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For Review Only

Public Health Goal for TCDD In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

July 2005

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than the general population.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above in items six and seven.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.

10. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DHS shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DHS must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

PHGs are not to be utilized as target levels for the contamination of environmental waters where additional concerns of bioaccumulation in fish and shellfish may pertain. Often environmental water contaminant criteria are more stringent than drinking water PHGs, to account for human exposures to a single chemical in multiple environmental media and from bioconcentration by plants and animals in the food chain.

OEHHA specifically requests comments on the following areas:

1. Considering the mandates of the California Health and Safety Code specifically Section 116300 (a), (d), (e), and (f) and Section 116365 (c), are the methods, assumptions and results of our analyses used in developing PHGs consistent with the intent of the statute? OEHHA solicits comments on toxicity study selection, no observed adverse effect levels (NOAELs), lowest observed adverse effect levels (LOAELs), uncertainty factors, severity of effect modifications, relative source contributions, multi-route exposure assumptions for volatile chemicals and other relevant assumptions and analyses for each chemical. Are the assumptions employed sufficiently health-protective in view of the statutory definition of the PHG (above)?
2. In proposing PHGs for carcinogens, OEHHA employed new methodology proposed by U.S. EPA in their 1996 Guidelines for Carcinogen Risk Assessment. These methods were applied to low-dose extrapolation and inter-species scaling and generally resulted in approximately two-fold lower estimated carcinogen potencies or slope factors as compared to earlier methods. We invite your comments on our methodology.
3. In developing the PHGs, OEHHA considered different levels of risk. Previously, when OEHHA developed Recommended Public Health Goals (RPHGs), the recommended levels were based on a 10^{-6} level of risk, a level that has been considered negligible or de minimis. This level corresponds to a theoretical extra

lifetime cancer risk of 1×10^{-6}, or one fatal cancer per million population exposed over 70 years. This risk level has been identified by federal and state agencies as a level at or below which there are no public health concerns. Higher risk levels of 1×10^{-5} and 1×10^{-4} were also considered and are provided in the supporting documentation for the aid of risk managers. State law allows PHGs to be set at zero. U.S. EPA policy employs zero as a numerical goal (Maximum Contaminant Level goal, or MCLG) for drinking water standards for selected carcinogens. OEHHA welcomes comments on the various options that were considered in identifying the proposed PHGs presented in these technical support documents.

OEHHA expects the following process to pertain to these PHG documents:

1. The Draft documents will be released for external peer review and public comment including a public workshop.
2. Public comments will be received and reviewed by OEHHA and the documents revised as may be appropriate.
3. In accordance with the Health and Safety Code Section 57003, the revised document drafts will be circulated for a period of 30 days following the public workshop.
4. Following this 30-day comment period the documents will be finalized and the PHGs adopted by OEHHA.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR TCDD IN DRINKING WATER

SUMMARY

A proposed public health goal (PHG) of 0.001 ng/L (0.001 ppt, or1 pg/L) has been developed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in drinking water, based on its carcinogenic effects in animals. This proposed health-protective level applies to TCDD alone, rather than TCDD plus all its congeners (dioxins and furans). The development of the proposed PHG follows the general approach of the United States Environmental Protection Agency (U.S. EPA) to estimate TCDD toxicity in humans, which uses body burden, as opposed to daily intake, as a dose metric. A multi-site oral cancer potency of 2.6×10^{-2} $(ng/kg\text{-}day)^{-1}$ is estimated based on increased incidences of neoplasms in lung, liver, oral mucosa, pancreas and uterus in female rats in a chronic oral gavage study (NTP, 2004). This is supported by findings of carcinogenicity in other animal bioassays and by the epidemiological data, which show an increased risk of cancer at multiple sites after exposure to dioxins.

A public health-protective concentration of 0.007 ng/L (0.007 ppt) for noncarcinogenic effects of TCDD in drinking water was also determined, based on the subchronic mouse study of Toth *et al*. (1979). In this study, a LOAEL of 1 ng/kg-day was associated with an increased incidence of amyloidosis and dermatitis. A default relative source contribution of TCDD from drinking water of 20 percent, and a total uncertainty factor of 1,000 were then applied to the LOAEL to derive the noncancer PHG value.

The Office of Environmental Health Hazard Assessment (OEHHA) concurs with the U.S. EPA that the combined animal and human data contribute to the overall weight of evidence for TCDD carcinogenicity. The voluminous body of evidence that exposure to TCDD increases the risk of cancer in animals and in humans at multiple sites justifies the conclusion that TCDD poses a carcinogenic risk to humans.

The proposed PHG is lower than the Maximum Contaminant Level (MCL) of 0.03 ng/L established by U.S. EPA, but well above the U.S. EPA ambient water criteria level of 0.005 pg/L (which includes consideration of consumption of organisms in the water). The proposed PHG is considered to provide an adequate margin of safety to protect potential sensitive subpopulations, and to protect against all of the noncarcinogenic effects of TCDD, including adverse effects on the immune system, cardiovascular system, liver, and reproductive/developmental effects.

INTRODUCTION

This document examines available data and evidence on the toxicity of the 2,3,7,8-tetrachlorodibenzo-p-dioxin congener, hereafter referred to as TCDD, for establishing a proposed public health goal (PHG) for TCDD in drinking water. The U.S. EPA, in its drinking water criteria documents (U.S. EPA, 1978, 1984, 2002) and in a recent Exposure and Human Health Reassessment of TCDD and related compounds (U.S. EPA, 2000),

concluded that exposure to dioxins increases the risk of cancer at multiple sites in humans and animals. Based on tumorigenic effects in both humans and animals, the National Toxicology Program (NTP), International Agency for Research on Cancer (IARC), and the Agency for Toxic Substances and Disease Registry (ATSDR) have come to similar conclusions regarding the carcinogenicity of dioxin.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), commonly referred to as dioxin, represents the reference compound for a class of halogenated aromatic compounds that produce similar patterns of toxicity and appear to have a common mechanism of action, though they differ in potency. These are commonly referred to as chlorinated dibenzodioxins, or dioxins. The chlorinated dibenzodioxins are tricyclic aromatic compounds with similar physical and chemical properties. Polychlorinated dibenzodioxins (PCDDs) are non-polar, largely water-insoluble, and are stable in the environment. The PHG document is based exclusively on the 2,3,7,8-isomer because this compound is specified for the California MCL in California regulations (Title 22, Div. 4, Chap. 15,Art. 5.5, Sec 64444, Table 64444A).

Dioxins are largely contamination by-products. They are inadvertently formed from the manufacture of chlorophenols and hexachlorophene, as well as various herbicides. They are primarily released to the atmosphere through municipal waste incineration, combustion of coal, wood, leaded gasoline, and chemical wastes, and improper disposal of certain chlorinated chemical wastes (ATSDR, 1999). Uncontrolled burning of household waste, the use of wood burning stoves and fireplaces, and accidental fires at landfills may also be important sources of dioxin releases. Natural sources of dioxin include forest fires and volcanic eruptions. Because of their widespread distribution, persistence, and accumulation within the food chain, it is likely that most humans are exposed to some level of dioxins.

Contamination of municipal drinking water may occur through industrial contamination of source water (in sewage from municipal wastewater and in effluents from pulp and paper mills), and through erosion of contaminated soil (from dumps and agricultural run-off). Industrial pollution has resulted in contaminated drinking water in Southeast Alaska and in the areas of Ufa and Chapaevsk, Russia.

Exposure to dioxins has been clearly associated with an increased risk of cancer at multiple sites in animals and humans. The U.S. EPA, International Agency for Research on Cancer (IARC), and the World Health Organization (WHO) list dioxin as a Group 1 human carcinogen.

CHEMICAL PROFILE

Chemical Identity and Properties

Polychlorinated dibenzo-p-dioxins (PCDDs) occur as 75 different isomers. There are twenty-two possible tetrachlorodibenzo-para-dioxins (TCDD) isomers. Only 7 of the 75 congeners of PCDDs are thought to have dioxin-like toxicity. These are ones with chlorine substitutions in at least the 2, 3, 7, and 8 positions. The chlorinated

dibenzodioxins are tricyclic aromatic compounds with similar physical and chemical properties. PCDDs are non-polar, largely water-insoluble, and are stable in the environment. These structurally-related compounds have the ability to bind to the aryl hydrocarbon receptor (AhR) and to elicit similar biological actions. Thusly, the congeners are commonly referred to as dioxin-like compounds (DLCs).

The CAS registry number for the 2,3,7,8-tetrachlorodibenzo-para-dioxin (2,3,7,8-TCDD) congener is 1746-01-6. Its molecular formula is $C_{12}H_4Cl_4O_2$ and its molecular weight is 322 g/mol. The chemical structure of 2,3,7,8-TCDD is shown in Figure 1, below. TCDD is a white crystalline solid with a melting point range of 302 to 305 ^{o}C. TCDD is lipophilic, exhibiting a high degree of solubility in fats, oils and other relatively non-polar solvents, and is only slightly soluble in water (0.2 to 0.6 µg/L). This compound, often called simply dioxin, represents the reference compound for a class of halogenated aromatic compounds that produce similar patterns of toxicity and appear to have a common mechanism of action, though they differ in potency.

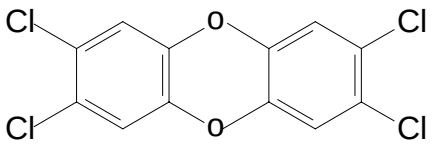

Figure 1. Structure of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

Uses and Occurrence

TCDD is largely produced by human activities, and has no uses as such. Dioxins are inadvertently formed as by-products from the manufacture of chlorophenols and hexachlorophene, as well as various herbicides. In the past, dioxins came primarily from production and use of chlorinated organics, including the pesticide Agent Orange, polychlorinated biphenyls (PCBs), and the wood preservative, pentachlorophenol (PCP), used on telephone poles and other wood products. Since the 1970s, many of the contaminated chemicals have been banned in the U.S. (e.g., PCBs and Agent Orange), or their use has been dramatically reduced (e.g., PCP). The U.S. EPA suspended the registration of most uses of 2,4,5-T in 1979, and banned it in 1989, but exposure to human populations continues as a result of past production, use, and disposal.

2,4-Dichlorophenoxyacetic acid (2,4-D), one of the top residential and commercial agricultural herbicides used in the U.S., and potentially other chlorinated pesticides, such as chlorthal-diethyl (dacthal), can be significantly contaminated with dioxins (and including the 2, 3, 7, 8-TCDD congener). Millions of pounds of 2,4-D are used in California agriculture annually. National data on 2,4-D use suggests that agricultural use is only slightly greater than non-agricultural use (Aspelin and Grube, 1999). Dioxin contamination has been detected in many other manufacturing processes including

production of polyvinyl chloride (PVC) and textile dyes. Dioxins in dyes may be removed during household washing and concentrated in sewage sludge.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

TEFs

Since human exposure to PCDDs always occurs as a complex mixture, a methodology referred to as the Toxic Equivalency Factor (TEF) was developed to assess the health risks posed by mixtures of these compounds. The TEF methodology is a relative potency scheme that ranks the dioxin-like toxicity of a particular PCDD congener relative to 2,3,7,8-TCDD, which is the most potent congener. Since 2,3,7,8-TCDD is the reference compound for the dioxin TEF scheme, it has been assigned a TEF of 1.0.

Air

Tetrachlorodibenzo-p-dioxin isomers are ubiquitous in soil, sediment and air. Naturally occurring sources of dioxin include forest fires and volcanic eruptions. Dioxins are primarily released to the atmosphere through municipal waste incineration, combustion of coal, wood, leaded gasoline, and chemical wastes, and improper disposal of certain chlorinated chemical wastes (ATSDR, 1999). They may also be released from fires of PVC-containing materials. Backyard trash burning, where PVC is incinerated, has been estimated to release substantial amounts (Lemieux *et al*., 2000). Other unregulated sources which may contribute significantly to dioxin releases include residential wood burning in stoves and fireplaces.

Inhalation exposure of the general population to dioxin primarily results from incineration processes. Occupational exposure and environmental contamination may result from the synthesis of 2,4,5-T and hexachlorophene and from metals reclamation (Papke *et al*., 1992). Other occupational exposures may result from workers involved with incineration operations, or from workers handling pesticides that may contain TCDD impurities. The U.S. EPA (2000) has estimated that the general human population is exposed to daily TCDD doses of ~0.3 pg/kg-day.

Soil

PCDDs can enter the soil system through pesticide and sewage sludge applications, leakage from waste dumps, atmospheric deposition of particulates, and gaseous-phase transport. In 1998, 460,000 dry tons of sewage sludge were applied to agricultural land in 26 California counties (Jones and Stokes Associates, 1999). TCDD is highly lipophilic and markedly hydrophobic, and can move through soil into lakes and rivers where it generally attaches to organic matter in sediment. Analysis of sediment cores throughout the U.S. suggests that dioxin deposition increased substantially between the 1930s and 1970s (Cleverly *et al*., 1996).

TCDD is generally resistant to biodegradation. Photodegradation of TCDD bound to fly ash is not an important atmospheric removal mechanism (Koester and Hites, 1992). The half life of 2,3,7,8-TCDD on soil surfaces may vary from less than 1 year to 3 years, but half-lives in soil interiors may be as long as twelve years (ATSDR/EPA, 1988). Nestrick *et al*. (1986) concluded that 2,3,7,8-TCDD occurs in U.S. urban soils at the level of 1-10 ng/kg. Kimbrough *et al*. (1984), on the basis of extrapolations from animal toxicity experiments, suggested that 1 ng/g of 2,3,7,8-TCDD in soil "is a reasonable level at which to begin considerations of action to limit human exposure to contaminated soil."

