# PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

# TCDD (DIOXIN)

**September 2010**

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# Public Health Goal for
# TCDD (Dioxin)
## in Drinking Water



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**September 2010**

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# PREFACE

**Drinking Water Public Health Goals**
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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 that 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 potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
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. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.
8. 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.

9. 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.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. 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 Public Health (DPH) 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 DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH 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 DPH must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DPH, 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 intended to be utilized as target levels for the contamination of other environmental media.

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 public health goal (PHG) of 0.00005 nanograms/liter (ng/L), or 0.05 picograms/liter (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 health protective level applies to the 2,3,7,8-isomer alone, rather than TCDD plus all its congeners (dioxins and furans), because this compound is specified for the California MCL in California regulations. The development of the PHG follows the general approach of the United States Environmental Protection Agency (U.S. EPA) to estimate TCDD toxicity in humans by using body burden rather than daily intake as a dose metric. A multi-site oral cancer potency of 0.77 $(ng/kg\text{-}day)^{-1}$ 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 studies, which report an increased risk of cancer at multiple sites after exposure to dioxins.

A public health-protective concentration of 0.002 ng/L (0.002 parts per trillion [ppt]) for noncarcinogenic effects of TCDD in drinking water was also determined based on the same NTP (2004) study described above. Body burden was used as a dose metric to account for significant differences in the half-life of TCDD in humans vs. rats. A default relative source contribution of TCDD from drinking water of 20 percent, and a total uncertainty factor of 100 were then applied to the LOAEL to derive the noncancer value.

The Office of Environmental Health Hazard Assessment (OEHHA) concurs with the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) that the combined mechanistic, animal and human data indicate that TCDD is a human carcinogen. In addition to observations of increased risk of cancer in animals and in humans at multiple sites, it has been established that TCDD causes cancer through a mechanism involving the Ah receptor, a receptor that functions the same way in humans as it does in experimental animals.

The PHG of 0.05 pg/L is much lower than the Maximum Contaminant Level (MCL) of 0.03 ng/L TCDD established by U.S. EPA, but well above the U.S. EPA ambient water criteria level of 0.005 pg/L TCDD (which includes consideration of consumption of organisms in the water). The 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

public health goal (PHG) for TCDD in drinking water. The World Health Organization's International Agency for Research on Cancer and the U.S. National Toxicology Program concluded that TCDD is a human carcinogen. The U.S. EPA, in its drinking water criteria documents (U.S. EPA, 1978, 1984, 2002) and in its draft Exposure and Human Health Reassessment of TCDD and related compounds (U.S. EPA, 2000, 2003), has reached the same conclusion. The Agency for Toxic Substances and Disease Registry (ATSDR) has also judged dioxin to be a human carcinogen (ATSDR, 1999).

TCDD, commonly referred to as dioxin, represents the reference compound for a class of halogenated aromatic compounds that produce similar patterns of toxicity and are considered to have a common mechanism of action, though they differ in potency. The chemical class is 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 by 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.

Drinking water is not considered to be a significant source of dioxin exposure; over 90 percent of adult human daily intake of dioxins is estimated to be from fat in fish and other animal products. However, 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. Because exposure to dioxins has been clearly associated with an increased risk of cancer at multiple sites in animals and humans, any additional source of TCDD, such as drinking water (which is unavoidable), should be minimized. The metabolism of TCDD in humans is very slow and the half-life of TCDD in humans is much longer than has been documented in any other animal species.

## CHEMICAL PROFILE

### *Chemical Identity and Properties*

Polychlorinated dibenzo-p-dioxins (PCDDs) occur as 75 different isomers. There are 22 possible tetrachlorodibenzo-para-dioxin 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. For this reason, the congeners are commonly referred to as dioxin-like compounds (DLCs).

The CAS registry number for the 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.

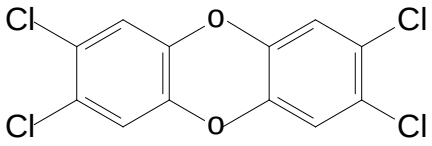


**Figure 1. Structure of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)**

### *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, and as a combustion by-product. 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.

Incidental production of dioxins during combustion has decreased as a result of decreased incineration of municipal waste. Controlling burning of chlorinated plastics such as polyvinyl chloride plastics is particularly important.

## ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

### *TEFs/TEQs*

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 (Birnbaum and DeVito, 1995; Van den Berg *et al.*, 1998). 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. TCDD is the major contributor to dioxin toxicity equivalent (TEQ) and many researchers have thusly chosen to measure only TCDD. OEHHA has accepted the World Health Organization TEF approach in its most recent cancer potency factor compilation (OEHHA, 2009a). The TEF/TEQ scheme has also recently been updated for estimating risks by oral exposure (Haws *et al.*, 2006, Van den Berg *et al.*, 2006).

### *Air*

PCDDs are ubiquitous in soil, sediment and air. 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; U.S. EPA, 2003). 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. Naturally occurring sources of dioxin include forest fires and volcanic eruptions.

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.

## *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) and decreased thereafter.

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*

PCDDs enter the aquatic environment from the atmosphere, agricultural runoff, and as direct discharges from industrial sources (e.g., pulp and paper mills) and municipal sewage treatment plants. 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 $2.4 \times 10^6$ (HSDB, 2004). Although dioxins have very low solubility in water (0.2 µg/L), other organic constituents present in the water may act as carriers. Particle-driven dispersion and solid-water partitioning of PCDD compounds has been shown to be significantly affected by their interaction with soot-carbon in addition to organic matter (Persson *et al*., 2002). 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). 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 |

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

Adapted from U.S. EPA, 2000.

**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 |

*congener specific emissions data were not available

Adapted from U.S. EPA, 2000.

A recent study undertaken to measure PCDDs and PCDFs in water and sediment samples taken from the Houston Ship Channel in Texas found that 100 percent of the samples (taken over the course of 1 year) exceeded the EPA water quality criterion of 0.014 pg/L (Suarez *et al.*, 2006). Average PCDD/PCDF concentrations in water were 0.32 pg TEQ/L for summer 2002, 0.63 pg TEQ/L for fall 2002, and 0.45 pg TEQ/L for spring 2003. The two sampling stations with the highest average TEQ concentrations include the San Jacinto River, home to paper and pulp mills, heavy shipping traffic and pipeline crossings. Most of the total 2,3,7,8-substituted PCDD/PCDF concentration can be attributed to octachloro-dibenzodioxins (OCDD) with an average contribution of 91 percent. However, 2,3,7,8-TCDD was the major contributor to the total TEQ (41 percent on average) for all three sampled media (dissolved, suspended sediment, bottom

sediment), followed by OCDD (36 percent on average). The particle bound fraction for PCDD/PCDFs increased with the degree of chlorination except for TCDDs.

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 (2,3,7,8-TCDD content 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 liquid chlorine acids, methyl chloroform, vinyl chloride and other pesticide-related 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 was analyzed in three drinking water samples from different areas of the town in 1998. High levels of the octa and hepta dioxin congeners (OCDD and HpCDD) were found (Table 3). The authors concluded that the situation was caused by wastes discharged from the production of pentachlorophenol. The PCDD and PCDF content 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 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.

Several studies have shown that most dioxins and dioxin-like compounds can be removed by drinking water treatment such as coagulation, sedimentation and filtration (Smirnov *et al.*, 1996; Kim *et al.*, 2002). (The more hydrophobic dioxins, such as TCDD, are adsorbed onto the suspended solids and can be more easily removed by coagulation and conventional sand-filtration). However, the concentrations of certain PCDDs, and particularly TCDDs, have shown significant increases following the water treatment process (Kim *et al.,* 2001). This is thought likely due to the chlorination process that influences the formation of dioxins.

