. Using the industrial hygiene measurements and product specification provided in Table 1 (p. 48) of that study, one can estimate a maximum exposure of TCDD of about 0.8 ng/m^3. This is about 20 times greater than the highest dose scenario in Section 9.4.

This dose could be applied directly to the unit risk for TCDD, given in Section 9.3 ($38 \times 10^{-3} [ng/m^3]^{-1}$) to derive the upper 95% confidence limit for excess risk. However, doing so would assume that all workers were exposed to 0.8 ng/m^3 for 24 hours a day for a 70 year lifetime. In fact, over 75% of study subjects worked in that environment for less than one year.

A refinement of this approach is shown in Table C-1, where the unit risk is applied to the distribution of exposure durations for the 204 workers. The "Proportion of 70 Year Lifetime Exposed" column assumes that workers were only exposed for 8 hours a day on weekdays.

As shown in Table C-1, the risk model we have used in Section 9.4 to estimate the upper 95% confidence limit of excess cancers per million in California would predict 0.06 excess cancers in the Ott et al. study. The number actually observed was 1 cancer, vs. 3.6 expected. Using a table of confidence limits for a Poisson variable (Pearson and Hartley, 1966), the 95% upper confidence limit for 1 observed case is 5.57 cases. Hence the 95% confidence limit for the predicted number of cancers (3.6 expected + 0.06 excess predicted) fits well within the 95% confidence limit for the number of cancers actually observed. Therefore, the model prediction is consistent with the observed human data. Furthermore, it is obvious that if the model prediction were correct, the study by Ott, et al. would never have detected as significant the predicted increased risk (cf. Section 7.2.5).

References

Ott MG, Holder BB, Olson RD. A mortality analysis of employees engaged in the manufacture of 2,4,5-Trichlorophenoxyacetic acid. J Occup Med 1980; 22(1):47-50.

Pearson ES, Hartley HO. Biometrika tables for statisticians. Cambridge: Cambridge University Press 1966; 227.

Table C-1

Excess Cancers Predicted by Multistage Model
from Study by Ott et al. (1980)

(1)	(2)	(3)	(4)	(5)	
Duration of Exposure	Proportion of 70 yr Lifetime Exposed*	Adjusted Unit Risk (2) X 70 yr Unit Risk (38×10^{-3} [ng/m3]$^{-1}$)	Upper 95% Conf. Limit of Risk (3) X Dose (0.8 ng/m3)	No. of Employees Exposed**	Upper 95% Conf. Limit of Predicted Excess Cancers (4) X (5)
< 1 yr	0.0034	0.00013 [ng/m3]$^{-1}$	0.00010	157	0.0162
1-2 yrs	0.0068	0.00026 [ng/m3]$^{-1}$	0.00021	30	0.0062
3-4 yrs	0.0136	0.00052 [ng/m3]$^{-1}$	0.00042	9	0.0037
5-40 yrs	0.1361	0.00517 [ng/m3]$^{-1}$	0.00414	8	0.0331
			Totals	204	0.06

* Assumes upper bound of duration of exposure interval, 8 hours per day, 5 days per week, 52 weeks per year

** From Table 3 in Ott et al. (1980), p. 49.

Appendix D

Structure Activity Considerations

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDF) are structually very similar. These two chemical classes contain a number of compounds that are some of the most potent toxicants known to exist. other compounds in these two classes are much less toxic and most have not been adequately tested. 2,3,7,8-TCDD is the most potent PCDD and is more potent than any PCDF thus far studied. Because of its high toxicity and the potential for human exposure to this compound, it is the most extensively investigated PCDD or PCDF.

Many of the adverse effects of 2,3,7,8-TCDD appear to be associated with the presence of a cytosolic receptor protein that has a high affinity for 2,3,7,8-TCDD. The structual requirements for binding have been examined by McKinney and McConnell (1982). They have found that the more toxic PCDDs and PCDFs are chlorinated in the 2,3,7, and 8 positions which allows the compounds to fit into a postulated binding site on the receptor protein. The absence of at least one of these chlorines can significantly reduce the toxicity of the PCDD or PCDF while addition of other chlorine may not seriously hinder receptor binding and toxicity of the compound. Thus, this common mechanism of action allows one to infer a toxicological potential on compounds that are structurally similar to 2,3,7,8-TCDD but have not been adequately tested. Such inferences, however, do involve a great deal uncertainty.

Such inferences may not be sound in the case of carcinogenicity since it is not known whether the cytosolic receptor is even associated with this effect. However, 2,3,7,8-TCDD and a mixture of two HexaCDD that are chlorinated in the 2,3,7, and 8 positions did induce neoplastic responses in mice and rats. Therefore, general structural inferences for other PCDDs and PCDFs could be made when there is a lack of other toxicological information.

Reference

McKinney J, McConnell E. Structural specificity and the dioxin receptor. In: Hutzinger O, Frei RW, Merian E, Pocchiari F, eds. Chlorinated dioxins and related compounds, impact on the environment. New York: Pergamon Press, 1982; 367-381.