Water

Tetrachlorodibenzo-p-dioxins may also be released in sewage from municipal wastewater and in effluents from pulp and paper mills. In the greater San Francisco Bay area, dioxins have been detected in filtered storm water outfall at levels above the U.S. EPA surface water guideline of 0.013 parts per quadrillion (ppq) (average levels between 10-25 ppq ITEQ) (Wenning *et al*., 1999). ITEQ refers to International Toxic Equivalency units (generally expressed as ITEQ/g fat, or ppt). When released to water, TCDD will adsorb strongly to sediments and suspended matter, based on the high Koc value of $2.4x10^6$ (HSDB, 2004). Although dioxins themselves have low solubility in water (0.2 µg/L), other organic constituents present in the water may act as carriers. Volatilization from water is expected to be slow. The persistence half-life of TCDD in lakes has been estimated to be in excess of 1.5 years (ATSDR, 1999). Polychlorinated dibenzo-p-dioxins (PCDDs) in waterways can bioaccumulate in fish, leading to human exposure via consumption of fish. Table 1, adapted from U.S. EPA (2000), shows a quantitative inventory of environmental releases of dioxins to water in the United States. Most sources of PCDDs released to the environment are not quantifiable.

Table 1. Releases (g TEQ/yr) to Water in the United States*

Emission Source Category	Reference Year 1995	Reference Year 1987
Chemical Manufacturing/Processing Sources Bleached chemical wood pulp and paper mills	19.5	356
Ethylene dichloride/vinyl chloride	0.43	
Total Quantified Releases to Water	19.93	356

Adapted from U.S. EPA (2000)

*Congener-specific emissions data were not available; the TEQ_{DF} emission estimate was used as a surrogate

Table 2. Preliminary Indication of the Potential Magnitude of *I-TEQ$_{DF}$ Emissions from "Unquantified" Sources in Reference Year 1995

Emission Source Category	Release Medium	Preliminary Release Estimate (g I-TEQ$_{DF}$/yr)	Estimated Activity Level	Estimated Emission Factor
Municipal Wastewater	Water	13	44.5 trillion L of wastewater	0.29 pg I-TEQ$_{DF}$/L water
Urban Runoff	Water	190	190 trillion L of urban runoff	1 pg I-TEQ$_{DF}$/L water
Rural Soil Erosion	Water	2,700	2.7 billion metric tons of soil	1 ng I-TEQ$_{DF}$/kg soil

Adapted from U.S. EPA (2000)
*congener specific emissions data were not available

While there is very little information in the literature about contamination of drinking water by PCDDs, there are instances where contamination of drinking water by PCDDs has been quantified. In one area of Southeast Alaska, because of a lack of suitable groundwater and surface water sources, drinking water for homes and businesses has almost exclusively been supplied by individual roof-catchment systems and stored in cisterns. This area is located downwind from a sulfite pulp mill that operated from 1954-1997. To supply power for mill operations, dewatered wastewater treatment plant sludge, fuel oil, and wood waste were burned in two power boilers. PCDDs (and polychlorinated dibenzo furans (PCDFs)) were apparently synthesized *de novo* during combustion of sludge that contained chlorinated effluent from the pulp bleaching operations and combustion of hog fuel from logs that had been stored in rafts in saltwater. In one analysis, conducted in 1998, PCDDs and PCDFs were detected in all four drinking water cistern sediment samples. Cistern sediments had maximum total PCDD/PCDF concentrations of 77 µg/kg (range 4.8-77 µg/kg) (Peek *et al*., 2002).

In addition to the U.S., a number of other industrialized countries have dioxin-contaminated drinking water. In the city of Ufa, in the Bashkortostan Republic of Russia, the drinking water supply has been contaminated over a period of 30 years as a result of industrial pollution. The city of Ufa is home to a number of factories and has had several industrial incidents which have released 2,3,7,8-TCDD and other PCDDs into the nearby Ufa river, where a total of 0.13 to 0.20 ng/L (ppt) of PCDDs are regularly present (Smirnov *et al*., 1996). Dioxins enter the Ufa river both with sewage and with underground water through contaminated soil (dumps and contaminated soil contain tens of kilograms of dioxins). Emergency situations, in which the concentration of dioxins in river or tap water exceeds their permissible level of 0.02 ng/L by ten to one hundred times, occur on a regular basis. Elevated dioxin levels have been found in blood from certain plant workers and their children as well as in pooled blood from the Ufa general population (Schecter *et al*., 1993; Schecter and Ryan, 1993).

In Chapaevsk, Russia, dioxins have been detected in the town's drinking water (28.4-74.1 pg/L), in cow's milk (the content of 2,3,7,8-TCDD was 17.32 pg TEQ/g fat), in air (0.116

pg/m^3) and in soil (8.9-298 ng/kg). From 1967-1987, the Middle Volga chemical plant in Chapaevsk produced lindane and its derivatives. Currently it produces crop protection chemicals (liquid chlorine acids, methyl chloroform, vinyl chloride and other chemicals). (Dioxins and similar compounds can be formed in the production of methyl chloroform, vinyl chloride, dichloropropionic acid, hexachloroethane, sodium pentachlorphenolate and polychloroform). The town's drinking water source is groundwater. Dioxin analysis of three samples of drinking water from different areas of the town was conducted in 1998. The results revealed high levels of the octa and hepta dioxin congeners (OCDD and HpCDD) (Table 3). The authors conclude that the situation was caused by wastes discharged from the chemical production of pentachlorophenol. The total content of PCDD and PCDF exceeds the maximum allowable concentration of dioxin in drinking water in the U.S. (0.013 pg/L), in Germany and Canada (0.01 pg/L), and in Italy (0.05 pg/L).

Table 3. Concentration of PCDDs (pg/L) in Chapaevsk, Russia drinking water, July 1998

Congeners	6-8 Kilometers From the Plant	City Center	
		Sample 1	Sample 2
2,3,7,8-TCDD	< 2	< 2	5.0
1,2,3,7,8-PeCDD	<5	<5	<5
1,2,3,4,7,8-HxCDD	<10	<10	<10
1,2,3,6,7,8-HxCDD	<10	<10	18.5
1,2,3,7,8,9-HxCDD	<10	<10	<10
1,2,3,4,6,7,8-HpCDD	166.4	291	70
OCDD	26,789	78,549	32,887
Other TCDD	<2	<2	<2
Other PeCDD	<5	<5	<5
Other HxCDD	<10	<10	<10
Other HpCDD	<20	106.9	76.5

Adapted from Revich *et al.*, 2001. PeCDD means pentachloro-, HxCDD means hexachloro-, HpCDD means heptachloro-, and OCDD means octachloro-dibenzodioxins.

Analyses performed in drinking water treatment plants (DWTP) in Sant Joan Despi and Cardedeu, Spain, which supply drinking water to the city of Barcelona and its surroundings, have detected the presence of high levels of many industrial contaminants, including PCDDs, PCDFs and PCBs (Riviera *et al.*, 1997). The PCDD profile is dominated by OCDD with levels ranging from 1,200-3,560 pg/g sludge. The HpCDDs are the second dominant congener group, with concentrations ranging from 300-1,200 pg/g. In this study, the 2,3,7,8-TCDD isomer was detected in only one sample at a concentration of 3.7 pg/g. The Llobregat River constitutes the main source of drinking water for Barcelona and its surroundings. The river is extremely polluted and receives

the discharges of many different industries including textile mills, metallurgic factories, pulp mills, salt mines and farms, in addition to domestic wastewater. The DWTP sampled in this study is 7 km from the mouth of the Llobregat River.

Food and Others

Excluding occupational or accidental exposures, most human exposure to PCDDs occurs as a result of eating meat, milk, eggs, fish and related products, as PCDDs are persistent in the environment and accumulate in animal fat. TCDD has been detected at concentrations ranging from 3-6 ppt in adipose tissue samples taken from cattle feeding on contaminated forage (Kocher *et al*., 1978; U.S. EPA, 1978). Direct exposure to TCDDs may also occur through inhalation of cigarette smoke (Mueller *et al*., 1993; Ono *et al*., 1987; Muto and Takizawa, 1989). Infants may be exposed to TCDDs through ingestion of contaminated milk (Noren, 1993). Studies in the Netherlands suggest that breast fed infants have a 50-fold higher daily dioxin intake than adults after adjusting for bodyweight (Patandin *et al*., 1999).

TCDD will bioconcentrate strongly in aquatic organisms based on bioconcentration factors (BCFs) of 1,225 and 2,238 in rainbow trout and fathead minnow, respectively (Muir *et al*., 1996). Normal dietary intake of 2,3,7,8-TCDD is quite variable depending primarily on consumption of contaminated fish. The maximum daily intake of 2,3,7,8-TCDD was estimated for residents of the Great Lakes region who regularly consume fish from the Great Lakes. The intake ranged from 0.39-8.4 µg/day (U.S. EPA, 1984). Representative intake for the average adult of 0.1 ng/day may be associated with a human body burden of 100 ng (~7 ng TCDD/kg adipose tissue) (Jones and Bennett, 1989). The inferred biological half-life of TCDD in the human body is approximately 7 years.

The daily intake of dioxins in humans in the United States is estimated at approximately 1 pg TEQ/kg-day (U.S. EPA, 2000). In human tissues, current mean background levels of TCDD are in the range of 2-3 ng/kg fat (McGregor *et al*., 1998). A single acute exposure from the environment results in the exposure of potential target tissues over many years.

METABOLISM AND PHARMACOKINETICS

Absorption

Rose *et al*. (1976) administered a single oral dose of 1.0 µg ^{14}C-TCDD/kg to Sprague-Dawley rats. Absorption from the gastrointestinal (GI) tract ranged from 66-93 percent, with a mean of ~83 percent. The response to repeated oral dosing (at 0.1 or 1.0 µg/kg-day, 5 day/week for 7 weeks) was also monitored and absorption (86 percent) was observed to be approximately the same as that observed for the single oral dose. Similar results by other investigators in a variety of species (Piper *et al*., 1973; Diliberto *et al*., 1996) indicate that oral exposure to TCDD in the diet or in an oil vehicle results in absorption of >50 percent of the administered dose. Lakshmanan *et al*. (1986), using thoracic duct cannulated rats, found that following GI absorption, TCDD is primarily

absorbed via the lymphatic route, and ninety percent of the TCDD in lymph is associated with the chylomicron fraction. The plasma disappearance of TCDD-labeled chylomicrons followed first-order delay kinetics, with 67 percent of the compound leaving the blood compartment very rapidly ($t_{½}$ = 0.81 minutes), partitioning into cellular membranes and tissues. The limited database in experimental animals suggests that there are no major interspecies differences in the GI absorption of TCDD.

Poiger and Schlatter (1980) investigated the absorption of TCDD in a forty-two year old man following ingestion of 105 ng ^{3}H-TCDD (1.4 ng/kg) in corn oil, and reported that >87 percent of the TCDD was absorbed from the GI tract. The half-life for elimination was estimated to be 2,120 days. Studies using human cadaver skin (Weber *et al*., 1991), and in rats (Birnbaum, 1991) show that the rate of dermal absorption of TCDD is very slow, even following a low-dose application of 200 pmol (1 nmol/kg). In humans, the stratum corneum acts as a protective barrier; the rate of penetration of TCDD into the dermis ranged from 6-170 pg/hour/cm^2 (Weber *et al*., 1991).

Studies by Nessel *et al*. (1990, 1992) in rats show that transpulmonary absorption of TCDD does occur following intratracheal instillation of the compound in corn oil vehicle. A study by Diliberto *et al*. (1993) in rats showed that transpulmonary absorption following intratracheal instillation resulted in almost complete absorption of TCDD (95 percent).

Distribution

Dioxins are extremely lipid soluble allowing for storage in body tissues. Once absorbed into the blood, TCDD readily distributes to all organs within the first hour(s) after exposure. Dioxins are stored in the fat of breast milk, and they cross the placenta. The average body burden in the U.S. population is estimated at 36-58 International Toxic Equivalency units (ITEQ)/g fat or parts per trillion (ppt) (Grassman *et al*., 1998).

Lakshmanan *et al*. (1986), using thoracic duct cannulated rats, found that TCDD distributes primarily to adipose tissue and liver. Piper *et al*. (1973) used a single oral dose of ^{14}C labeled TCDD to study distribution and excretion of TCDD in male Sprague-Dawley rats. Tissue analysis showed liver and adipose tissue contained the highest percent of the dose per gram of tissue, 3.18 and 2.6 percent, respectively, after three days. Studies performed by Van Miller *et al*. (1976) on rhesus monkeys and rats using tritiated TCDD showed that while rats had over 40 percent of the TCDD in liver, the monkeys had only about 10 percent in the same organ. Following a single i.p exposure of rats to TCDD, liver, adipose tissue, skin and thyroid were the only tissues to show an increased concentration of TCDD 4 days post-exposure (Pohjanvirta *et al*., 1990). This general pattern of distribution, with the liver and adipose tissue being the primary disposition sites, is similar in mice, rats, rhesus monkeys, hamsters and guinea pigs. Abraham *et al*. (1988) studied the tissue concentration of TCDD in liver and adipose tissue of rats following a single s.c. exposure to 300 ng/kg TCDD. The maximum concentration of TCDD in the liver was reached at 3 days, that of adipose tissue, 7 days post–exposure. The concentration of TCDD was found to decrease more rapidly in liver than in adipose tissue. Pegram *et al*. (1995), in a study using mice, showed that age is an important factor affecting distribution of TCCD; liver concentrations of TCDD were approximately

25 percent greater in young mice than in old. Dose has also been shown to be a factor in the tissue distribution of TCDD. Exposure to higher doses results in a disproportionately greater hepatic concentration than in adipose tissue.