A study was undertaken in Japan to determine the effect of the drinking water treatment process on the levels of PCDDs/Fs and Co-PCBs (Kim *et al.,* 2002). A total of 40

surface water and 5 ground water treatment plants were included in the study. Raw water and treated water were sampled twice, summer and winter. The mean concentration of dioxins in raw and treated water was 56.45 pg/L (0.15 pg TEQ/L) and 4.24 pg/L (0.019 pg TEQ/L), respectively. For raw water, the survey found that 76 out of a total of 90 samples had dioxin concentrations below 100 pg/L and 14 samples had values 100-600 pg/L. The highest dioxin concentration found in one sample was 540 pg/L. The average dioxin surface water concentration reported in this study is lower than that found in several European countries (Smirnov *et al.*, 1996). As for the congener distribution profile, total PCDDs made up 39.51 pg/L (70 percent) on average of the total concentration of surface and ground water before water treatment; total PCDFs were 4.23 pg/L (7.5 percent) and total Co-PCBs were 12.7 pg/L (22.5 percent) (the concentration of PCDFs in terms of pg *TEQ*/L is much more predominant than PCDDs in treated water). The octachloro-dibenzodioxins (OCDD) congener was predominant in terms of pg/L, followed next by TCDDs. This study showed that most dioxin congeners are well removed (93 percent removal efficiency) by water treatment. In the removal patterns of homologues, with increasing chlorine substitution, the removal rate also increases (except for OCDD). In terms of pg/L, the concentration of total dioxins in treated water was one tenth of that in the raw water. The percentage of OCDD (42.1 percent as pg/L) to total dioxins in raw water decreased to 3.7 percent after water treatment; in contrast, the percentage of TCDDs (17.4 percent in raw water) *increased* significantly to 28.01 percent after water treatment. According to the authors, this finding shows that the chlorination process in water treatment influences the formation of dioxins. Other investigators have suggested that the increase may be attributable to the reaction of chlorine with the precursors of dioxins such as dichlorophenol and trichlorophenol in the water treatment process (Luthe and Berry, 1996). In the current study, the location of the water treatment plants significantly influenced the concentration of dioxins and also resulted in different patterns of dioxin homologues. Levels of dioxins in ground water were much less than that of surface water in both raw and treated water.

## *Food and Other Sources*

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). One study in China that analyzed the concentration of PCDDs in green tea reported that in certain Chinese populations that drink a large amount of tea, tea consumption can contribute up to ten percent of the TDI recommended by the WHO (Fiedler *et al.*, 2002). 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). This effect is somewhat offset by a faster TCDD elimination rate in infants and their rapidly expanding body weight and lipid volume (U.S. EPA, 2003; Howd, 2010).

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 adult human is approximately 7.1 years (U.S. EPA, 2000, 2003).

The daily intake of dioxins in humans in the United States is estimated at approximately 1 pg TEQ/kg-day (U.S. EPA, 2000, 2003). 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.

## *Temporal Trends in TCDD/TEQs*

Time trends analyses of sediment cores of lakes and rivers show that dioxin levels in the environment have been declining since the 1970s, both in the U.S. and abroad (Alcock *et al*., 1997; Cleverly *et al.*, 1996; Czucwa and Hites, 1984; Czucwa *et al.*, 1985; Smith *et al.*, 1992, 1993, 1995; Marvin *et al.*, 2007; Zennegg *et al.*, 2007). The highest dioxin concentrations have been found in core segments corresponding to the 1930's through the 1960's. Higher core concentrations likely resulted from higher dioxin depositions to the land surface and to water bodies as a result of increases in industrial and combustion practices during this period (U.S. EPA, 2000, 2003). According to U.S. EPA (2003), factors that led to the decline in environmental levels and exposures to dioxin were the Clean Air Act of 1970 and implementation of air pollution controls, the phase-out of leaded automobile fuels and the use of catalytic converters, process changes at pulp and paper mills, and reductions in the manufacture and use of chlorinated phenolic intermediates and products, such as the ban of 2,4,5-T.

In humans, most dioxin exposure results from the consumption of animal fats. National surveys on beef, pork, poultry and milk show that current TEQ concentrations are in the range of 0.8 to 1.0 ppt TEQ lipid (U.S. EPA, 2000). Several European dietary intake studies have reported declines in dioxin concentrations measured in food over the past few decades (Furst and Wilners, 1995; Harrison *et al*., 1998).

There is a considerable volume of literature on human TEQ burdens over the past several decades; only a very few provide congener-specific concentrations, and fewer still provide information on body burden concentrations in infants or young children. The data derive largely from measurements in Vietnam veterans although some data on civilians are available from U.S. EPA's National Human Adipose Tissue Repository and National Human Adipose Tissue Surveys (NHATS) from 1982 and 1987. Kang *et al.* (1991) and U.S. EPA (1990) compared tissue samples (n = 195) from civilians and Vietnam veterans (males only; samples dated from between 1972 and 1981) and found that TEQ concentrations among the groups were indistinguishable. TEQ concentrations

were 71.9, 65.4, and 72.0 pg/g lipid for the Vietnam veterans, the non-Vietnam veterans and the civilians, respectively. The TCDD concentrations followed the same trend with concentrations of 13.4, 12.5 and 15.8 pg/g lipid for the same three groups. Within this data subset, TEQ concentrations declined overall from approximately 80 ppt TEQ to approximately 50 ppt TEQ between the years 1972 and 1981; several samples from various years had TEQ concentrations in excess of 100 ppt.

Pinsky and Lorber (1998) summarized studies measuring 2,3,7,8-TCDD in human blood and adipose tissue from Vietnam veterans. Using a single compartment, first-order PK model, they calculated a range of 10-20 pg TCDD/g (ppt) lipid during the 1970s, and 2-10 ppt lipid during the 1990s.

In 1982, and again in 1987, the U.S. EPA (U.S. EPA, 1991) analyzed composite samples of adipose tissue taken from individuals to characterize average background levels of dioxins and furan congeners. The overall average 1,2,3,7,8-PCDD concentration for NHATS '82 was 73.6 ppt (this included all three age groups: 0-14, 15-45, and > 45 years). The 1,2,3,7,8-PCDD concentration for the 15-45 age group alone was 125.0 ppt. For NHATS '87, composite samples from 2 age groups, 15-45 and > 45 years, showed an average concentration of 28 and 53 ppt TEQ, respectively. Data for the 10 composite samples representing the <15 age group were not provided.

A study by Graham *et al*. (1986), which reported adipose tissue TEQ concentrations from 35 autopsy patients (16 male, 19 female) who died suddenly or violently during 1985, showed a clear age trend in the data set, with concentrations ranging from <20 ppt lipid for the youngest individual (aged 21) to over 200 ppt for the oldest individual (aged 88). The average TEQ concentration from this population was 47 ppt lipid. Stanley *et al.* (1989) analyzed a total of 57 adipose tissue samples taken from surgical patients who were in the hospital for reasons other than cancer. The average age of the patients was 50 years; the average TEQ concentration was 31 ppt lipid.

U.S. EPA compiled results from six site-specific studies to represent average background body burdens in the United States during the mid-to latter 1990s for their draft dioxin reassessment document (U.S. EPA, 2000). The average TEQ concentration from this grouping of 214 individuals from 5 different states (age range, 20-70 years) was 20 ppt lipid. Petreas *et al.* (2000) conducted the only TEQ study comprised solely of women. Breast tissue samples taken from women undergoing breast surgery during 1998 (n = 45), who had an average age of 45 years (range: 28-67), had an average TEQ concentration of 25 ppt lipid. Results for individual women were not provided.

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, despite the decline in environment levels since the 1970s. 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 draft dioxin document, U.S. EPA (2000) estimated current average background body burdens at 5 ng/kg, and about 25 ng/kg on a lipid basis (U.S. EPA, 2003. The current estimated average dose to the U.S. population is ~1 pg TEQ/kg-day. The U.S. EPA (2000) estimated that the general human population is exposed to daily TCDD doses of ~0.3 pg/kg-day (from all

sources). Over ninety percent of adult human daily intake of dioxin-like compounds was estimated to be from fat in fish and other animal products.

## 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 also 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 is a relatively 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 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 ≤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*

The rate of excretion of TCDD is species specific. TCDD is most persistent in human and nonhuman primates. 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. 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.

One study in rats using a single radiolabeled congener indicated 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.

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 male volunteer. Elimination rates for TCDD, with estimates of half-life ranging from 5-12 years, have also been have been estimated from epidemiologic studies (Hooiveld *et al*., 1998; Michalek *et al.*, 2002; Steenland *et al*., 2001). Median TCDD half-lives of 8.7 years (95 percent confidence interval 8.0-9.5 years) and 7.2 years have been calculated in blood fat from 213 Ranch Hand veterans (Michalek *et al.*, 1996) and 43 Boehringer workers (Flesch-Janys *et al.*, 1996), respectively. A median half-life of 7.8 years (95 percent confidence interval 7.2-9.7 years) was calculated in 27 adults from Seveso, Italy with initial TCDD concentrations between 130 and 3,830 pg/g; a faster decline of TCDD concentrations in blood fat was reported for a few of the Seveso children with a very high exposure (Needham *et al.*, 1997). The highest TCDD blood level reported in these children was 56,000 pg/g.

In most of these cases, serum samples were taken many years following the initial exposure and the studies did not examine the initial elimination of TCDD. Considerably shorter overall half-lives of 1.5 and 2.9 years were observed in two women with very high exposures to TCDD (Geusau *et al.*, 2002). The marked difference in half-lives observed in this latter study are likely due in part to enhanced fecal TCDD excretion following administration of Olestra, a non-digestible, non-absorbable dietary fat substitute. The U.S. EPA, in its most recent assessment of 2,3,7,8-TCDD (U.S. EPA, 2000), utilized an elimination rate constant (i.e., half-life) for TCDD in humans of 7.1 years.

Several studies, both in humans (Michalek *et al.*, 2002) and laboratory animals (Abraham *et al.*, 1988; Dilberto *et al.*, 2001) suggest that the elimination rate of TCDD is dose dependent and is a function of the aryl hydrocarbon receptor-mediated induction of cytochrome P450 1A2 (CYP1A2). In both the human and animal data it was shown that as the exposure dose increases the apparent half-life decreases, indicating an inducible elimination of TCDD.

Edmond *et al*. (2005) compared the use of a physiologically based pharmacokinetic (PBPK) model that used a body burden-dependent elimination rate, with a classical pharmacokinetic model for a dioxin exposure assessment using two human data sets. The first data set came from studies of U.S. Air Force veterans from Operation Ranch Hand in which individuals were responsible for spraying Agent Orange and other herbicides contaminated with TCDD during the Vietnam War from 1962-1971. The second data set comprised 2 women with clinical signs of TCDD intoxication. Their findings indicate that the rate of TCDD elimination varies with the severity of exposure. In the case of the two highly exposed women (Geusau *et al.*, 2002), the first blood samples showed TCDD concentrations of 144,000 and 26,000 ppt (lipid-adjusted). The elimination rates in these women suggest that the overall half-life of TCDD during the first two years of exposure is <3 months. The authors suggest that previous exposure assessments may have underestimated peak blood TCDD concentrations.

Pinsky and Lorber (1998) analyzed the relationship between body fat fraction and the rate of TCDD dissipation. The analysis was conducted by using blood concentration data (taken over time) from Vietnam veterans with high TCDD body burdens. Although the database comprised only men and did not involve a wide age range, the analysis showed that the first-order elimination rate, *k*, of TCDD is a function of body fat fraction. That is, as body fat increases, the elimination rate decreases (equivalently, the elimination half-life increases).

Gender differences in TCDD excretion have been observed. In men and women exposed to TCDD in the Seveso industrial accident, shorter half-lives were found in Seveso men compared with women; on average, half-lives were 6.5 years in men (n=9, decay rate =0.1066/year) compared to 9.6 years in women (n=13, decay rate = 0.0722/year) (Michalek *et al.*, 2002). Several studies in rats have reported similar findings. Jackson *et al.* (1998) found that female rats eliminate TCDD more slowly than adult male rats. Li *et al.* (1995) observed that toxicokinetic differences in Sprague-Dawley rats, including higher tissue concentrations and longer half-lives in females than males, likely account for the sex differences in the acute toxicology of TCDD. However, in females, lactation can also serve as a relatively efficient route for excretion of TCDD.

Several studies in humans and experimental animals indicate that the elimination rate of TCDD is influenced by age. Flesch-Janys *et al.* (1996) in their analysis of occupationally-exposed persons, found that younger people metabolize TCDD more rapidly than older persons, on average. A similar relationship has also been reported in animal studies with both sexes of rats (Jackson *et al.*, 1998).

# 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 and 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). Apart from the Seveso, Italy industrial accident, there are few reports of human exposure uniquely to TCDD. A 20-year follow-up study of the Seveso population found an excess of lymphohemopoietic neoplasms in both men and women (RR=1.7, 95 percent CI 1.2-2.5). Hodgkin's disease risk was elevated in the first 10 year observation period (RR=4.9, 95 percent CI 1.5-16.4) whereas the highest increase for non-Hodgkin's lymphoma (RR=2.8, 95 percent CI 1.1-7.0) and myeloid leukemia (RR=3.8, 95 percent CI 1.2-12.5) occurred after 15 years. For men in the highest exposure zone, increases in all cancers (RR=1.3, 95 percent CI 1.0-1.7), rectal cancer (RR=2.4, 95 percent CI 1.2-4.6), and lung cancer (RR=1.3, 95 percent CI 1.0-1.7) were found. Three case control studies have shown relative risks of 5.7 (95 percent CI 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 is related to the occupation of the four lateral positions of the molecule by chlorine, which results 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 studies and no inhalation exposure toxicity studies are available in the literature.

**Table 5. Toxic Responses Following Exposure to 2,3,7,8-TCDD: Species Differences (adapted from U.S. EPA 2000)**

| 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 | | | | ++ | | | | |
| Cervix | ++ | | | | | | | |
| Hypoplasia, atrophy, or necrosis | | | | | | | | |
| Thymus | + | + | + | + | + | | + | + |
| Bone marrow | + | + | | | $\pm$ | | + | |
| Testicle | + | + | | + | + | | | |
| Other responses | | | | | | | | |
| Liver lesions | + | $\pm$ | ++ | + | ++ | + | + | $\pm$ |
| Porphyria | 0 | 0 | | + | ++ | | + | 0 |
| Edema | + | 0 | | 0 | + | | ++ | + |

0= lesion not observed; + = lesion observed (number of "+" denotes severity); $\pm$ = 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 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 (CYP1A2) isoenzymes (Diliberto *et al*., 1997; Vogel *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. Vogel *et al.* (1997) calculated a benchmark dose of about 0.03 ng TCDD/kg-day for EROD/MROD activities in the livers of female C57BL/6 mice (EROD and MROD activities are taken as surrogates for CYP1A1 and CYP1A2 expression, respectively). 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). Exposure of adult male rats to TCDD has also been shown to alter testicular steroidogenesis and reduce total Leydig cell volume in the testis (Johnson *et al.*, 1994). 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**

The immune system is a sensitive target organ for the action of TCDD. 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, injection of a single dose of 10 ng TCDD/kg in marmosets led to a decreased ratio of helper-inducer T cells as indicated by the ratio of $CD4^+CD29^+/CD4^+CD45RA^+$ cells. The NOAEL for this effect was 3 ng/kg TCDD. In addition, the number and percentage of certain B cells ($CD20^+$) were reduced, while an increase in the percentage of $CD8^+$ cells was seen (Neubert *et al*., 1990). 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).