The distribution of TCDD in humans has been examined. Poiger and Schlatter (1986) estimated that ~90 percent of the body burden of TCDD was stored in adipose tissue after a volunteer ingested 1.4 ng/kg TCDD ^{3}H in corn oil. The study duration was for 135 days; radioactivity in the blood was only detected during the first two days following treatment. Geyer *et al*. (1986) estimated a bioconcentration factor (BCF) of between 104 and 206 for TCDD in human adipose tissue. A number of researchers have reported adipose tissue TCDD levels averaging from 5-10 ppt for background populations in various parts of the U.S. The mean serum TCDD level in Vietnam veterans with exposure to herbicides was 49 ppt in 1987 (n= 147), while the mean serum level of the controls was 5 ppt (MMWR, 1988).

Metabolism

TCDD appears to be a poor substrate for detoxification systems such as the microsomal cytochrome P-450 enzymes, which oxygenate other lipophilic compounds to inactive derivatives during their metabolic processing. Because of its relative resistance to metabolism, TCDD persists in the body with a half-life in humans of up to 8.7 years (Michalek *et al*., 1996). Although no metabolites of TCDD have been identified in humans, samples of human feces suggest that humans do metabolize TCDD (Wendling *et al*., 1990). Studies on the metabolism of TCDD in animals suggest that reactive epoxide intermediates may be formed (Poland and Glover, 1979). Mason and Safe (1986) synthesized two metabolites of TCDD, 2-hydroxy-3,7,8-TCDD and 2-hydroxy-1,3,7,8-TCDD, and assessed their toxicity in male Wistar rats. While the metabolite 2-hydroxy-3,7,8-TCDD did induce hepatic microsomal enzymes, the compounds produced no significant effect on body weight gain, thymus, liver or spleen weights at a dose of $\leq$5,000 µg/kg. Structure activity studies of TCDD support the evidence that the parent compound is the active species, and that biliary and urinary excretion of these monohydroxylated metabolites is dependent on metabolism. The relative rate of TCDD metabolism can be estimated from tissue and excretion half-life data (U.S. EPA, 2000).

Excretion

Once inside the body, there are few metabolic pathways for dioxins, and they tend to accumulate in human tissues over time, making body burden (bioaccumulation) a reliable indicator of absorbed dose and potential effects. Studies in animals using a single radiolabeled congener indicate that excretion of dioxins follows a first-order elimination process. Piper *et al*. (1973), using a single oral dose of ^{14}C-labeled TCDD to study distribution and excretion of TCDD in male Sprague-Dawley rats, found that most of the radioactivity (53 percent) was excreted via the feces, but urine and expired air accounted for 13 and 2 percent, respectively. Gender differences in TCDD excretion have been observed. Male rats given 1.0 µg/kg/day of TCDD for seven weeks excreted an average of 3.1 percent of the cumulative dose in the urine while the female rats excreted an

average of 12.5 percent in the urine (Rose *et al*., 1976). The rate of excretion of TCDD is species specific. TCDD is most persistent in human and nonhuman primates. Factors which may regulate the rate of TCDD excretion include: percent body fat, hepatic and extrahepatic binding proteins, and direct intestinal elimination of the parent compound. In females, lactation can also serve as a relatively efficient route for excretion of TCDD. Poiger and Schlatter (1986) estimated that the half-life for elimination of TCDD in humans was 2,120 days based on fecal excretion over a 125-day period following a single exposure of 1.4 ng/kg TCDD^{3}H in a 42-year old man.

TOXICOLOGY

Although there are many congeners among the polychlorinated dibenzo-dioxins, the 2,3,7,8-tetrachloro-p-dioxin congener is the most toxic. TCDD is extremely toxic to some animal species, as indicated by its acute oral LD_{50}s of 0.022 and 0.045 mg/kg for male and female rats, and only 0.0006 mg/kg (0.6 μg/kg) for guinea pigs (Casarett and Doull, 1986). A more than 8,000-fold difference exists between the dose of TCDD reported to cause 50 percent lethality (LD_{50}) in male Hartley guinea pigs, the most sensitive species tested (Schwetz *et al*., 1973) and the LD_{50} dose in male Syrian golden hamsters (Henck *et al*., 1981). Polymorphism in the Ah locus is thought to account for many of the differences in sensitivity of the different species/strains to TCDD.

Table 4 shows LD_{50}s for TCDD in various species of animals. One of the characteristics of TCDD-induced toxicity is delayed manifestation of lethality after acute exposure, with time to death after exposure being several weeks. This delay is seen in all species. Progressive hypoglycemia from feed refusal and inhibition of gluconeogenesis seems to be the ultimate cause of death (Gorski *et al*, 1990).

Table 4. Lethal TCDD Doses (LD_{50}s) in Various Animal Species

Species and Sex	Route of Administration	LD_{50} (μg/kg)	Reference
Rat, male	Oral	22	Schwetz *et al*., 1973
Rat, female	Oral	45	Schwetz *et al*., 1973
Mice, male	Oral	114	Vos *et al*., 1974
Guinea pig, male	Oral	0.6-2.1	Schwetz *et al*., 1973
Rhesus monkey, female	Oral	<70	McConnell *et al*., 1978
Rabbit, mixed	Oral	115	Schwetz *et al*., 1973
Rabbit, mixed	*Skin	275	Schwetz *et al*., 1973
Rabbit, mixed	Oral	10	Schulz, 1968

Adapted from U.S. EPA, 1978.
*Death was sometimes delayed as long as 40 days.

In animals, TCDD elicits a wide range of biological effects, including alterations in metabolic pathways, immunological changes, reproductive and developmental abnormalities, and neoplasia. These toxicological endpoints are discussed in greater detail in the animal toxicology section that follows.

Accidental exposures indicate that TCDD has low acute toxicity for man as compared with that for certain species (e.g., guinea pigs) (Gilman *et al*., 1991). In humans, acute exposure to TCDD results in irritation of the eyes, skin and respiratory tract (U.S. EPA, 1985). The most commonly reported symptom related to TCDD exposure in man has been chloracne (acneform lesions of the skin). Other reported skin problems include hyperpigmentation, hirsutism, increased skin fragility and vesicular eruptions on exposed areas of the skin (HSDB, 2004). Other less consistently reported effects from dioxin exposure in humans include: asthenia (weakness), headaches, pain in the extremities, peripheral neuropathy, ulcers, altered liver function, enzyme induction, altered lipid metabolism, and abnormal urinary porphyrin patterns (Andrews, 1992).

TCDD is a multi-site carcinogen in experimental animals and the International Agency for Research on Cancer (IARC) has listed 2,3,7,8-TCDD as a Group 1 carcinogen (carcinogenic to humans) (IARC, 1997). There is no report of human exposure only to TCDD. Three case control studies have shown relative risks of 5.7 (95 percent confidence limits, 2.9-11.3) and 5.1 (2.5-10.4) for soft tissue sarcoma and 6.0 (3.7-9.7) for lymphoma in association with exposure to phenoxyacetic acids or chlorophenols, in which TCDD was a likely contaminant (IARC, 1982). Fingerhut *et al* (1991b), in a retrospective cohort mortality study of 5,172 chemical workers from 12 facilities in the U.S., found that mortality due to soft tissue sarcoma, respiratory system cancer, as well as all other cancers combined, was significantly elevated for workers with histories of exposure to phenoxy herbicides and chlorophenols contaminated with TCDD.

The toxicity of TCDD appears to depend on the fact that the four lateral positions of the molecule are occupied by chlorine, resulting in high-affinity binding to an intracellular protein known as the aromatic hydrocarbon receptor (AhR). The Ah receptor is a member of a family of proteins, and the genes regulated by this receptor are involved not only in xenobiotic metabolism, but also in cell growth and differentiation. The AhR has been identified in numerous mammalian species, including humans, as well as in several non-mammalian vertebrates. Studies in Ah receptor-deficient mice have demonstrated that most, if not all, of the toxic responses elicited by TCDD are mediated by the ability of this chemical to bind to the AhR (Fernandez-Salguero *et al*., 1996).

Toxicological Effects in Animals

Acute, Subacute, and Chronic Noncancer Effects

A number of authoritative bodies have reviewed and summarized the toxic effects of TCDD. TCDD toxicity involves many different types of symptoms, which vary from species to species and from tissue to tissue. The toxic responses of various species to TCDD are summarized in Table 5. Most of the toxicity data available for TCDD are from oral experiments in animals. Very few percutaneous and no inhalation exposure toxicity data are available in the literature.

Table 5. Toxic Responses Following Exposure to 2,3,7,8-TCDD: Species Differences

Response	Monkey	Guinea Pig	Cow	Rat	Mouse	Rabbit	Chicken	Hamster
Hyperplasia or metaplasia								
Gastric mucosa	++	0	+	0	0			0
Intestinal mucosa	+							++
Urinary Tract	++	++	++	0	0			
Bile duct or gall bladder	++	0	+	++	++			0
Lung				++				
Skin	++	0	+	0	0	++		0
Gingival				++				
Cortical				++				
Oval Cell				++				
Hypoplasia, atrophy, or necrosis								
Thymus	+	+	+	+	+		+	+
Bone marrow	+	+			±		+	
Testicle	+	+		+	+			
Other responses								
Liver lesions	+	±	++	+	++	+	+	±
Porphyria	0	0		+	++		+	0
Edema	+	0		0	+		++	+

Source: Adapted from U.S. EPA (2000)
0= lesion not observed; + = lesion observed (number of "+" denotes severity); ± = lesion observed to a very limited extent; blank = no evidence reported in literature

The liver is extremely sensitive to TCDD toxicity in all animals, regardless of duration of exposure. Significant hepatotoxicity has been observed in a number of animal studies (Kociba *et al*., 1978; NCI, 1980; NTP, 1982, 2004). The degree of severity of pathological alterations in the liver seems to be species-specific. Thymic atrophy has been found in all animal species given lethal doses of TCDD. In addition to those listed in Table 5, other signs and symptoms that have been demonstrated in various species include: hepatic porphyria, hepatocyte hypertrophy, hemorrhages in various organs, testicular atrophy, reduced prostate weight, reduced uterine weight, increased thyroid weight, increased liver weight, increased relative lung weight, lesions of the adrenal glands, inhibited bone marrow hematopoiesis, histiocytic infiltration, decreased thyroxine (T_4), and increased serum total triiodothyronine (T_3) and thyroid stimulating hormone (TSH), decreased serum albumin, and increased serum triglycerides and free fatty acids. Exposure to TCDD also affects various physiological equilibrium processes such as vitamin A storage, plasma membrane functions, and the formation of keratin and cell differentiation. The specifics of all underlying studies for these observations have been extensively reviewed (U.S. EPA, 1984, 1985; WHO/IPCS, 1989; NTP, 2004).

Enzyme Induction

TCDD has repeatedly been found to increase the activities of various enzymes, and particularly the cytochrome P4501A1 (CYP1A1) and P4501A2 isoenzymes (Diliberto *et al*., 1997; NTP, 2004), which catalyze oxygenation of polycyclic aromatic substrates to their more water-soluble derivatives. Increases in CYP1A1 and CYP1A2 are characteristic responses to dioxin-like compounds. On a molecular basis, TCDD is the most potent mixed-function oxidase (MFO)-inducing compound known (U.S. EPA, 2000). According to Kitchin and Woods (1979), induction in the rat takes place at doses as low as 0.002 µg TCDD/kg bodyweight. Several investigators have reported that enzyme induction has also been observed in the offspring of various species after prenatal and postnatal (milk) exposure to TCDD (Lucier *et al*., 1975; Korte *et al*., 1991; Waern *et al*., 1991). A number of other hepatic enzymes have also been shown to be affected by TCDD exposure (U.S. EPA, 1984, 1985; WHO/IPCS, 1989). Based on data from a number of studies (Kitchin and Woods, 1979; Abraham *et al*., 1988; Kruger *et al*., 1991; Neubert *et al*., 1991), a NOAEL of 1 ng/kg-day can be calculated for enzyme induction for both rats and marmoset monkeys.

Endocrine Effects

Exposure to TCDD has been shown to interfere with normal endocrine function by disrupting natural hormones. TCDD induces the expression of a large number of genes involved in growth regulation, hormonal signaling and signal transduction, and hormone metabolism. Van der Kolk *et al*. (1992) and Van Birgelen *et al*. (1995a,b) observed dose-dependent reductions in plasma thyroid hormones levels in TCDD-exposed animals. NTP (2004) observed significant changes in thyroid hormones of female rats exposed via gavage to TCDD for two years: decreased thyroxine (T_4) and increased serum total triiodothyronine (T_3) and thyroid stimulating hormone (TSH). TCDD induces several enzymes related to testosterone metabolism (Moore *et al*., 1991). Mittler *et al*. (1984)

demonstrated a decreased activity of testicular 16-alpha-testosterone hydroxylase, 6-beta-hydroxytestosterone, and 7-alpha-hydroxytestosterone in young Sprague-Dawley rats 90 hours after exposure to single i.p. doses of 0.2, 1 or 5 µg TCDD/kg. Maternal exposure to TCDD has been shown to affect the male reproductive system at low doses (the lowest dose tested was 64 ng/kg) (Mably *et al*., 1992a,b,c). Estrogen, glucocorticoid, prolactin, insulin, gastrin, melatonin and other hormones are affected by TCDD either by its activity on the hormone or the receptor.

The importance of estrogens as modulators of TCDD-induced toxicity has been investigated by Lucier *et al*. (1991), who found that by removing the ovaries from female rats before exposure to TCDD, the tumor promoting effects of TCDD could be prevented. Several long term bioassays have demonstrated that female rats are more sensitive to TCDD-induced neoplasms than are males, and that this is likely due to the hormonal status of the animals (Kociba *et al*., 1978; NTP, 1982). Although the precise mechanism of the interactions between TCDD and estrogens are not fully known, TCDD decreases uterine estrogen receptor (ER) concentrations in cytosolic and nuclear fractions of rats and mice, and these changes are associated with diminished estrogen action in both *in vivo* and *in vitro* studies. TCDD has also been shown to increase estrogen metabolism (Shiverick and Muther, 1982). Fernandez and Safe (1992) have shown that TCDD is anti-mitogenic in human breast cancer cells.