Vogel *et al.* (1997) observed changes in a number of biochemical parameters in mice exposed subchronically to low doses of TCDD. Female C57BL/6 mice were administered TCDD i.p. for 135 days. The initial doses were 1, 10, and 100 ng TCDD/kg, followed by weekly injections of 0.2, 2 and 20 ng TCDD/kg (this was done to keep the tissue levels of TCDD nearly constant). At days 23, 79, and 135 of treatment, ten animals each from the control and TCDD groups were killed. At the end of the study, the treated animals had received total doses of 4.6, 46, and 460 ng TCDD/kg, which are equivalent to daily dose rates of 0.034, 0.34, and 3.4 ng TCDD/kg, respectively. Liver, lung and thymus were excised, weighed and frozen in liquid nitrogen for analysis (the

thymus is the control organ for generation of T cells). TCDD content in the liver was determined. Body weights of the animals were recorded weekly. No overt toxicity or increased mortality was observed at the doses tested, nor were body weights or organ weight ratios altered by the different TCDD doses. Exposure to TCDD at all dose levels led to changes in some thymocyte subsets; at the lowest dose, only moderate changes in the percentage of $CD4^{+}CD8^{+}$ cells were observed (these were not dose-dependent); a significant effect was seen in the medium dose group at day 23 and day 79 compared with controls, but no this parameter was not measured at day 135. Low doses of TCDD significantly enhanced the mRNA expression of interleukin (IL-1b) in liver, lung and thymus. At day 23, IL-1b mRNA contents in the liver, lung and thymus were significantly higher in the high dose group than in vehicle-treated controls. On average, IL-1b mRNA levels of liver, lung and thymus were 2 to 3-fold above the control values. IL-1b belongs to a family of soluble polypeptide mediators which have a wide variety of biological functions in the regulation of proinflammatory processes. IL-1b has also been shown to inhibit apoptosis in hepatic cell lines (Leist *et al*., 1995) and apoptosis inhibition is well-established as an important step in the tumor-promotion process (Schulte-Hermann *et al.*, 1990).

Vogel *et al.* (1997) also found a significant induction of EROD activity at a dose of 0.34 ng TCDD/kg-day (EROD is a marker for CYP1A1 activity; MROD is a marker for CYP1A2 activity). On average, in TCDD treated animals, CYP1A1 mRNA content in livers was increased 25-fold and in lungs 3-fold compared to control animals. Whereas CYP1A1 and CYP1A2 mRNA expression was dose-dependently enhanced, CYP1B1 mRNA expression remained unchanged at any of the TCDD doses tested. According to the authors, this finding agrees well with previous studies showing that in the livers of C57BL/6 mice, CYP1B1 is about 16-fold less responsive to TCDD than CYP1A1, indicating that CYP1B1 is of minor importance in the metabolism of xenobiotics. The results of the present study show that for CYP1A2, EROD- and MROD-activities there is neither an apparent threshold nor strong evidence of a sigmoidal dose-response relationship. The same finding holds true for IL-1b mRNA expression. For most of the parameters tested, the dose-effect relationship is effectively linear and the slopes estimated from all dose levels mostly tended to underestimate the slope at the lower dose. A benchmark dose of about 0.03 ng TCDD/kg-day was calculated for effects on EROD/MROD activities in the liver.

**Reproductive/Developmental Effects**

The potential for dioxins to cause reproductive and developmental toxicity in animals has been recognized for many years. Prenatal exposure to 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, 2003). Studies of male exposures have not provided evidence of paternally mediated effects on the offspring. TCDD is listed as a developmental toxicant in California under Proposition 65.

According to U.S. EPA (2003), 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.

The U.S. EPA, in its detailed evaluation of 2,3,7,8-TCDD and related compounds, has extensively reviewed maternal and developmental responses produced following gestational exposure to TCDD in various species of laboratory mammals (U.S. EPA, 2003). 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). 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 can be 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 resulted in delay of the onset of puberty, reduction in testis weight, sperm parameters and sex accessory gland weights. Most of these effects occurred in a dose-related fashion, some occurring at 0.05 µg/kg and 0.064 µg/kg, the lowest TCDD doses tested.

A number of recent studies have found that exposure to TCDD causes adverse reproductive and developmental effects in primates (Guo *et al.*, 2000; Moran *et al.*, 2001; Moran *et al.*, 2004; Scott *et al.*, 2001). In several of these studies, a single exposure to 4 µg TCDD/kg resulted in the observed adverse effect. Ten of twelve female Cynomolgus macaques administered single doses of 1, 2 or 4 µ/kg TCDD on gestational day (GD) 12 had early fetal loss (EFL) ten to twenty days later (Guo *et al.*, 2000). Seven control animals treated only with the vehicle had normal pregnancies. Blood samples were repeatedly collected for hormone evaluation, from two days before treatment to thirty-one days following treatment. Immunoreactive monkey chorionic gonadotropin (mCG) was measured in serum using ELISA, and bioactive mCG was measured using a luminescence LH/CG bioassay. No change in immunoreactive mCG levels was detected as a result of TCDD treatment, but bioactive mCG levels were significantly lower in TCDD-treated animals compared to controls. This change in bioactivity of mCG was also reflected in the ratio of mCG bioactivity to mCG immunoreactivity (B/I ratio) which began to rise in normal pregnancies by GD 20, but did not rise in TCDD treated animals. These results demonstrated that normal pregnancy in the monkey, as in humans, is

characterized by a post-implantation change in the B/I ratio of CG (several studies have shown that a decrease in the CG B/I ratio has been associated with early pregnancy loss in humans) (Ho *et al.,* 1997; Irwin and Giudice, 1998). Guo *et al.* (2000) suggested that changes in the production of bioactive CG may provide a marker for environmental toxicant exposures leading to EFL.

Results from a related study using human trophoblasts support the notion that TCDD acts directly on placental trophoblasts and reduces the B/I ratio of secreted CG (Chen *et al.,* 2003). Primary cultures of cytotrophoblast cells were incubated under differentiation-inducing and nondifferentiation-inducing conditions in the presence or absence of different concentrations of TCDD. These *in vitro* findings support earlier *in vivo* studies in macaques suggesting that the trophoblast is a target for TCDD and that TCDD-induced early pregnancy loss is accompanied by a decrease in the CG B/I ratio.

Female Cynomolgus macaques (n = 7) were treated with a single dose of 4 µg/kg TCDD on gestational day (GD) 15 or 20 via nasogastric intubation (Moran *et al.,* 2004). Pregnancies were terminated on GD 24-26 and embryos were examined to determine morphology of the developing neural tube. Compared to controls, all TCDD-exposed embryos exhibited cellular changes, including increased cell death and intercellular spaces in the neural tube, suggestive of an adverse effect on the developing nervous system (enlarged intracellular spaces and increased apoptosis during neurulation are indicative of neurotoxicity). These anatomical malformations in TCDD-treated embryos were closely correlated with significant decreases in fatty acid composition found in some of the eight classes of lipids analyzed. In particular, a significant decrease in maternal levels of the essential fatty acids docosahexaenoic acid (DHA) and arachidonic acid (AA) was seen. DHA and AA are in the n-3 and n-6 fatty essential fatty acid families, and are considered necessary for normal development in mammals. These adverse effects were more prevalent in the embryos treated with TCDD on GD 15 compared to those exposed on GD 20, suggesting that the earlier, less differentiated stages of neurulation may be particularly vulnerable to toxic insult. The authors concluded that since neural tube formation is dependent, in part, on n-3 and n-6 fatty acids, limitation of these fatty acids in plasma resulted in the observed detrimental effects on early neural (brain) development. The suggestion is that TCDD acts by antagonizing the estrogen-induced rise in maternal lipids during embryonic development.

Female Cynomolgus macaques (n = 11) were treated orally with graded doses of 2,3,7,8-TCDD (Scott *et al.*, 2001). Cervical tissue was recovered at necropsy 1.2-2.7 years later and examined using routine histopathology. Results were compared histologically with cervical tissue from untreated, age- and parity-matched controls. Significant squamous epithelial metaplasia was observed in the endocervix of 9 of 11 TCDD-treated animals, and the degree of severity was TCDD dose-dependent. In contrast, minimal or no pathological changes were observed in eight of nine control animals and one animal had only mild squamous metaplasia.

Ovarian function was evaluated in mature female Cynomolgus macaques 443 to 625 following a single oral exposure of 1, 2 or 4 µg/kg BW TCDD (Moran *et al.*, 2001). Urinary estrone conjugates, pregnanediol-3-glucuronide and follicle stimulating hormone (FSH) were measured. In the high dose group, three of four animals had no evidence of menstrual cycles. Treated animals in the low and medium dose groups (plus one from the

high dose group) had cycles similar to control animals. Mean FSH concentrations during the midfollicular phase of the medium dose group and during the entire cycle of the high dose group were elevated compared to controls, and the endometria of the noncycling animals were inactive. These data demonstrate that a single exposure of 4 µg/kg TCDD leads to long-term adverse effects on ovarian function in primates.

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 in rodents 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).

In addition to mediating the toxicological response to compounds such as TCDD, the Ah receptor (AhR) has been shown to be responsible for guiding the resolution of fetal vascular architecture, specifically, the closure of the ductus venosus (DV) in the heart (Walisser *et al.*, 2004). Failure of the DV to close at parturition decreases the portal blood supply to the liver and, as a consequence, liver size is reduced. Walisser *et al.* (2004) showed that mice harboring a hypomorphic aryl hydrocarbon receptor nuclear translocator (ARNT) allele demonstrated attenuation to two classic TCDD toxic endpoints, thymic involution and hepatotoxicity. ARNT is a dimeric partner for the AhR, and plays a pivotal role in cellular adaptation to low oxygen environments. Thus, AhR ARNT dimerization seems to be an essential feature for both the toxic endpoints of dioxin exposure as well as the AhR-dependent closure of the DV.

### *Genotoxicity*

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 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). 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). There is no consistent evidence for increased frequencies of chromosomal aberrations in human populations exposed accidentally or occupationally to TCDD (Shu *et al*., 1987). TCDD 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), which could be through epigenetic mechanisms.

## 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) 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 incidence of hepatocyte hypertrophy. The increases in hepatocyte hypertrophy were significant in all dosed groups except the lowest group, 3 ng/kg, at fifty-three weeks; severity of this lesion increased with increasing dose. The incidence of pigmentation of the liver was 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 were 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.

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).

**Table 7. Summary of Chronic Non-cancer Effects of TCDD in Harlan Sprague-Dawley Female Rats (gavage), adapted from NTP (2004)**

| 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) | | | | √ | √ |

* = denotes a significant increase over vehicle controls

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 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 of TCDD 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). Findings from several key animal cancer bioassays are summarized in Table 8.

**Table 8. Sites for Increased Cancer in Key Animal Bioassays of 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, B6C3$F_1$ | 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) 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 in a corn oil:acetone vehicle (99:1). 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 for the remainder of the study. The cancer findings are reported in this section,

while the non-cancer and nonneoplastic lesions are summarized in Table 7 above. Administration of TCDD under the conditions of this two-year 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 also have been related to TCDD administration. The tumor incidence data are summarized in Table 9.

**Table 9. Summary of Female Rat Tumor Incidence from NTP (2004) TCDD Study**

| **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 to calculate 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).

Subsequent to the Kociba and Squire evaluations, the criteria for the diagnosis of hepatocellular proliferative lesions in the rat changed (Maronpot *et al.*, 1986; NTP/NIEHS 1989). In light of this, the paper industry requested Pathco, Inc. to review the slides (liver sections) from the original Dow Chemical study (Kociba *et al.*, 1978). Some of the hyperplastic nodules originally seen in the Kociba *et al*. (1978) study were thus reclassified as non-neoplastic. 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. Although the tumor incidence (refers to hepatocellular neoplasms only) was lower than either Kociba or Squire, the Goodman and Sauer (1982) results indicated a similar trend (*i.e.*, increased number of female rats with hepatocellular adenomas in the middle dose group and increased numbers of female rats with hepatocellular adenoma and carcinoma in the high dose group when compared with controls. There was also a dose-related increase in the incidence and severity of hepatotoxicity in treated animals compared to controls). A Pathology Working Group (PWG), comprised of pathologists from Pathco, Inc., the National Institute of Environmental Health Sciences (NIEHS), the National Cancer Institute (NCI), the U.S. Food and Drug Administration (FDA), U.S. EPA and John Hopkins University reviewed the findings and concluded that this study demonstrated a weak oncogenic effect of TCDD in the livers of female Sprague-Dawley rats. The incidence of hepatocellular neoplasms from the Goodman and Sauer (1982) re-evaluation is shown in Table 11 below (of note: the re-evaluation focused uniquely on hepatocellular neoplasms. Only the most malignant tumor observed in an animal was diagnosed).

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

| 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 |

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

**Table 11. Comparison of Female Rat Tumor Incidence in the Kociba *et al.* (1978) Squire (1980), and Goodman and Sauer* (1982) Reports**

| 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 (Kociba and Squire include both hyperplastic nodules and carcinomas; Goodman and Sauer include adenomas and carcinomas only) | Kociba | 9/86 | 3/50 | 18/50 | 34/48 |
| | Squire | 16/86 | 8/50 | 27/50 | 33/47 |
| | Goodman and Sauer | 2/86 | 1/50 | 9/50 | 18/45 |

Adapted from U.S. EPA (1985)
SCC = squamous cell carcinoma
**only the incidence of hepatocellular neoplasms (adenoma and carcinoma) was evaluated*

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 that using the unadjusted data.

**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 B6C3$F_1$ 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/kg 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 mouse strains. Della Porta *et al.* (1987) administered TCDD in corn oil i.p at 0, 1, 20, or 60 µg/kg to groups of 89-186 B6C3 and B6C infant mice once weekly for five weeks, starting on postnatal day 10. 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 hybrid strains, 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 at 0, 2.5 or 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 the mice at both exposure levels. Comparison of the carcinogenic potency of TCDD in female B6C3 mice exposed as infants (once per week for five weeks, starting on postnatal day 10) to that in female B6C3 mice exposed during the juvenile and adult lifestages (once per week for 52 weeks, starting at six weeks of age) indicates an increased susceptibility of infant mice to TCDD (OEHHA, 2009b).

*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.

Low-dose subchronic exposure to TCDD in female C57BL/6 mice has been shown to significantly induce interleukin (IL-1b) mRNA content in liver, lung and thymus (Vogel *et al.*, 1997). The authors suggest that an increase in reactive oxygen species (ROS) production via IL-1b induction by TCDD could be a mechanism by which TCDD initiates tumor formation. Such initiation activity of TCDD has been demonstrated in tumor promotion studies in rats (Moolgavkar *et al.*, 1996; Portier *et al.*, 1996). IL-1b has also been shown to inhibit apoptosis in hepatic cell lines (Leist *et al.*, 1995) and apoptosis inhibition is well known as an important step in the tumor-promotion process (Schulte-Hermann *et al*., 1990).

Additionally, 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. One study in China that analyzed the concentration of PCDDs in green tea reported that in certain Chinese populations that drink a large amount of tea, tea consumption can contribute up to ten percent of the TDI recommended by the WHO (Fiedler *et al.*, 2002). 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

Results from accidental exposures to high levels of TCDD show that compared with other species, humans are one of the less sensitive species to dioxins with regard to the $LD_{50}$ (Caramaschi *et al.*, 1981; Geusau *et al.*, 2001). (Polymorphism in the Ah locus is thought to account for many of the species differences in sensitivity to TCDD). 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 in humans.

Too little human data exist to determine the threshold level of TCDD at which chloracne occurs. Results from several epidemiological studies indicate that chloracne may occur at dioxin blood levels of approximately 1,000 pg/g blood fat (Needham *et al.*, 1997; Sweeney *et al.*, 1997; Coenraads *et al.*, 1999). 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).

In the spring of 1998, two women who were severely contaminated with 2,3,7,8-TCDD were diagnosed with chloracne (Geusau *et al.*, 2001). Autumn 1997 was the presumed time of TCDD intoxication; the cause of the exposure has not been fully explained. The initial blood concentrations, measured in the spring 1998 (many months after the appearance of the first symptoms), were 144,000 pg/g blood fat in patient 1 and 26,000 pg/g in patient 2, the highest levels ever measured in adults. For patient 1, this corresponded to a calculated body burden of 1.6 mg TCDD, and a dosage of 25 µg/kg body weight (BW); for patient 2, this corresponded to a calculated body burden of 0.4 mg TCDD and a dose of 6 µg/kg. In addition to chloracne, patient 1 experienced GI symptoms, including nausea, vomiting, epigastric pain and loss of appetite in the months preceding diagnosis. Moderately elevated levels of blood lipids, a normocytic, normochromic anemia, and leukocytosis were the most prominent pathologic changes. The patient was thrombopenic in the first three months of observation; antiplatelet

antibodies were negative. Histology carried out in October 1999 revealed normocellular bone marrow with prominent myelopoiesis; no chromosomal abnormality was detected. Menstruation in this patient, age 30, ceased in late autumn 1997. The second patient, a 27-year old woman who worked in the same office as the first patient, also had been suffering from GI symptoms from autumn 1997 to early 1998. Apart from marginally elevated values of cholesterol and lipase, an elevated number and percentage of B lymphocytes, and a decreased percentage of natural killer cells, her routine laboratory and immunologic parameters were within the normal range.