In laboratory rats, high doses of TCDD have been related to decreased testosterone levels (Kleeman *et al*., 1990; Mebus *et al*., 1987; Moore and Peterson, 1988; Moore *et al*., 1985).

Cardiovascular Effects

Data on animals indicates that exposure to TCDD affects cardiac and vascular integrity (Allen *et al*., 1977; Norback and Allen, 1973), causes damage to the myocardium and heart valves in rats (Kociba *et al*., 1978), and to the arterial wall in rabbits (Brewster *et al*., 1987). A recent study by NTP (2004) observed a significantly increased incidence of cardiomyopathy in female rats administered 10 ng TCDD/kg or greater.

Neurological Effects

Elovaara *et al*. (1977) found anomalous CNS function in some rats exposed to a single dose of TCDD. Creso *et al*. (1978) reported CNS symptoms of irritability, restlessness, and increased aggression in rats administered TCDD. Hassoun *et al*. (1998) exposed $B6C3F_1$ mice to TCDD orally for 13 weeks and observed a dose-dependent increase in superoxide anions (indicated by reduction in cytochrome c), lipid production and DNA single–strand breaks in brain tissue. Adult male and female Sprague-Dawley rats exposed maternally to 100 ng/kg-day TCDD showed a deficit in learning a visual discrimination-reversal activity (Seo *et al*., 1999)**.**

NTP (2004) recently reported that female rats exposed to as low as 10 ng/kg TCDD had increased incidences of cortical atrophy and hyperplasia (treatment-related changes in the adrenal cortex). The incidences of cytoplasmic vacuolization were increased in the 22 ng/kg or greater exposed groups. Cortical cystic degeneration was seen in all groups

(including controls); the incidence was higher in treated groups, and was significantly increased in the 10 and 22 ng/kg groups.

Immunological Effects

Animal toxicological studies have demonstrated numerous immunologic effects following exposure to TCDD. Several studies of note include Vos *et al*. (1973), which entailed an assessment of cell-mediated immunity, and a study by Smialowicz *et al*. (1994) on humoral immune responses in rats and mice. Several studies have examined immune function in mice, rats and guinea pigs following exposure to TCDD or PCB during fetal development (Moore *et al*., 1973; Vos *et al.*, 1974; Thomas and Hinsdill, 1979; Luster *et al*., 1980). Perinatal exposure to TCDD results in persistent suppression of immune response in rats (Badesha *et al*., 1995; Gehrs *et al*., 1997). A number of studies provide evidence that prenatal or neonatal exposure to TCDD enhances sensitivity to immune suppression compared with adult exposures (Vos *et al.*, 1974; Faith and Moore, 1977; Luster *et al*., 1980). Exposure to TCDD has been shown to decrease host resistance to certain infectious agents: TCDD exposure increases susceptibility to challenge with bacteria (Vos *et al*., 1978), viruses (Clark *et al*., 1983), parasites (Tucker *et al*., 1986) and tumors (Luster *et al*., 1980).

In nonhuman primates, a single injection of TCDD in marmosets resulted in a delayed decrease in lymphocyte populations, CD4+ T cells and CD20+ B cells in the blood, and an increase in the percentage of CD8+ cells (Neubert *et al*., 1990). Significant effects were observed after a dose of 10 ng/kg. The NOAEL for this effect was 3 ng/kg TCDD. In a subsequent study, chronic exposure of young marmosets to low levels of TCDD (0.3 ng/kg per week for 24 weeks) produced the opposite effect in the CD4+CDw29+ subset, resulting in a significant increase in this population. A higher dose of TCDD (1.5 ng/kg per week) for 3 weeks reversed this enhancement effect and suppression of the CD4+Cdw29+ subset was observed (Neubert *et al*., 1992).

Reproductive/Developmental Effects

The potential for dioxins to cause reproductive and developmental toxicity in animals has been recognized for many years. Prenatal exposure to 2,3,7,8-TCDD has been associated with increased pre- and postnatal mortality, cleft palate and kidney abnormalities, altered sexual development, and reduced fertility in studies of maternal exposure in a number of species (U.S. EPA, 2000). Studies of male exposures have not provided evidence of paternally mediated effects on the offspring.

According to U.S. EPA (2000), the manifestations of developmental toxicity from exposure to TCDD encompass primarily three categories: death/growth/clinical signs, structural malformations (e.g. cleft palate formation and hydronephrosis), and postnatal functional alterations (e.g. effects on male and female reproductive system and object learning behavior). Added to these effects are other effects that are highly species-specific.

Gestational exposure to TCDD produces a characteristic pattern of fetotoxic responses in most laboratory mammals consisting of thymic hypoplasia, subcutaneous edema,

decreased fetal growth and prenatal mortality. These can occur at dosages that have no overt toxicity to the pregnant dam. A number of researchers have reported increased prenatal death following a single exposure to TCDD during gestation that did not cause maternal toxicity (Bjerke *et al*., 1994; Roman *et al*., 1995; Gray *et al*., 1995). Olson and McGarrigle (1990, 1992) reported that a maternal dose of 1.5 µg TCDD/kg increases the incidence of prenatal mortality in the guinea pig, while a maternal dose of 18 µg TCDD/kg increases the incidence of prenatal mortality in the hamster embryo/fetus. In mice, TCDD exposure has been shown to induce as much as a 10-fold increase in cleft palate over controls (Birnbaum *et al*., 1985). Concentrations of TCDD as low as 0.8 ng/g in the murine embryonic palate have been shown to result in cleft palate (Abbott *et al*., 1996). Males exposed to TCDD during gestation are demasculinized. Malby *et al*. (1992) reported that a single exposure of the maternal rat to as low as 0.064 µg/kg TCDD could alter normal sexual development in the male offspring. Exposure during the prenatal and lactational periods results in delay of the onset of puberty, reduction in testis weight, sperm parameters and sex accessory gland weights. Most of these effects occur in a dose-related fashion, some occurring at 0.05 µg/kg and 0.064 µg/kg, the lowest TCDD doses tested.

The U.S. EPA, in its recent document on 2,3,7,8-TCDD and related compounds, has extensively reviewed those maternal and developmental responses that are produced following gestational exposure to TCDD in various species of laboratory mammals (U.S. EPA, 2000). Gestational treatment of rats with CDD congeners that do not bind the Ah receptor do not cause TCDD-like effects on development (Khera and Ruddick, 1973).

Rhesus monkeys exposed to dioxin for four years in their feed developed a dose-related increase in both the incidence and severity of endometriosis compared with their non-exposed controls (Rier *et al*., 1993). The induction of endometriosis occurred at body burdens near background human exposure levels. Studies using rodent models have also shown the ability of TCDD to promote similar lesions in a dose-related manner (Cummings *et al*., 1996, 1999; Johnson *et al*., 1997).

Genotoxicity

There is considerable evidence that TCDD does not damage DNA directly through the formation of DNA adducts (Randerath *et al*., 1988; Turteltaub *et al*., 1990; NTP, 2004). TCDD is negative in short-term tests for genotoxicity, and is a potent promoter and weak initiator in multi-stage models for chemical carcinogenesis (Pitot *et al*., 1980; Graham *et al*., 1988; Lucier *et al*., 1991; Clark *et al*., 1991; Flodstrom and Ahlborg, 1991; NTP, 2004). It has been suggested that TCDD, though not directly genotoxic, may be indirectly genotoxic through the formation of potentially reactive oxygen species. Higher levels of oxidative DNA damage (80OH-dG adducts) have been observed in chronically exposed female rats (Tritscher *et al*., 1996).

Mutagenicity

TCDD is negative in the *Salmonella*/Ames test with or without the presence of a mixed-function oxidase activating system. These negative studies have encompassed 13 different bacterial strains with tests performed in 9 laboratories (Wassom *et al*., 1977; IARC, 1982; Giri, 1987; Shu *et al*., 1987). The NTP (1984, and again in 2004) concluded that TCDD was not mutagenic. There is no consistent evidence for increased

frequencies of chromosomal aberrations in human populations exposed accidentally or occupationally to TCDD (Shu *et al*., 1987).

Chronic Toxicity

The results of chronic toxicity studies performed on laboratory animals exposed to TCDD are summarized in Table 6. Details for many of the studies have been reviewed by the U.S. EPA (1984, 1985) and WHO/IPCS (1989). Several key studies are discussed in further detail below.

Table 6. Chronic Non-cancer Studies of TCDD in Laboratory Animals

Species, Strain	Sex and no. per group	Doses tested	Treatment Schedule	Parameters monitored	References
Rats, Harlan Sprague-Dawley	F/81-82	3, 10, 22, 46, 100 ng/kg-day	Gavage 5d/week for 2 yrs	Extensive histopathology, thyroid hormones	NTP, 2004
Rats, Sprague-Dawley	M/10	0, 1, 5, 50, 500, 1,000, 5,000, 50,000, 500,000, 1,000,000 ng/kg	Continuous in diet for 65 wks	Survival	Van Miller *et al.*, 1977
Rats, Sprague-Dawley	M, F/10	0.001, 0.01, 0.1 µg/kg-day	Continuous in diet for 2 yrs	Extensive histopathology, hematology, and clinical chemistry	Kociba *et al.*, 1978, 1979
Mice, Swiss	M/38-44	0, 0.0007, 0.7, 7.0 µg/kg-week	Gavage weekly for 1 yr	Histopathology	Toth *et al.*, 1979
Mice, $B6C3F_1$	M/50,F/50	0.01, 0.05, 0.5 µg/kg-week (males) 0.04, 0.2, 2.0 µg/kg-week (females)	Gavage biweekly for 2 yrs	Extensive histopathology	NTP, 1982
Monkey	F/8	500 ng/kg	Continuous in diet for 9 months	Extensive histopathology, hematology, and clinical chemistry	Allen *et al.*, 1977

Adapted from U.S. EPA (2000)

The National Toxicology Program (NTP) recently conducted long-term toxicology and carcinogenesis studies of TCDD in Harlan Sprague-Dawley rats (NTP, 2004). Females only (81-82 per group) were exposed via gavage 5 d/week to doses of 3, 10, 22, 46 or 100 ng TCDD/kg-week for up to 105 weeks. Up to 10 rats/group were evaluated at 14, 31 or 53 weeks. A stop-exposure group of 50 female rats was administered 100 ng TCDD/kg by gavage for 30 weeks, and then the corn oil:acetone vehicle only, for the remainder of the study. The non-cancer findings are summarized here and in Table 7 below (refer to the cancer section for the cancer findings). Survival of dosed groups was similar to the vehicle controls. Mean body weights of the 22 ng/kg rats were less than those of the vehicle controls the last 10 weeks of the study; mean body weights of the 46 ng/kg rats were lower than controls during year two of the study; mean body weights of the 100 ng/kg core study and stop-exposure groups were less than controls following week 13 of the study. Serum total and free thyroxine (T_4) concentrations were significantly decreased in the 22, 46, and 100 mg/kg dose level groups relative to vehicle controls at 31 weeks. Serum total triiodothyronine (T_3) and thyroid stimulating hormone (TSH) levels were significantly higher than controls in the 46 and 100 ng/kg-week dose groups; serum T_3 concentrations were significantly higher than controls in the 10, 22, 46 and 100 ng/kg groups. Hepatic cell proliferation, as measured with the 5-bromo-2'-deoxyuridine (BrdU) labeling index, was significantly higher in all dosed groups compared with controls. Both hepatic and pulmonary cytochrome P450 enzyme activities were significantly higher in all experimental dosed groups compared with controls. Liver weights were significantly increased at all dose levels; liver weight increases were correlated with increased incidences of hepatocyte hypertrophy. The increased incidences of hepatocyte hypertrophy were significant in all dosed groups, except the lowest group, 3 ng/kg, at fifty-three weeks; the severities of this lesion increased with increasing dose. The incidences of pigmentation of the liver were significantly increased in rats administered 10 ng TCDD/kg or greater. Toxic hepatotrophy was significantly increased in the 46 and 100 ng/kg-week exposure groups. An increased incidence of cardiomyopathy was seen at all but the lowest dose level.

In this study (NTP, 2004), TCDD administration caused increased incidences of non-neoplastic lesions of the liver, lung, oral mucosa, pancreas, thymus, adrenal cortex, heart, clitoral gland, kidney, forestomach, and thyroid gland. A dose-related increased incidence of hepatic necrosis, oval cell hyperplasia, and bile duct hyperplasia was seen in the 22, 46 and 100 ng/kg-week exposure groups. At two years, the incidence of hepatocyte hypertrophy, multinucleated hepatocytes, eosinophilic focus, inflammation, pigmentation, diffuse fatty change and toxic hepatopathy, and an increased incidence of adrenal cortical hyperplasia was observed at the top four dose levels. An increased incidence of gingival squamous hyperplasia was observed at *all* dose levels. There was also a significant increase in histiocytic infiltration at dose levels of 22, 46 and 100 ng/kg-week.

Table 7. Summary of Chronic Non-cancer Effects of TCDD in Harlan Sprague-Dawley Female Rats (gavage)

Adverse Effect	TCDD Dose Level (ng/kg-week)				
	3	10	22	46	100
Serum Total and free T_4 (lower than vehicle controls)			√	√	√
*Serum Total T_3 and TSH				√	√
*Hepatic cell proliferation	√	√	√	√	√
*Cytochrome P450 enzyme activities	√	√	√	√	√
*Liver weights	√	√	√	√	√
*Liver pigmentation		√	√	√	√
Hepatocyte hypertrophy		√	√	√	√
Toxic hepatopathy				√	√
*Relative lung weights	√	√	√	√	√
*Histiocytic infiltration			√	√	√
Non-neoplastic lesions:					
*Cardiomyopathy		√	√	√	√
*Cystic dilation of clitoral gland ducts			√		√
*Nephropathy					√
Hypertrophy of thyroid follicular cells (increased incidence)				√	√

Reference: NTP (2004)
* = denotes a significant increase over vehicle controls

Kociba *et al*. (1978, 1979) exposed male and female Sprague-Dawley rats (50/sex) to daily doses of 0.001, 0.01 and 0.1 µg TCDD/kg for 2 years in the diet. Control rats (86/sex) received diets containing the vehicle only. Survival was poor in all groups of exposed and control rats; at two years, only 8-22 percent of the males and 8-32 percent of the females were still alive. The mortality in the high dose females (0.1 µg/kg-day) was significantly greater than the controls. The mean body weights of both males and females were decreased at all dose levels, although those in the low-dose group were comparable to the controls towards the end of the study. An increase in urinary porphyrins was found in female rats at the mid- and high-dose levels. Analyses of blood serum collected at necropsy revealed increased enzyme activities related to impaired liver function in female rats given 0.1 µg/kg-day. Histological examination revealed multiple degenerative, inflammatory and necrotic changes in the liver in both males and females,

though the damage was more extensive in the females. No damage to the liver was observed at the 0.001 µg/kg-day level (1 ng/kg-day). Similar results have been described by other authors (Cantoni *et al*., 1981).

Toth *et al*. (1979) exposed male Swiss mice to weekly oral doses of 0, 0.007, 0.7 and 7.0 µg TCDD/kg for 1 year. Amyloidosis and dermatitis were seen in all dose groups. The incidence of these lesions was 0 of 38 in the control group, 5 of 44 at the 0.007 dose level, 10 of 44 at the 0.07 dose level, and 17 of 43 at the high dose level of 7.0 µg/kg. The LOAEL in this study was estimated to be 0.001 µg/kg-day.

In the National Toxicology Program (NTP, 1982) study in which male and female $B6C3F_1$ mice were exposed to TCDD biweekly via gavage for two years**,** no adverse effects were seen at the lowest dose level tested (0.01 and 0.04 µg/kg per week for males and females, respectively, corresponding to ~1.4 and 6 ng/kg-day).

Cancer

TCDD as a Carcinogen

It is unequivocal that TCDD is a carcinogen at multiple sites in both sexes of rats and mice (U.S. EPA, 1985; IARC, 1997; NTP, 2004). It has been shown to cause carcinomas of the skin in hamsters, which are considered to be the species most resistant to the acute toxic effects of TCDD (Rao *et al*., 1988). Indeed, all long-term carcinogenicity studies on TCDD have produced positive results (van Miller *et al*., 1977; Kociba *et al*., 1978; NTP, 1982a; Rao *et al*., 1988; Johnson *et al*., 1992; NTP, 2004). Animal carcinogenesis is thought to arise from Ah receptor-mediated alteration of gene expression, although other possible mechanisms, such as increased oxidative DNA damage or immune suppression, have been proposed (IARC, 1997; Tritscher *et al*, 1996). Significant animal cancer bioassays are summarized in Table 8.

Table 8. Sites for Increased Cancer in Animal Bioassays for TCDD

Species, Strain	Sex	Sites	Reference
Rats, Harlan Sprague-Dawley	Female	Liver, lung, oral mucosa, uterus, pancreas	NTP, 2004
Rats, Sprague-Dawley	Male	Tongue, nasal turbinates/hard palate	Kociba *et al*., 1978
	Female	Lung, nasal turbinates/hard palate, liver	
Rats, Osborne-Mendel	Male	Thyroid, adrenal cortex	NTP, 1982
	Female	Liver, adrenal cortex, subcutaneous fibrosarcoma	
Mice, $B6C3F_1$	Male	Liver	NTP, 1982
	Female	Liver, thyroid, subcutaneous fibrosarcoma	
Mice, B6C3 and B6C	Male	Thymic lymphomas	Della Porta *et al*., 1987
	Female	Liver	
Hamsters, Syrian Golden	Male	Facial skin carcinoma	Rao *et al*., 1988

Adapted from U.S. EPA (2000)

The National Toxicology Program (NTP) recently conducted long-term toxicology and carcinogenesis studies of TCDD in Harlan Sprague-Dawley rats (NTP, 2004). Females only (81-82 per group) were administered by gavage doses of 3, 10, 22, 46 or 100 ng TCDD/kg 5 d/week for up to 105 weeks. Up to ten rats/group were evaluated at 14, 31 or 53 weeks. A stop-exposure group of 50 female rats was administered 100 ng TCDD/kg-week by gavage for 30 weeks and then the vehicle (corn oil:acetone, 99:1) for the remainder of the study. The cancer findings are reported in this section. The non-cancer and nonneoplastic lesions are summarized in Table 7 above. TCDD administration under the conditions of this two year gavage study resulted in increased incidences of cholangiocarcinoma and hepatocellular adenoma of the liver, epithelioma of the lung, gingival squamous cell carcinoma of the oral mucosa, squamous cell carcinoma of the uterus, and pancreatic acinar neoplasms; increased incidences of hepatocholangioma and cholangioma of the liver may have been related to TCDD administration. The tumor incidence data are summarized in Table 9.

Table 9. Summary of the TCDD Tumor Incidence Data from the NTP (2004) Study in Female Rats

Tumor Site/Type	Dose (ng/kg)						
	0	**3**	**10**	**22**	**46**	**100**	**100 (stop-exposure)**
Liver/hepatocellular adenoma	0/53	0/54	0/53	0/53	1/53	13/53	2/50
Liver/cholangiocarcinoma	0/53	0/54	0/53	1/53	4/53	25/53	2/50
Liver/hepatocholangioma	0/53	0/54	0/53	0/53	0/53	2/53	0/50
*Liver/cholangioma	0/53	0/54	0/53	0/53	0/53	0/53	1/50
Lung/cystic keratinizing epithelioma	0/53	0/54	0/53	0/52	0/53	9/52	0/50
Oral mucosa/gingival squamous cell carcinoma	1/53	2/54	1/53	0/53	4/53	10/53	5/50
Uterus/squamous cell carcinoma	0/53	0/54	0/53	0/53	5/53	0/53	2/50
Pancreas/acinar adenoma or carcinoma	0/51	0/54	0/52	0/53	0/52	3/51	1/49

*data not used in the calculation of the cancer slope factor, due to sole tumor found in the recovery group

One of the most cited cancer bioassays for TCDD is that conducted by Dow Chemical (Kociba *et al*., 1978). Male and female Sprague-Dawley rats (50/sex) were exposed to 0, 1, 10 and 100 ng TCDD/kg-day for two years in their feed. The most significant finding was an increase in hepatocellular hyperplastic nodules and hepatocellular carcinomas in

female rats. The incidence of hepatocellular carcinomas was significantly elevated above the control incidence at the 100 ng/kg-day dose, whereas increased incidence of hyperplastic nodules was evident in the 10 ng/kg-day dose group. No increase in liver tumors at any of the dose groups was observed in male rats. It is important to note that survival was poor in all groups of control and exposed rats: at two years, only 8-22 percent of males, and 8-32 percent of females were alive. The mortality in the high dose females was significantly greater than controls. This early mortality reduced the sensitivity of this study for determining the actual number of neoplasms induced by two years of exposure to TCDD.

A re-evaluation of the slides of liver sections from the Kociba study (Squire, 1980), requested by U.S. EPA, showed significant increases in the incidence of hyperplastic nodules of the liver in female rats (27/50) in the high dose group. In addition to the liver nodules, an increased incidence of stratified squamous cell carcinoma (SCC) of the tongue and nasal turbinates/hard palate, and keratinizing SCC of the lung were also observed in female rats in the 100 ng/kg-day dose group. In male rats at the 100 ng/kg-day dose level there was an increased incidence of stratified SCC of the hard palate/nasal turbinate, stratified SCC of the tongue, and adenoma of the adrenal cortex. In addition, the Squire (1980) re-evaluation of the slides identified two male rats in the lowest dose group, 1 ng/kg-day, with SCC of the nasal turbinates/hard palate; one of these male rats had a SCC of the tongue. The initial study, by Kociba *et al*. (1978), reported that no chemically-related increases in preneoplastic or neoplastic lesions were found in the 1 ng/kg-day dose group. U.S. EPA concluded that these are both rare tumors in Sprague-Dawley rats and these sites are targets for TCDD. Tumor incidences for the two evaluations in both sexes of rats are provided in Tables 10 (male) and 11 (female).

Table 10. Comparison of Tumor Incidence in the Kociba *et al.* (1978) and Squire (1980) Reports (Male Rats)

Tumor Site/Type	Pathological Assessment	Dose (ng/kg-day)			
		0	**1**	**10**	**100**
Tongue (stratified SCC)	Kociba	0/76	1/49	1/49	3/42
	Squire	0/77	2/44	1/49	3/44
Nasal turbinates/hard palate (SCC)	Kociba	0/51	1/34	0/27	4/30
	Squire	0/55	1/34	0/26	6/30
Tongue, nasal turbinates or hard palate (SCC)	Kociba	0/65	1/49	1/49	7/42
	Squire	0/77	2/44	1/49	9/44

Source: Adapted from U.S. EPA (1985)
SCC = squamous cell carcinoma

Table 11. Comparison of Tumor Incidence in the Kociba *et al.* (1978) and Squire (1980) Reports (Female Rats)

Tumor Site/Type	Pathological Assessment	Dose (ng/kg-day)			
		0	**1**	**10**	**100**
Lung (keratinizing SCC)	Kociba	0/86	0/50	0/49	7/49
	Squire	0/86	0/50	0/49	8/47
Nasal turbinates/hard palate (keratinizing SCC)	Kociba	0/51	1/34	0/27	4/30
	Squire	0/55	1/34	0/26	6/30
Liver (hyperplastic nodules or carcinomas)	Kociba	9/86	3/50	18/50	34/48
	Squire	16/86	8/50	27/50	33/47

Adapted from U.S. EPA (1985)
SCC = squamous cell carcinoma

TCDD induced tumors in multiple sites in this study. Table 12 below provides a comparison of the tumor incidence data reported by Kociba *et al.* (1978) and Squire (1980), adjusted for early mortality. U.S. EPA considers the adjustment for early mortality to yield a better estimate of upper bound lifetime risk than the unadjusted risk.

Table 12. Comparison of Tumor Incidence in the Kociba *et al*. (1978) and Squire (1980) Reports, Adjusted for Early Mortality

Dose (ng/kg-day)	*No. animals with tumors/No. examined	
	Squire	**Kociba**
0	16/85	9/85
1	8/48	3/48
10	27/48	18/48
100	34/40	34/40

*The number of tumors refers to the number of animals with at least one liver, lung, hard palate and/or nasal turbinate tumor. Adjustment for early mortality refers to eliminating from the analysis those animals that died during the first year of study.

The National Toxicology Program (NTP, 1982) conducted a two-year gavage study in Osborne-Mendel rats (50/sex) and B6C3F_1 mice (50/sex). TCDD was administered by gavage twice weekly as a suspension in corn oil:acetone to achieve doses of 0, 10, 50 or 500 ng TCDD/kg-week; groups of female mice were treated similarly to achieve doses of 0, 40, 200 or 2,000 ng/kg-week. These exposures correspond to daily averaged doses of 1.4, 7.1, or 71 ng/kg-day for rats and male mice, and to doses of 5.7, 28.6, or 286 ng/kg-day for female mice. TCDD induced tumors at multiple sites, and statistically significant

increases in neoplasia were observed at every dose level administered to either rats or mice. Malignant liver tumors incidences were increased in both sexes of mice and in high-dose female rats (286 ng/kg-day). The incidences of thyroid gland (follicular cell) tumors were significantly increased in all three dose groups in male rats. TCDD-induced neoplasms of the adrenal gland were observed in the 7.1 ng/kg-day dose group in male rats, and in the high-dose female rats. Fibrosarcomas of the subcutaneous tissue were significantly elevated in high-dose female mice and female rats. In addition, one additional tumor type, lymphoma, was seen in high–dose female mice. A dose-related increase in lung tumors (Cochran-Armitage trend test, p=0.004), though not significant, was observed in high-dose female mice. There were no statistically significant dose-related decreases in survival in any sex-species group.

Rao *et al*. (1988) administered groups of 10 to 24 male Syrian golden hamsters two or six i.p or s.c. injections once every four weeks containing either dioxane (control) or TCDD in dioxane at 50 or 100 µg/kg over a period of 12-13 months. By both routes of exposure, the 100 µg groups (total exposure equaled 600 µg/kg) developed SCC of the skin in the facial region, 4/18 in the i.p. groups and 3/14 in the s.c. group. These lesions were large, showed extensive necrosis, and had metastasized to the lungs. Neoplasms were first observed 8 months after the first exposure. No neoplasms were seen in hamsters that received two i.p injections of 100 µg/kg TCDD, six s.c. injections of 50 µg TCDD, or in controls.

TCDD exposure early in life by the i.p. route resulted in thymic lymphomas in two strains of mice. Della Porta *et al.* (1987) administered TCDD in corn oil i.p at 0, 1, 20, and 60 µg/kg to groups of 89-186 B6C3 and B6C infant mice once weekly for five weeks, starting on the tenth day of life. Mice were observed for 78 weeks. Histopathological examination was limited to the liver, kidney and "organs with apparent or suspected pathological changes." Thymic lymphomas were induced at the high dose level in both sexes of both hybrids, and at the 30 µg/kg dose level in both sexes of B6C mice and in male B6C3 mice. Neoplasms of the liver occurred in male B6C3 mice at 30 µg/kg, and in female B6C3 mice at 60 µg/kg. In a separate study, groups of 42-50 B6C3 mice were exposed to TCDD 0, 2.5 and 5.0 µg/kg in corn oil by gavage once weekly for 52 weeks starting at 6 weeks of age. The study duration was 110 weeks. Increased incidences of liver neoplasms were reported in both sexes of mice at both exposure concentrations.