In Medina, Italy in 1976 an explosion of a trichlorophenol (TCP) reactor in a 2,4,5-T production facility caused the contamination by TCDD of the neighboring city of Seveso, Italy. The most evident adverse health effect ascertained was chloracne (193 cases). Chloracne was observed in chemical workers between 2 weeks and 2 months after the reactor release, and among Seveso schoolchildren, who were outdoors and in the path of the toxic cloud, after 6 months (Reggiani, 1980). Other reversible early effects noted were peripheral neuropathy and liver enzyme induction (Bertazzi, 1989). In a follow-up study of the Seveso population 20 years later, an increase in diabetes (notably among women, RR= 2.4, 95 percent CI 1.2-4.6) and chronic ischemic heart disease was observed (Bertazzi *et al.,* 2001). Mortality from respiratory disease, particularly chronic obstructive pulmonary disease (COPD), was elevated immediately after the incident as well as in the latest observation period; rates of COPD were significantly increased in the zones with the highest exposure. (A summary of the cancer findings from this study can be found in the cancer section that follows).

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; Vena *et al.,* 1998), in Seveso, Italy (Pesatori *et al.,* 1998; Bertazzi *et al.,* 2001), 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 in primates (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). TCDD has also been shown to increase serum triglycerides and cholesterol, well-established risk factors for cardiovascular disease, in both experimental animals (Brewster and Matsumura, 1984; Lovati *et al.,* 1984) and humans (Martin, 1984; Pazderova-Vejilupkova *et al.,* 1981).

In a 20-year follow-up study of the population exposed to dioxin after the 1976 TCP accident in Seveso, Italy, investigators reported an increase in chronic obstructive pulmonary disease (COPD), particularly among males living in the most contaminated zone (Bertazzi *et al.,* 2001). It also affected women in the two most contaminated zones. The authors hypothesize that the most plausible way in which TCDD might have contributed to this finding is through its recognized immunotoxic activity.

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.

A number of epidemiologic studies provide evidence that exposure to TCDD causes alterations in glucose metabolism. In a twenty year follow-up of the population exposed to dioxin after the 1976 TCP accident in Seveso, Italy, an increase in diabetes mellitus was present among females in all exposure zones (Bertazzi *et al.*, 2001). In one case report of 55 trichlorophenol (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). An elevated prevalence of diabetes and a positive association between TCDD serum levels and fasting serum glucose levels were found in a survey of U.S. chemical workers exposed to dioxin, but confounding by other variables could not be ruled out (Sweeney *et al.*, 1992). 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; the veterans were found to have a high prevalence of diabetes and a decrease in time-to-diabetes onset with dioxin exposure. 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 large volume of epidemiologic literature on dioxins and cancer, with both negative and positive results. 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 nearly 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. One important exception to this is the 1976 Seveso, Italy accident in which several thousand local residents were exposed to relatively pure TCDD (the contents of a TCP reactor from a chemical plant were vented directly into the atmosphere). To date, the only other female cohort study with good TCDD exposure surrogate information is that of Manz *et al*. (1991), which found a narrowly statistically significant increase in breast cancer. Animal and mechanism studies suggest that males and females may respond differently to TCDD exposure.

Of the cohort mortality studies that have been published since EPA's 1988 review, 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 (U.S. EPA, 2000). 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. Two other cohort mortality studies, Bertazzi *et al.* (2001) and Bodner *et al*. (2003), were published subsequent to the release of the 2000 U.S. EPA Draft Dioxin Reassessment Document. Results of the Bertazzi study, a follow-up of the population exposed to dioxin in the 1976 accident in Seveso, Italy, support the evaluation of dioxin as carcinogenic to humans, while Bodner *et al.* (2003) found no significantly increased risk of cancer in a followup of a male chemical production cohort exposed to "substantial levels of dioxin."

Bertazzi *et al.* (2001) conducted an extended follow-up of the population exposed to dioxin after the 1976 industrial accident in Seveso, Italy. Several thousand people were potentially exposed to relatively pure TCDD. The level and the extent of the environmental contamination were documented by dioxin soil measurements, and three contamination zones were delimited, A, B and R. The most heavily contaminated was zone A; zone B was its natural continuation along the fallout path of the chemical cloud, and zone R was designated as an area of low-level and patchy contamination. A fourth zone, the reference zone, comprised individuals from the surrounding non-contaminated area. The earliest accident-related health effect was chloracne in children who were outdoors and in the path of the chemical cloud. The initial (collected in 1976-1977)

median lipid-adjusted plasma concentrations of subjects in zones A (n=296), B (n=80), and R (n=48) were 447, 94 and 48 ppt TCDD, respectively. The median TCDD blood concentration for individuals living in the reference zone (n =52) was 5.5 ppt.

Causes of death in these populations were taken from death certificates. For persons living in zones A and B, deaths from rectal cancer were elevated, with a nearly two-fold increase in zone B. In zone B, a significant increase in lymphohemopoietic neoplasms occurred, particularly Hodgkin's disease, multiple myeloma, and myeloid leukemia. Regarding nonmalignant causes of death, chronic obstructive pulmonary disease (COPD) was significantly increased in zone A, and less so in zone B. Hypertension was nonsignificantly in excess in zone A; the zone B population exhibited moderate increases in diabetes and chronic ischemic heart disease. Results were also analyzed separately by gender. Among males in zone A, cancer mortality was slightly elevated after 15 years. Lung cancers, rectal cancer, and non-Hodgkin's lymphoma showed a significant increase. Males in zone B had moderately increased mortality from cancer causes. Rectal cancer increased significantly, digestive cancer deaths represented a borderline significant excess, and lung cancer exhibited a slight persistent elevation 5 or more years after first exposure. Lymphatic and hemopoietic neoplasms showed a nearly two-fold borderline significant increase; Hodgkin's disease and leukemia mainly contributed to this finding. Among females in zone A, an excess of colon and other digestive cancers and of melanoma was found in the 5-9 year latency period. Stomach cancer was increased in the second decade. For females in zone B, in the 10-14 year period since first exposure, digestive cancer mortality was elevated, and stomach and liver cancer showed statistically significant increases. Twelve cases of lymphatic and hemopoietic neoplasms made up a twofold statistically significant excess; the increase involved Hodgkin's disease, non-Hodgkin's lymphoma, and multiple myeloma. An excess of leukemia deaths – although not significant – was found 15 or more years after first exposure. In zone R, no excess cancer deaths were found for any of the cancer sites. A special group within the cohort, composed of 182 persons (57 in zone A, 11 in zone B, 69 in zone R and 45 in the reference area), was diagnosed with chloracne after the accident. All were traced; two had died by the time of this follow-up study, one zone A resident from myocarditis and one zone R resident from suicide.

In the cohort mortality study of chemical production workers of Bodner *et al*. (2003) there was no increase in SMR for all cancers. The SMR for soft tissue sarcoma and non-Hodgkin's lymphoma were non-significantly elevated with large confidence intervals; the authors point out the "wide range of cancer rates and the lack of consistency across dioxin studies."

Fingerhut *et al*. (1991b) 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) re-reviewed 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) (Revich *et al.*, 2001). From 1967-1987, the Middle Volga chemical plant in Chapaevsk produced lindane and its derivatives. Currently it produces crop protection chemicals involving use of liquid chlorine acids, methyl chloroform, vinyl chloride and other intermediates. (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 PHG for TCDD is based on a study in animals (NTP, 2004). For species extrapolation, body burden, rather than daily intake, was selected as the dose metric. The half-life of TCDD is approximately 100-fold greater in humans (2,593 days) than in rats (25 days). The calculation of human equivalent doses from rat adipose tissue concentrations in the animal study accounts for interspecies pharmacokinetic differences. 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 (U.S. EPA, 2000, 2003). 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).

In addition to the pharmacokinetic adjustment (*i.e.*, body burden approach), an adjustment was made for interspecies pharmacodynamic differences, as specified in OEHHA's Hot Spots Guidelines (2009a).

**Rate of TCDD Elimination**

The assumption of a single TCDD half-life (7.1 years) is uncertain because it is possible that in humans the apparent half-life may be shorter at higher exposure levels. Several studies in humans (Michalek *et al.,* 2002) and laboratory animals (Abraham *et al.,* 1988; Dilberto *et al.*, 2001) suggest that the elimination rate of TCDD is dose dependent and is a function of the aryl hydrocarbon receptor-mediated induction of cytochrome P450 1A2 (CYP1A2). In both the human and animal data, as the exposure increases the apparent half-life decreases, indicating an inducible elimination of TCDD. In the case report of two Austrian women with very high exposures to TCDD, the blood levels of TCDD were approximately 20-100 fold higher than the concentrations that are predicted to cause maximal induction (Geusau *et al.,* 2002). Application of a human PBPK model to the elimination rates in these women suggests that the half-life of TCDD during the first 2 years of exposure was <3 months (Edmond *et al.*, 2005). Because limited data exist to validate a PBPK model that incorporates an inducible elimination of TCDD, the decision is made to use the TCDD human half-life of 7.1 years recommended by the U.S. EPA (2000, 2003) for the PHG cancer calculation.

## *Noncarcinogenic Effects*

Dose response data are very sparse for *human* noncancer endpoints. In contrast, animal studies on the effects of TCDD following multiple exposures provide a wealth of data, enabling the determination of effective dose/responses far below the usual 10 percent adverse effect level, or $ED_{10}$. For that reason, $ED_{01}$ is commonly chosen as an estimated response level for TCDD. The range of $ED_{01}$ values is highly variable within and across response categories. 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 endpoint as the "critical effect" for non-cancer risk assessment. The lowest $ED_{01}$ values tend to be for biochemical effects, followed by hepatic responses, immune responses, and changes in organ weights. Results from the analysis of $ED_{01}$s and LOAELs suggest that non-cancer effects occur at about the same body burden levels as 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 (Schantz *et al*., 1979). In a 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 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 TCDD is almost always found in association with other PCDDs and other materials (e.g., chlorophenols, combustion products, etc.), several of which are also carcinogens, it is difficult to develop quantitative dose response relationships from human studies of TCDD. Estimates derived from human data (U.S. EPA, 2000; Portier, 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 6-161 ng/kg (U.S. EPA, 2000). Estimates from the animal studies, which ranged from 14 to 1,190 ng/kg (most were in the range of 14 to 500 ng/kg), and 2.7 ng/kg for the single mechanism-based model, overlap the estimates derived from human studies (U.S. EPA, 2000).

Debate continues on the most appropriate cancer potency calculation method for TCDD (Portier, 2000; U.S. EPA, 2001; Aylward *et al.*, 2003; Cole *et al.*, 2003; Hays and Aylward, 2003; Mackie *et al.*, 2003; Popp *et al.*, 2006; NAS, 2006). OEHHA has chosen to utilize the default linear multistage approach, as used by U.S. EPA (2003) and others (Crump *et al.*, 2003; Mackie *et al.*, 2003), as recommended in the U.S. EPA cancer guidelines when evidence is inconclusive (U.S. EPA, 2005).

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 (although the accident in Seveso, Italy exposed the local population to relatively pure TCDD, the exposure characterization was ecologic).

In its reassessment document on TCDD and related compounds (U.S. EPA, 2000, 2003), 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 used 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 has no effective threshold, that potency is a function of "average" TCDD levels in the body, and that twenty-five percent of human body weight is comprised of lipid (fat).

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 $1.4 \times 10^{-3}$. 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. Although body burden was used to account for interspecies pharmacokinetic differences, no interspecies adjustment was included to account for pharmacodynamic differences between rats and humans. 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.)

The NTP (2004) chronic gavage study in female Harlan Sprague-Dawley rats provides a superior basis for risk assessment, due to its careful design and conduct, as well as improved survival rate, compared to Kociba *et al*. (1978). In the latter study, which U.S. EPA used to estimate human cancer risk, survival was poor in all groups of control and exposed rats; at 2 years, only 8-22 percent of males, and 8-32 percent of females were still alive. The early mortality reduced the sensitivity of this study for determining the actual number of neoplasms induced by two years of exposure to TCDD.

The study design, species, and dose range of 3 to 100 ng/kg per day selected for the NTP (2004) 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 to TCDD in previous studies of Sprague-Dawley rats. TCDD induced tumors at several different sites (and in the case of the liver, tumors arose from multiple cell types) 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 estimated as a pharmacokinetic adjustment from the rat adipose tissue 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 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:

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

where:

D = daily intake (pg/day);

t½ = half-life of TCDD (in days) = 7.1 years x 365 days/year

V = volume of body fat (kg) = 70 kg x 0.25 = 17.5 kg

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

A = fraction of dose that is absorbed = 0.5

Thus, $D = [\ln 2/(365 \times 7.1)] \times 17.5 \text{ kg} \times C/0.5 = 9.495 \times C$

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 14. The trapezoid rule was used to estimate the overall average, assuming a linear increase between timepoints. To calculate Human Equivalent Doses as a pharmacokinetic adjustment for a 70 kg human, dose is divided by 70 kg; picograms are converted to milligrams using $10^9$ scaling to express results in mg/kg-day.

**Table 14. TCDD Human Pharmacokinetic 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) | Human Equivalent Doses (mg/kg-day) |
|---|---|---|
| 0 | 0 | 0 |
| 3 | 345.6 | $4.69 \times 10^{-8}$ |
| 10 | 656.5 | $8.9 \times 10^{-8}$ |
| 22 | 1275.3 | $1.73 \times 10^{-7}$ |
| 46 | 2337.4 | $3.17 \times 10^{-7}$ |
| 100 | 5244.9 | $7.11 \times 10^{-7}$ |

*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 or at the same site, but arising from different cell types, within a given sex, species and study, a methodological approach using Monte Carlo analysis has been used to sum potency estimates across sites, as shown in Table 15. 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 or type, 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 $39.1 \times 10^4$ $(mg/kg\text{-}day)^{-1}$, or $3.9 \times 10^{-1}$ $(ng/kg\text{-}day)^{-1}$. This value was then adjusted by a factor to account for interspecies pharmacodynamic differences between rats and humans, to generate the cancer potency estimate. This pharmacodynamic factor is (human body weight/female rat body weight)$^{1/8}$ or (70 kg / 0.328 kg)$^{1/8}$ * or 1.96. As discussed previously, the use of human equivalent doses in the potency calculations accounts for interspecies pharmacokinetic differences. For TCDD, the multisite cancer potency derived from the NTP (2004) study is therefore $76.6 \times 10^4 (mg/kg\text{-}day)^{-1}$, or $7.7 \times 10^{-1} (ng/kg\text{-}day)^{-1}$.

**Table 15. Human Cancer Potency Estimates for TCDD extrapolated from the NTP (2004) Study in Female Rats**

| Tumor Site/Type | Human Cancer Potency $(mg/kg\text{-}day)^{-1}$ | |
|---|---|---|
| | **Adipose Equivalence $q_1$*** | **Applied Dose $q_1$*** |
| Liver/hepatocellular adenoma | $7.66 \times 10^4$ | $0.493 \times 10^4$ |
| Liver/cholangiocarcinoma | $19.0 \times 10^4$ | $1.15 \times 10^4$ |
| Liver/hepatocholangioma | $4.16 \times 10^4$ | $0.265 \times 10^4$ |
| Lung/cystic keratinizing epithelioma | $4.16 \times 10^4$ | $0.266 \times 10^4$ |
| Oral mucosa/gingival squamous cell carcinoma | $25.3 \times 10^4$ | $1.40 \times 10^4$ |
| Uterus/squamous cell carcinoma | $9.13 \times 10^4$ | $0.832 \times 10^4$ |
| Pancreas/acinar adenoma or carcinoma | $4.16 \times 10^4$ | $0.268 \times 10^4$ |
| Combined site estimate for TCDD | $39.1 \times 10^4$ | $2.63 \times 10^4$ |

OEHHA has chosen this study and cancer potency derivation for development of the PHG for TCDD because these are judged to be superior to earlier approaches.