TCDD as a Co-carcinogen or a Promoter

TCDD has been shown to be a potent tumor promoter in mouse skin, as well as rat liver (Maronpot *et al.*, 1993; Teegarden *et al.*, 1999). Lucier *et al*. (1991) reported a tenfold increase in tumor promotion capacity of TCDD in female rats receiving 100 ng TCDD/kg for 30 weeks, whereas liver lesions, characterized by increases in altered hepatocellular foci, are significantly reduced in the livers of ovariectomized rats. The observations by Lucier *et al*. (1991) of the ovarian hormone-dependent increase in hepatocyte replication parallel the observed sex-dependent induction of liver tumors in rats. Clark *et al.* (1991), using ovariectomized rats, demonstrated an increase in lung tumors in initiated

(diethylnitrosamine), TCDD-treated rats. No tumors were seen in diethylnitrosamine (DEN) only, TCDD only, control or DEN/TCDD intact rats.

In addition, the effect of TCDD on the endocrine system may potentially play a role in susceptibility to carcinogenesis induced by other compounds. A study by Brown *et al.* (1998) showed that prenatal exposure of female rats to TCDD resulted in an increased susceptibility to DMBA-induced mammary adenocarcinomas. This was believed to be due to an increase in mammary gland terminal end buds as a result of prenatal exposure.

Toxicological Effects in Humans: Oral Exposure

Exposure to TCDD by the oral route may occur through drinking water, recreational water, or consumption of foods and beverages contaminated with dioxins. Direct exposure to TCDDs may also occur through inhalation of cigarette smoke (Lofroth and Zebuhr, 1992; Ono *et al*., 1987; Takizawa and Muto, 1987), and infants may be exposed to TCDDs through ingestion of contaminated milk (Noren, 1993).

Acute, Subacute, and Chronic Noncancer Effects

Human exposure to TCDD has been associated with many noncancer effects, including dermatological lesions, gastrointestinal effects, cardiovascular effects, neurologic effects, immune system effects, endocrine effects, and other metabolic disturbances.

Chloracne, a persistent acneform condition characterized by comedones, keratin cysts, and inflamed papules with hyperpigmentation, is a common consequence of acute or chronic exposure to TCDD-contaminated chemicals. Too little human data exist to determine the threshold level of TCDD at which chloracne occurs. In Medina, Italy in 1976 an explosion of a trichlorophenol reactor in a 2,4,5-T production facility caused the contamination by TCDD of the neighboring city of Seveso, Italy. Chloracne was observed in chemical workers between 2 weeks and 2 months after the reactor release, and among Seveso schoolchildren after 6 months (Reggiani, 1980). In chemical workers involved in the TCP reactor release at BASF in Ludwigshafen, Germany, most cases of chloracne developed within 2 days after first exposure (Ott *et al*., 1994).

Case reports and epidemiologic studies show that exposure to TCDD-contaminated materials is associated with neurologic abnormalities. Symptoms include fatigue, nervousness, anxiety and decreased libido (Ashe and Suskind, 1950; Bauer *et al*., 1961; Goldman, 1972; Jirasek *et al*., 1974; Oliver, 1975).

TCDD has been implicated as a possible cause of heart disease. Elevated rate ratios for mortality from ischemic heart disease (1.8, 95 percent CI, 0.9 to 3.6) were found in a large multicounty cohort study (Hooiveld *et al*., 1998), and elevated cardiovascular disease has been noted in several of the occupational cohorts (Steenland *et al*., 1999; Sweeney *et al*., 1997), in Seveso (Pesatori *et al*., 1998), and in the Yusho rice oil poisoning incident. In addition**,** data on animals indicates that high doses of 2,3,7,8-TCDD affect cardiac and vascular integrity (Allen *et al*., 1977; Norback and Allen, 1973), cause damage to the myocardium and heart valves in rats (Kociba *et al*., 1978) and to the arterial wall in rabbits (Brewster *et al*., 1987).

Several case reports of hepatomegaly and hepatic enzyme changes have been reported among exposed human populations (Ashe and Suskind, 1950; Suskind *et al*., 1953; Jirasek *et al*., 1974). (Changes in liver function and structure, and increased liver size have consistently been reported in animal studies). In Seveso, Italy, 5 of 22 residents with severe chloracne had temporary liver enlargement (Reggiani, 1980). The hepatomegaly lasted "several" months without concomitant increases in liver enzymes. Epidemiologic studies and case reports have observed elevations in hepatic enzyme levels among exposed TCP production workers (Roegner *et al*., 1991) and among Seveso residents (Mocarelli *et al*., 1986).

The hepatic enzyme gamma glutamyl transferase (GGT) has been found in a number of studies to be chronically elevated in adults exposed to high levels of TCDD (animal data on TCDD-related effects on GGT are sparse, whereas statistically significant changes in hepatic enzyme levels of AST, ALT and ALK have been observed following exposure to TCDD in rats and hamsters). In humans, increased levels of GGT may suggest activity such as cholestasis, liver regeneration, or drug or xenobiotic metabolism.

Epidemiologic studies provide some evidence that exposure to TCDD causes alterations in glucose metabolism. In one case report of 55 TCP workers, evaluated 10 years after cessation of exposure, approximately 50 percent of the study subjects had either confirmed cases of diabetes or abnormal glucose tolerance tests (Pazderova-Vejlupkova *et al*., 1981). Results from the Ranch Hand study (Henriksen *et al*., 1997), in which participants had exposure to Agent Orange, suggest that serum TCDD levels may be positively associated with diabetes. Two studies of nursing infants suggest that ingestion of breast milk with a higher dioxin TEQ may alter thyroid function (Pluim *et al*., 1993; Koopman-Esseboom *et al*., 1994).

Exposure to TCDD has been shown in animals to decrease testosterone levels. Several studies of human subjects offer evidence of alterations in male reproductive hormone levels in association with occupational exposure to TCDD. In two separate studies of West Virginia TCP workers, exposed subjects reported reduced libido approximately 50 percent more frequently than the unexposed controls (Moses *et al*., 1984; Suskind and Hertzberg, 1984). A NIOSH study of TCP production workers (Egeland *et al*., 1994) found that the prevalence of abnormally low testosterone was two to four times higher in exposed workers with serum TCDD levels above 20 pg/g (range: 20 to > 244 pg/g) than in unexposed referents (mean serum TCDD = 7 pg/g). A study of Vietnam veterans found that subjects with current serum dioxin levels exceeding 33 pg/g have a lower mean serum testosterone level (515 ng/dL) compared with the nonexposed comparison group (525 ng/dL), though the differences were not statistically significant.

A number of studies have reported a correlation between women's body burdens of TCDD and endometriosis, an endocrine disorder. Several investigators have reported that Belgium women, who have the highest levels of dioxins in their background populations, have a higher incidence of endometriosis than other populations (Koninckx *et al.*, 1994; Pauwels *et al.*, 2001). Mayani *et al*. (1997) demonstrated a correlation between women with surgically confirmed endometriosis and TCDD levels, in Israel.

Cancer

Epidemiological Data

There is a tremendous volume of epidemiologic literature on dioxins and cancer. Most of the epidemiological information concerning TCDD toxicity results from occupational studies, in which workers were exposed primarily via the dermal or inhalation route. A major weakness in nearly all of these studies is the lack of good exposure information that does not provide for a quantitative estimate of exposure. And in all cases, the workers were exposed concurrently to other chemicals that were contaminated with TCDD. The vast majority of the cancer epidemiological data in humans (all of the case-control studies and the majority of the cohort study analyses) comprise uniquely male subjects. Animal and mechanism studies suggest that males and females may respond differently to TCDD exposure. The only reported female cohort with good TCDD exposure surrogate information is that of Manz *et al*. (1991), which found a narrowly statistically significant increase in breast cancer.

Of the cohort mortality studies that have been published since EPA's last review in 1988, three studies, Fingerhut *et al*. (1991b), Hooiveld *et al*. (1996, 1998), and Steenland *et al*. (1999), are considered by U.S. EPA to be the most important new TCDD cancer epidemiology studies. This is due to their attention to cohort selection, to TCDD exposures or exposure surrogates (chloracne), and to the fact that exposure to dioxin is associated with an increasing risk of cancer at multiple sites.

Fingerhut *et al*. (199b) conducted a retrospective cohort study of mortality among the largest and most highly exposed of four industrial cohorts considered by IARC in their classification of TCDD as a human carcinogen. The cohort is comprised of 5,172 U.S. chemical workers from 12 plants that produced chemicals contaminated with TCDD. Occupational exposure was documented by reviewing job descriptions and by measuring TCDD in serum from a sample of 253 workers. Causes of death were taken from death certificates. Mortality from all cancers combined was slightly but significantly elevated in the overall cohort (SMR, 115; 95 percent confidence interval (CI), 102 to 130). The cohort had a nonsignificant increase in mortality from cancers of the trachea, bronchus and lung (SMR 111; 95 percent CI, 89 to 137). In a subcohort of 1,520 workers with one year or more of exposure and at least 20 years of latency, mortality was significantly increased for soft tissue sarcoma (3 deaths; SMR, 922; 95 percent CI, 190 to 2,695) and for cancers of the respiratory tract (SMR, 142; 95 percent CI, 103 to 192). The mean serum TCDD level in the sample of 253 workers from two plants was 233 pg/g of lipid (range, 2 to 3,400). A mean level of 7 pg/g lipid was found in a comparison group of 79 unexposed persons, all of whose levels were under 20, a range found in other unexposed populations (Patterson *et al*., 1989). All of the workers had received their last occupational exposures 15 to 37 years earlier.

Steenland *et al*. (1999) did an extended follow-up of the same industrial cohort that Fingerhut *et al*. (1991b) had evaluated previously. For this study, Steenland *et al*. (1999) rereviewed all of the data and restricted the original cohort of 5,172 male workers to a subcohort of 3,538 workers, eliminating those that lacked adequate data to characterize duration of exposure, who had never worked in TCDD-exposed departments, or who had

concomitant exposure to pentachlorophenol (which is contaminated with the higher chlorinated dioxins, which are considered less toxic than TCDD). They also analyzed another subcohort of 608 workers taken from all 12 plants who had chloracne and no exposure to pentachlorophenol. These workers were likely to have had higher TCDD exposures. The SMR for all cancers combined was 1.13 (95 percent CI, 1.02 to 1.25). The SMR for all cancers combined for the highest exposure group was 1.6 (95 percent CI, 1.15 to 1.82). The excess of all cancers in the subjects with highest exposure was not specific for any type of cancer. SMRs for heart disease showed a weak increasing trend with higher exposure (p=0.14). Diabetes showed a negative response trend. Cox regression, using an internal comparison group with low exposure, found a statistically significant positive trend between all cancers (after a 15-year lag time) and cumulative exposure.

Hooiveld *et al*. (1998) conducted a retrospective cohort mortality study of 1,167 workers exposed to phenoxy herbicides, chlorophenols, and contaminants (TCDD and other polychlorinated dioxins and furans) at a chemical factory in the Netherlands. Classification of exposure was based on individual job histories and additional information from company questionnaires. Serum levels of PCDDs, PCDFs and polychlorinated biphenyls were measured in a sample of surviving cohort members (n = 47). Serum concentrations ranged from a geometric mean of 40.8 ppt in exposed workers in nonproduction departments, to a geometric mean of 2,148 ppt in workers exposed as a result of a TCDD explosion reaction and who worked in main production. Among nonexposed workers, all but one had serum TCDD levels below 20 ppt. Male workers exposed to phenoxy herbicides or chlorophenols showed increased relative risks for total mortality (RR = 1.8, 95 percent CI, 1.2 to 2.5), cancer mortality (RR = 4.1, 95 percent CI 1.8 to 9.0), respiratory cancer (RR = 7.5, 95 percent CI, 1.0 to 56.1), non-Hodgkin's lymphoma (RR = 1.7, 95 percent CI, 0.2 to 16.5), and ischemic heart diseases (RR = 1.8, 95 percent CI, 0.9 to 3.6), compared with an internal referent group of nonexposed workers. An elevated risk for bladder and kidney cancer (SMR = 3.9, 95 percent CI, 1.7 to 7.6) was found, but the relative risk compared with nonexposed workers was unstable because there were no cases in the referent group. Workers exposed as a result of the accident in 1963 showed a statistically significant increased risk for prostate cancer.

In Chapaevsk, Russia, dioxins have been detected in the town's drinking water (28.4-74.1 pg/L), in cow's milk (the content of 2,3,7,8-TCDD was 17.32 pg TEQ/g fat), in air (0.116 pg/m^3) and in soil (8.9-298 ng/kg). From 1967-1987, the Middle Volga chemical plant in Chapaevsk produced lindane and its derivatives. Currently it produces crop protection chemicals (liquid chlorine acids, methyl chloroform, vinyl chloride and other chemicals). (Dioxins and similar compounds can be formed in the production of methyl chloroform, vinyl chloride, dichloropropionic acid, hexachloroethane, sodium pentachlorphenolate and polychloroform).

Elevated levels of dioxins have been found in human milk and blood samples taken from residents of Chapaevsk, Russia. The mean content of dioxins in seven pooled samples of human milk (40 individual trials) was 42.26 pg TEQ/g fat, in four female worker's blood samples, 412.4 pg TEQ/g fat, in six resident's blood samples (those who lived 1-3 km from the chemical plant), 75.2 pg TEQ/g fat, and in four resident's blood samples (5-8 km from the plant), 24.5 pg TEQ/g fat. The incidence and mortality analysis in

Chapaevsk showed an increased occurrence of cancer at all sites including lung, gastrointestinal, urinary organs, female breast cancer, cervix, leukemia, and lymphoma. The mean frequency of spontaneous abortions in the last seven years was higher (24.4 percent in Chapaevsk) than in other towns of the region. The average rate of premature labor was 45.7 per 1,000 women, significantly higher than most other towns of the area. The frequency of newborns with low birth weight was 7.4 percent. The average number of congenital morphogenetic conditions per child was significantly higher, 4.5 for boys and 4.4 for girls.

DOSE-RESPONSE ASSESSMENT

Dose Metric

The U.S. EPA, in its recent reassessment document on TCDD and related compounds (2000), has selected body burden as a more appropriate dose metric than daily intake for dioxin in species extrapolation. According to U.S. EPA, body burden (estimated at steady state conditions), provides for a reasonable description of dose because tissue concentrations of TCDD are directly related to the concentration of TCDD in the body. The half-life of TCDD is approximately 100-fold greater in humans (2,593 days) than in rats (25 days). Body burden takes into account differences in half-life between various species, as well as the uncertainty in the window of sensitivity for various endpoints (e.g. enzyme induction, cancer, developmental toxicities). The uncertainty of the steady state approach is that it does not account for variations in exposure (dose) over time. It provides for an average dose that could account for a given body burden (over time).

Noncarcinogenic Effects

Dose response data are very sparse for human noncancer endpoints. In animal studies examining the effects of TCDD following multiple exposures, the range of 1 percent effective dose (ED_{01}) values is highly variable within and across response categories (although effective dose evaluation at the ten percent response level is usually the norm, the observed range for many key events for TCDD extends down to or near the 1 percent response level). In studies in rats and mice following a single exposure, the median ED_{01} is above 10 ng/kg for all endpoints examined (U.S. EPA, 2000). U.S. EPA has chosen not to identify any particular non-cancer endpoint as the "critical effect." The lowest ED_{01} values tend to be for biochemical effects, followed by hepatic responses, immune responses, and responses in tissue weight. Results from the analysis of ED_{01}s and from examining LOAELs suggest that non-cancer effects occur at body burden levels in animals equal to or less than body burdens calculated for tumor induction in animals.

A chronic NOAEL of 1 ng/kg-day for hepatotoxicity is estimated for Sprague-Dawley rats from a two-year study (Kociba *et al*., 1978). In addition to liver toxicity, chronic exposure to TCDD has been associated with amyloidosis and dermatitis in Swiss mice (Toth *et al*., 1979). A LOAEL of 1 ng/kg-day for both these endpoints has been estimated for mice. Chronic exposure to 1.5 ng/kg-day in the diet results in hair loss, edema and pancytopenia in Rhesus monkeys (Allen *et al*., 1977). In a recent 2-year chronic NTP (2004) study, the lowest administered dose of 3 ng/kg-day via gavage in

female rats resulted in significant increased incidences of cell proliferation, gingival squamous hyperplasia, cytochrome P450 induction, as well as significant increases in lung and liver weights. No NOAEL was observed in this study. Based on data from several studies (Kitchin and Woods, 1979; Abraham *et al*., 1988; Kruger *et al*., 1990; Neubert, 1991), a NOAEL of 1 ng/kg-day can be calculated for enzyme induction for rats and marmoset monkeys. Table 13 shows the *lowest doses* demonstrated to cause biological responses following chronic exposure in animal studies.

Table 13. Lowest Effect Levels for Biological Responses to TCDD in Animals

Species	Dose or concentration and duration	Effect	Reference
Guinea pigs	0.6 µg/kg, single oral dose	Lethality (single dose LD_{50})	Schwetz *et al*., 1973
Rhesus monkey	1.0 µg/kg, single oral dose	Acute toxicity	McNulty, 1977
Sprague-Dawley rat	2.0 ng/kg, single oral dose	Induction of AHH	Kitchin and Woods, 1979
Marmoset monkey	3.0 ng/kg, single oral dose	Induction of N-demethylation (CYP1A2)	Kruger *et al*., 1990
Guinea pig	1 ng/kg-day for 8 wks	Immunosuppression	Zinkl *et al*., 1973
Swiss mouse	1 ng/kg-day for 1 yr	Amyloidosis and dermatitis	Toth *et al*., 1979
Rhesus monkey	500 ppt in diet for 9 mo. (12 ng/kg-day); 2 ppb in diet for 61 days (50 ng/kg-day)	Chronic lethality	Allen *et al*., 1977; McNulty, 1977
Rhesus monkey	50 ppt in diet for 20 mo. (1.5 ng/kg-day)	Chronic toxicity (hair loss)	Schantz *et al*., 1979
Sprague-Dawley rat	10 ng/kg-day for 2 yrs. in feed	Porphyrin metabolism	Kociba *et al*., 1978
Harlan Sprague-Dawley rat (female)	3 ng/kg-day, 5 d/week for 104 weeks (gavage)	Gingival hyperplasia, hepatocyte replication, alteration in cytochrome P450 enzymes, thyroid hormone, and increased liver and lung weights	NTP, 2004

Adapted from U.S EPA (2000).

Carcinogenic Effects

Because 2,3,7,8-TCDD is almost always found in association with other materials (e.g. chlorophenols, combustion products, etc.), several of which are themselves carcinogens, it may never be possible to evaluate the carcinogenicity of 2,3,7,8-TCDD by itself in humans. Estimates derived from human data (U.S. EPA, 2000) suggest an effective dose (ED_{01}) based on body burden in the range of 6-80 ng/kg for all cancers combined, and in the range of 36-250 ng/kg for lung cancer. Restricting the analysis to linear models results in cancer ED_{01} values ranging from 6 ng/kg to 161 ng/kg (U.S. EPA, 2000). Comparisons of human and animal ED_{01}s for cancer response on a body burden basis show approximately equal potential for the carcinogenic effects of TCDD. Dose-response data for cancer in animals following exposure to TCDD are limited to only three experimental dose groups. Estimates from the animal studies, which ranged from 14 ng/kg to 1,190 ng/kg (most estimates were in the range of 14-500 ng/kg), and 2.7 ng/kg for the single mechanism-based model, are therefore similar to those in humans (U.S. EPA, 2000).

As has been shown in laboratory studies, sex hormones exert a profound influence on the carcinogenic action of TCDD. Males and females may respond differently to the carcinogenic effects of dioxin, especially to hormonally-mediated tumors. Several studies have demonstrated that female rats are more susceptible to TCDD-induced liver neoplasms than males (Lucier *et al*., 1991; Kociba *et al*., 1978; NTP, 1982). In addition, studies by Brown *et al*. (1998) demonstrate that prenatal exposure of rats to TCDD enhances their sensitivity as adults to chemical carcinogenesis. As most TCDD exposure data resulting from human exposures comprise primarily adult males, more information on TCDD exposures in females and perinatal exposures are needed.

While the cancer findings in the epidemiologic literature are generally consistent with results from experimental animal studies in which dioxin has clearly been identified as a multisite carcinogen and tumor promoter, the epidemiologic data are not sufficient by themselves to infer a causal association between TCDD and increased cancer in humans (IARC, 1997; ATSDR, 1999). In the human studies, dosages must be extrapolated, as serum samples were often taken decades after the last known exposures. U.S. EPA has back-calculated body tissue burden levels using an assumed human elimination half–life for TCDD of approximately 7 years, which differs by 100-fold from the half-life of TCDD in rats (25 days). Another limitation with using human data to derive a potency estimate for dioxin is that none of the cohorts were exposed uniquely to TCDD.

In its recent reassessment document on TCDD and related compounds (U.S. EPA, 2000), U.S. EPA derived cancer slope factors for dioxin based on both human and animal data, using body burden as a dose metric. U.S. EPA's current upper bound slope factor estimate for estimating human cancer risk based on *human* data using average body burden as a dose metric is $1x10^{-3}$ risk/pg TEQ/kg-day. This cancer slope factor is based on a statistical estimate of risks from occupational exposures, principally to healthy, adult, male workers. This slope factor was derived using a meta-analysis of several human epidemiologic data sets, as the individual studies had particular strengths and weaknesses. The ED_{01} for all cancers combined from a meta-analysis of the three major occupational cohorts is 47 ng TCDD/kg, with a lower confidence limit of 30 ng

TCDD/kg. U.S. EPA uses 30 ng/kg as the point of departure for its slope calculation. In U.S. EPA's analysis, all excess cancers were attributed to TCDD exposure, despite significant levels of other dioxin-like compounds in blood measurements of some of the cohorts (e.g., the Hamburg cohort). Several additional assumptions inherent in this calculation are that cancer from TCDD is a function of "average" TCDD levels in the body, and that twenty-five percent of human body weight is comprised of lipid (fat).

Upper bound slope factors for human cancer risk calculated from lower bounds in ED_{01}s (LED_{01}s) for the animal cancers presented in Table 14 range from $3x10^{-3}$ to $1x10^{-4}$. This spans a range from 0.5 to 19 times the previous U.S. EPA (1985) upper bound estimate on cancer slope. The previous U.S. EPA slope factor, based on a re-read of the Kociba *et al.* (1978) data, which utilizes the standard default dose metric methodology of daily intake, is $1.6x10^{-4}$ per pg TCDD/kg-day.

Table 14. Doses Yielding 1 Percent Excess Risk (95 Percent Lower Confidence Bound) Based On 2-Year Animal Studies and Simple Multistage Models

Tumor/Study	Sex and Species	Shape	Intake for 1% excess risk (ng/kg-day)	Steady-state body burden at ED_{01} (ng/kg)
Liver cancer (Kociba)	Female Rats	Linear	0.77 (0.57)	14 (10)
SCC of tongue (Kociba)	Male Rats	Linear	14.1 (5.9)	254 (106)
SCC nasal turbinates / hard palate (Kociba)	Male Rats	Cubic	41.4 (1.2)	746 (22)
SCC of lung (Kociba)	Female Rats	Cubic	40.4 (2.7)	730 (48)
SCC nasal turbinates / hard palate (Kociba)	Female Rats	Linear	5.0 (2.0)	90 (36)
TFCA (NTP)	Male Rats	Linear	4.0 (2.1)	144 (76)
TFCA (NTP)	Female Rats	Cubic	33.0 (3.1)	1,190 (112)
Liver adenomas and carcinomas (NTP)	Female Rats	Quadratic	13.0 (1.7)	469 (61)
Liver adenomas and carcinomas (NTP)	Male Mice	Linear	1.3 (0.86)	20.6 (13.6)
Liver adenomas and carcinoma (NTP)	Female Mice	Linear	15.1 (7.8)	239 (124)
TFCA and carcinomas (NTP)	Female Mice	Linear	30.1 (14.0)	478 (222)
STS (NTP)	Female Mice	Lin-Cubic	43.2 (14.1)	686 (224)
Leukemias and lymphomas (NTP)	Female Mice	Linear	10.0 (5.4)	159 (86)

Adapted from U.S. EPA (2000). SCC = squamous cell carcinoma; TFCA = thyroid follicular cell adenoma; STS = subcutaneous tissue sarcomas

U.S. EPA's current slope factor for human cancer risk based on *animal* data, calculated from a revised estimate of the cancer slope from the Kociba *et al.* (1978) data, is $1.4x10^{-3}$. This number reflects an increase in slope factor based on the use of body burden dose metric and the use of the Goodman and Sauer (1992) study, which constitutes a second re-evaluation of the original Kociba study. This review confirmed only approximately one third of the tumors of the previous review. (Subsequent to the Kociba study, the nomenclature for hepatocellular proliferative lesions changed. Some of the hyperplastic nodules originally seen in the Kociba *et al*. (1978) study were reclassified as non-neoplastic. Thus, the incidence of hepatocellular adenoma (47 percent) at the highest dose of 100 ng/kg TCDD originally reported in the Kociba *et al.* (1978) study was reduced to 31 percent in the Goodman and Sauer re-evaluation.)

CALCULATION OF PHG

Noncarcinogenic Effects

Based on the study in mice reported by Toth *et al*. (1979), a LOAEL of 1 ng/kg-day was selected for calculation of a public health-protective concentration for noncarcinogenic effects of TCDD in drinking water. Calculation of a health-protective concentration (C, in µg/L) for noncarcinogenic endpoints follows the general equation:

$$C = \frac{LOAEL \times BW \times RSC}{UF \times L_{eq}/day}$$

where,

LOAEL = lowest-observed-adverse-effect-level (amyloidosis and dermatitis);

BW = adult body weight, a default of 70 kg for adults;

RSC = relative source contribution (generally values in the range of 20 percent to 80 percent, with a default of 20 percent [0.2] for chemicals with significant sources other than water);

UF = combined uncertainty factor (typical defaults are 10 for estimation of a NOAEL from a LOAEL, 10 to account for the uncertainty in inter-species extrapolation, and 10 for human variability); and

L_{eq}/day = adult daily water consumption rate (a default rate of 2 L/day, plus additional equivalent amounts where applicable to account for inhalation and dermal exposures from use of contaminated tap water.

It was assumed for the calculation that other sources of TCDD would be significant, so a 20 percent (0.2) default relative source contribution of TCDD from drinking water was chosen. Uncertainty factors of 10 each would be applicable to account for extrapolation of a LOAEL to a NOAEL, interspecies extrapolation, and human variability, for a total uncertainty factor of 1,000. Because of the endpoint under evaluation (amyloidosis and dermatitis), body burden was not used as the dose metric for species extrapolation in

calculating the non-cancer value, as the relationship between tissue concentrations and body burden in short-term animal studies may not be the same as under steady-state conditions.

$$C = \frac{1 \text{ ng/kg-d} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ L/d}} = 0.007 \text{ ng/L} \quad (0.007 \text{ ppt or } 7 \text{ pg/L})$$

Thus the public health protective concentration for TCDD in drinking water based on noncarcinogenic effects is proposed to be 0.007 ng/L (0.007 ppt).

Carcinogenic Effects

OEHHA agrees with U.S. EPA's use of body burden as the dose metric for carcinogenic effects because of the considerable difference in half-life of TCDD in humans vs. rats, 2,593 days vs. 25 days, respectively. The proposed PHG was derived using an animal study, as opposed to human epidemiological data, because the epidemiological data are still not definitively quantitative. In addition, U.S. EPA's cancer slope factor (CSF) of $1x10^{-3}$ risk/pg TEQ/kg-day is based on a statistical estimate of risks from healthy, adult male workers. Animal studies suggest that females and children may be more or especially susceptible to the toxic effects of TCDD. Also, segments of the population that consume many times the average level of fat per day, the principal exposure pathway for dioxins in the general population, may in actuality be at higher risk.

The proposed PHG for TCDD is based on a recent chronic NTP (2004) gavage study in female Harlan Sprague-Dawley rats. The study design, species, and dose range of 1 to 100 ng/kg per day selected for this study was based on the earlier dosed-feed studies conducted by the Dow Chemical Company (Kociba *et al.*, 1978). Female Sprague-Dawley rats were chosen because of the high incidence of hepatocarcinogenicity in females in this species and strain. Male rats were not studied because of the lack of neoplastic response in previous studies of Sprague-Dawley rats with TCDD. TCDD induced tumors at seven anatomic sites in the NTP (2004) study: liver (hepatocellular adenoma, cholangiocarcinoma, hepatocholangioma), lung, oral mucosa, uterus and pancreas. Table 9 shows the tumor incidence data that, with the exception of the liver cholangioma data (due to the single tumor found in the recovery group), were used to calculate the cancer slope factor.

Species Extrapolation

Using the body burden approach initially outlined by U.S. EPA (2000), human equivalent doses were calculated (estimated) from the rat tissue (adipose) levels reported in the NTP (2004) study. The body burden approach takes into account the approximately 100-fold difference in half-life of TCDD in humans vs. rats. The elimination rate constant (i.e., half-life) for TCDD in humans was assumed to be 7.1 years, and the fat volume was assumed to be 17.5 kg (i.e., 70 kg body weight x 0.25 fat) (U.S. EPA, 2000). The equation for calculating human equivalent doses is shown below:

$D = [(\ln 2/t_{½}) \times (V \times CF_1) \times (C \times CF_2)]/A$

where:

D = daily intake (pg/day);

T ½ = half-life of TCDD (years);

V = amount of body fat (kg);

C = concentration of TCDD in tissue (pg/g);

CF_1 = conversion factor (1,000 g/kg);

CF_2 = conversion factor (year/365 days);

A = fraction of dose that is absorbed.

The rat adipose tissue concentrations for the corresponding TCDD dose levels reported in the NTP (2004) study, and their equivalent calculated human intake estimates, are shown below in Table 15. The trapezoid rule was used to estimate the overall average, assuming a linear increase between timepoints. Based on available data (U.S.EPA, 2000), the assumption is made that TCDD adipose tissue concentrations in rats would produce the same risk in humans at equivalent doses.

Table 15. TCDD Human Equivalent Doses Calculated from Rat Adipose Tissue Levels Reported in NTP (2004)

Administered Dose in Rats (ng TCDD/kg BW)	*Rat Adipose Tissue Concentrations (pg/g tissue)	Human Equivalent Doses (mg/kg-day)
0	0	0
3	345.6	4.69×10^{-8}
10	656.5	8.9×10^{-8}
22	1275.3	1.73×10^{-7}
46	2337.4	3.17×10^{-7}
100	5244.9	7.11×10^{-7}

*Concentrations were averaged over the duration using an area under the curve (AUC) approximation

Multi-Site Analysis

For chemicals such as TCDD that significantly increase tumor incidence at multiple sites within a given sex, species and study, a methodological approach using Monte Carlo

analysis has been used to combine potency estimates across sites. For each tumor site, we generated a distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model with the MSTAGE 2.01 computer program (created by Edmund Crouch), modified to tabulate percentile values. A combined probability distribution was created by adding q_1 for each tumor site, according to its distribution, through one million Monte Carlo trial simulations (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound of the combined distribution was taken as the basis of the cancer potency estimate for the combined tumor sites. For TCDD, the combined potency for the seven tumor sites identified in the NTP (2004) study is 2.6×10^{-2} $(ng/kg\text{-}day)^{-1}$.

To derive the proposed PHG, the public health-protective concentration (C) associated with a one in one million cancer risk level for TCDD is then calculated as follows:

$$C = \frac{R \times BW}{CSF \times L/day} = ng/L$$

where:

R = *de minimis* lifetime extra risk of one in a million, or 1×10^{-6};

BW = adult body weight (default of 70 kg);

CSF = cancer slope factor; derived from the upper 95 percent confidence limit on the **one percent** tumor dose LED_{01}, where $CSF = 0.01/LED_{\mathbf{01}}$ $(ng/kg\text{-}day)^{-1}$;

L/day = drinking water consumption rate in liters per day (2 L/day default).

Therefore,

$$C = \frac{10^{-6} \times 70\ kg}{2.6 \times 10^{-2} \times 2\ L/day} = 0.001346\ ng/L = 0.001\ ng/L\ (rounded)$$

Based on the 95 percent upper bound of the lifetime individual excess risk of cancer of one in a million (10^{-6}), the public health goal for TCDD in drinking water is therefore proposed to be 0.001 ng/L (1 pg/L). Risks of 10^{-5} and 10^{-4} are associated with lifetime exposure to concentrations of 10 pg/L and 100 pg/L, respectively.

The proposed PHG is approximately an order of magnitude lower than the current U.S. EPA Maximum Contaminant Level (MCL) of 0.03 ng/L (or 30 pg/L). However, the current MCL does not reflect U.S. EPA's revised approach to utilizing body burden as the dose metric for TCDD risk assessments, nor the results of the newest and arguably the best animal cancer study (NTP, 2004). In 1984, U.S. EPA promulgated a much lower guideline of 0.013 pg/L TCDD for ambient surface water (U.S. EPA, 1984). In the most recent ambient water quality criteria document, U.S. EPA has established a human health protective level of 0.005 pg/L, based on consumption of water plus organisms (U.S. EPA,

2002). In its current dioxin re-assessment document (U.S. EPA, 2000), U.S. EPA stated that, based on animal data, current margins of exposure are too low, especially for more highly exposed human populations.

RISK CHARACTERIZATION

Although dioxin levels in the environment have been declining since the 1970s, given the widespread distribution, persistence, and accumulation of TCDD within the food chain, it is likely that most humans are exposed to some level of dioxin. At present, estimates of national background levels of dioxins in tissues are uncertain because current data cannot be considered statistically representative of the general U.S. population. In its latest draft document on dioxin (U.S. EPA, 2000), U.S. EPA estimated average current background body burdens at 5 ng/kg. The current estimated average dose to the U.S. population is ~1 pg TEQ/kg-day. Over ninety percent of adult human daily intake of dioxins is estimated to be from fat in fish and other animal products.

Occupational epidemiological studies show an association between 2,3,7,8-TCDD exposure and increases in all cancers combined (Fingerhut *et al*., 1991; Revich *et al.*, 2001; Steenland *et al.*, 1999), primarily in adult male populations. TCDD has been shown to be carcinogenic in both sexes of multiple species of animals at multiple sites, and at doses well below the maximum tolerated dose. Indeed, all long-term studies for the carcinogenicity of TCDD have produced positive results (van Miller *et al*., 1977; Kociba *et al*., 1978; NTP, 1982a; Johnson *et al*., 1992; NTP, 2004), including studies in hamsters (Rao *et al*., 1988), a species which has been shown to be relatively resistant to the lethal effects of TCDD. Exposure to dioxin has been shown in animal studies to result in both male and female reproductive effects, as well as effects on development. Prenatal death has been observed in a number of animal studies in which no maternal toxicity was evident (Olson and McGarrigle, 1990; Schantz *et al.*, 1989). In humans, data on developmental effects are limited to a few studies of populations exposed to a complex mixture of potentially toxic compounds. However, epidemiological findings do provide evidence that alterations in human male reproductive hormone levels are associated with serum 2,3,7,8-TCDD levels (Egeland *et al*., 1994; Grubbs *et al*., 1995; Thomas *et al.*, 1990). The immune system is a target for toxicity of TCDD. Numerous studies in animals suggest that perinatal development is a critical and sensitive period for TCDD-induced immunotoxicity.

Limited data in both humans and animals suggest that developing organisms, both prenatal and postnatal, are especially sensitive to the adverse effects of dioxin. Recent studies by Brown *et al*. (1998) suggest that prenatal exposure of rats to dioxin and related compounds may enhance their sensitivity as adults to chemical carcinogenesis from other carcinogens. Nursing infants represent a special subset of the population that may have elevated exposures on a body-weight basis compared to non-nursing infants and adults. Intake estimates of PCDDs are over three times higher for a young child on a body weight basis compared to those for an adult (U.S. EPA, 2000).

Animal laboratory data and mechanism studies suggest that males and females may respond differently to TCDD. Gender differences in the acute toxicology of TCDD are

likely due to toxicokinetic differences; higher tissue concentrations and longer half-lives in females than males (Li *et al*., 1995). Human studies have focused on males. The epidemiological data examining the association between exposure of adult women to TCDD and cancer is limited. Several researchers have reported a statistically significant increase in breast cancer in TCDD-exposed females (Manz *et al*., 1991; Kogevinas *et al.*, 1997).

Consumption of a diet that is disproportionately high in animal fats, and particularly diets that include a lot of freshwater fish, can lead to elevated exposures compared to the general population. The geographical locations of agricultural areas may be an important consideration concerning dioxin contamination levels. One study in the U.S. showed elevated levels of dioxin in chicken and eggs near a contaminated soil site (Harnly *et al.*, 2000). Elevated PCDD levels in milk and other animal products have also been found near combustion sources.

The U.S. EPA's Dioxin Reassessment Review Subcommittee (DRSS) of the Science Advisory Board (SAB) reviewed the U.S. EPA's draft 2000 dioxin risk assessment, and could not reach agreement on some of the specific conclusions, including carcinogenic mechanism and cancer risk extrapolation methods (U.S. EPA, 2001). The report summary states:

> "There was a lack of consensus among the Panel Members regarding the strength of weight of evidence for supporting the classification of TCDD as a human carcinogen, reflective of the limitations of the available scientific data and disagreements and confusion about the EPA cancer risk assessment guidelines, discussed below. However, the Panel was satisfied that the document reviews the relevant epidemiological studies and characterizes their findings appropriately, and the Panel agreed with EPA's conclusion that causal associations have been established between exposure to TCDD and increased cancer in laboratory animals. The Panel agreed that the treatment of the range of upper bound risks obtained for the general population in this assessment is consistent with past EPA practice. However, members differed in their confidence that animal experiments establish a hazard for specific endpoints or that the postulated mechanisms for those endpoints are well enough established to be similar in humans and laboratory animals. Members also differed regarding the likelihood that effects observed in the laboratory would be observed at lower levels of exposure."

However, the SAB DRRS panel also acknowledged that the various issues are not resolvable with current data, and concluded:

> "Since neither knowledge breakthroughs nor fully developed and widely accepted techniques for producing improved risk assessment procedures can be expected to be available in the near future, the DRRS recommends that the Agency proceed expeditiously to complete and release its Dioxin Risk Reassessment, taking appropriate note of the findings and recommendations of this report and other public comments.
>
> "Consistent with sound environmental and public health policy, the Panel believes that it is important that EPA continue to limit emissions and human exposure to

this class of chemicals in view of the very long biological and environmental persistence of these chemicals."

OEHHA concurs with this point, and believes that despite the uncertainty associated with risk extrapolation to low environmental levels for this (or any other) chemical, public health protection requires prudent assumptions, such as the use of the linearized multistage method for cancer risk assessment, as in this case.

REGULATORY STANDARDS

Maximum Contaminant Level and Other Drinking Water Standards

In 1997, the International Agency for Research on Cancer (IARC) upgraded TCDD to the status of "known human carcinogen" (IARC, 1997). The U.S. National Toxicology Program (NTP) also upgraded TCDD to "known human carcinogen" status in its 2001 Report on Carcinogens document (NTP, 2001). In 2000, the U.S. EPA concluded in its Draft Dioxin Reassessment document that TCDD, as well as other closely related structural analogs, are carcinogenic to humans and can cause immune system alterations, reproductive, developmental and nervous system effects, endocrine disruption, altered lipid metabolism, liver damage and skin lesions in humans (U.S. EPA, 2000).

The U.S. EPA has established a maximum contaminant level (MCL) of 0.03 ng/L (or 30 pg/L) TCDD based on potential health effects from ingestion of water. In 1984, U.S. EPA promulgated a much lower guideline of 0.013 pg/L TCDD for ambient surface water (industrial effluent). In the most recent compilation of National Water Quality Criteria (U.S. EPA, 2002), the value for TCDD for protection of human health for consumption of water plus organisms is listed as 0.005 pg/L. The California Department of Health Services (CDHS) drinking water standard for TCDD is 30 pg/L. The reporting limit of 5 pg/L is below the standard. Other states that have set guidelines for TCDD in drinking water include Maine at 0.2 ng/L and Minnesota at 0.002 ng/L. A concentration of 0.0039 ng/L was estimated to provide an upper confidence limit cancer risk of one in a million by U.S. EPA in 1980.

Other Regulatory Standards

U.S. EPA has declined to derive a reference dose (RfD) for dioxin, as any RfD the Agency would recommend under the traditional approach for setting an RfD is likely to be 2-3 orders of magnitude below current background intakes and body burdens. ATSDR (1999) set a minimal risk level (MRL), which is defined similarly to the U.S. EPA's RfD, for dioxin and related compounds of 1.0 pg TEQ/kg-day. The World Health Organization has set a tolerable daily intake of 1-4 pg TEQ/kg-day. The non-significant risk level for TCDD calculated for California's Proposition 65 is 5 pg/day (OEHHA, 2004).

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