* The female rat body weight estimate was derived from the time-weighted average body weight of NTP's three reported means in the control female rats for weeks 1-13, 14-52, and 53-101 (NTP, 2005).

# CALCULATION OF PHG

## *Noncarcinogenic Effects*

A LOAEL of 3 ng/kg-day was selected for calculation of a public health-protective concentration for noncarcinogenic effects of TCDD in drinking water, based on the NTP (2004) toxicology/carcinogenesis gavage studies of TCDD in female Sprague-Dawley rats. These studies were not specifically designed to determine a NOAEL or LOAEL (dose selection was based on a prior carcinogenicity study), and no NOAEL was observed in this study. The female Sprague-Dawley rat has been used frequently in chronic and subchronic studies of the action of dioxins. Male rats were not studied due to the lack of tumor response in prior studies. In the 2-year chronic NTP (2004) study, the lowest administered dose of 3 ng/kg-day resulted in significant increased incidences of cell proliferation, gingival squamous hyperplasia, cytochrome P450 induction, as well as significant increases in lung and liver weights. Results from the analysis of $ED_{01}$s and LOAELs suggest that non-cancer effects occur at about the same body burden levels as for tumor induction in animals.

The NTP study measured adipose tissue concentrations of TCDD in the rats over the course of the study duration. Average rodent tissue concentrations (pg/g) for each dose level are shown in Table 14 of this document, above. At the 3 ng/kg-day dose level, the human equivalent dose level (calculated from rat adipose tissue levels) is $4.69 \times 10^{-8}$ mg/kg-day. Because the NTP (2004) study was a chronic study, body burden was used as the dose metric for species extrapolation to calculate the non-cancer value. The body burden approach takes into account the approximately 100-fold difference in half-life of TCDD in humans vs. rats. The 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). It is noted that in the event of an acute (less than lifetime) exposure, the accuracy of body burden as the dose metric can vary because the relationship between tissue concentrations and body burden in short-term animal studies may not be the same as under steady-state conditions. This value was then adjusted by a pharmacodynamic factor of 1.96 (human body weight/female rat body weight)$^{1/8}$ or (70 kg / 0.328 kg)$^{1/8}$ to account for interspecies pharmacodynamic differences between rats and humans.

Calculation of a health-protective concentration (C, in µg/L) for noncarcinogenic endpoints follows the general equation:

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

where,

LOAEL = lowest-observed-adverse-effect-level;

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); |
| PD | = | pharmacodynamic factor of 1.96 (human body weight/female rat body weight)$^{1/8}$ or (70 kg / 0.328 kg)$^{1/8}$ to account for interspecies pharmacodynamic differences between rats and humans. |

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. Inhalation and dermal exposures were assumed to be negligible because of the low volatility and low dermal absorption estimated for TCDD in drinking water. Uncertainty factors of 10 each would be applicable to account for extrapolation of a LOAEL to a NOAEL, and for human variability, for a total uncertainty factor of 100. A health-protective level for non-cancer effects can therefore be calculated as follows:

$$C = \frac{4.69 \times 10^{-8} \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{100 \times 2 \text{ L/d} \times 1.96} = 1.7 \times 10^{-9} \text{ mg/L (0.002 ppt or 2 pg/L)}$$

Thus the public health protective concentration for TCDD in drinking water based on noncarcinogenic effects (increased lung/liver weights) is calculated to be 0.002 ng/L (2 pg/L or 0.002 ppt). This value is considerably lower than the maximum contaminant level (MCL) of 30 pg/L TCDD established by U.S. EPA but higher than U.S. EPA's guideline for ambient surface water (industrial effluent) of 0.013 pg/L TCDD. 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. 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.

## *Carcinogenic Effects*

OEHHA has used 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 PHG was derived using a cancer potency estimate based upon data from an animal study. Animal cancer dose response data were considered more robust for purposes of potency estimation than the available human epidemiological data. 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 (Della Porta *et al.*, 1987; Brown *et al.*, 1998; OEHHA, 2009b). Also, segments of the population that consume many times the average level of animal-derived fat per day,

the principal exposure pathway for dioxins in the general population, may be at higher risk.

The PHG for TCDD is based on the NTP (2004) chronic 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 demonstrated susceptibility to chemical-induced hepatocarcinogenicity in females in this species and strain. TCDD induced tumors at multiple sites in the NTP (2004) study: liver (hepatocellular adenoma, cholangiocarcinoma, hepatocholangioma), lung, oral mucosa, uterus and pancreas. The cancer potency factor was calculated based on a combined-sites approach. Human equivalent doses used in the calculation were based on the U.S. EPA's recommended body burden approach, thus taking into account interspecies pharmacokinetic differences. An interspecies pharmacodynamic adjustment factor of 1.96 was also applied. The combined cancer potency for the seven tumor sites/ types identified in the NTP (2004) study is 0.77 $(ng/kg\text{-}day)^{-1}$. To derive the 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 $1x10^{-6}$;

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

CSF = cancer slope factor, derived from the probability distribution of the multisite analysis, of 0.77 $(ng/kg\text{-}day)^{-1}$;

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

Therefore,

$$C = \frac{10^{-6} \times 70\ kg}{0.77\ (ng/kg\text{-}day)^{-1} \times 2\ L/day} = = 0.00005\ ng/L\ (rounded)$$

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 calculated to be 0.00005 ng/L (0.05 pg/L). Risks of $10^{-5}$ and $10^{-4}$ are associated with lifetime exposure to concentrations of 0.5 pg/L and 5 pg/L, respectively.

The PHG developed here is 600-fold lower than the current U.S. EPA Maximum Contaminant Level (MCL) of 0.03 ng/L (or 30 pg/L) TCDD. 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 TCDD, based on consumption of water plus organisms (U.S. EPA, 2002). U.S. EPA stated in its draft dioxin re-assessment document (U.S. EPA, 2003) 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, 2003), U.S. EPA estimated average current background body burdens at 5 ng/kg. The 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. Although these data are not adequate for calculation of human cancer potency factors, they do indicate the apparent lack of a threshold for TCDD carcinogenicity at environmental exposure levels. In animal bioassays, 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 (Della Porta *et al.*, 1987; OEHHA, 2009b). 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, 2003).

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).

Another potential subpopulation of concern may be older adults. PCDDs are lipophilic compounds that tend to accumulate in the lipid stores of the body and are resistant to metabolism. One study that looked at a total of 588 serum dioxin samples from participants in the U.S. with no known exposure to dioxin-like compounds (other than exposure to background levels of dioxin) found that the 95$^{th}$ percentile for dioxin TEQ levels in the oldest age-group, defined as 60$^{+}$ years, was about six times larger than the 95$^{th}$ percentile for the youngest age-group, aged 15-29 years (Patterson *et al.*, 2004).

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.

NTP in 2001 and the International Agency for Research on Cancer in 1997 found TCDD to be a human carcinogen after systematically reviewing the evidence from mechanistic, animal and human studies. According to the NTP:

> "2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD or TCDD) is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans involving a combination of epidemiological and mechanistic information that indicates a causal relationship between exposure to TCDD and human cancer."

NTP further noted that:

> "There is scientific consensus for a common mode of action of TCDD and other chlorinated dibenzodioxins, dibenzofurans, and planar polychlorinated biphenyls (PCBs). In humans and rodents, this mode of action involves events that stem from the initial binding of TCDD to the aryl or aromatic hydrocarbon (Ah) receptor."

IARC in making its finding that "2,3,7,8-Tetrachlorodibenzo-para-dioxin is carcinogenic to humans (Group 1)" took into consideration the following supporting evidence:

> "(i) 2,3,7,8-TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor;
>
> "(ii) this receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals;
> "(iii) tissue concentrations are similar both in heavily exposed human populations in which an increased overall cancer risk was observed and in rats exposed to carcinogenic dosage regimens in bioassays."

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 U.S. EPA published it with minor changes as the 2003 draft. In 2004 they submitted it to the National Academy of Sciences/National Research Council (NAS/NRC) for another lengthy review process. The NAS completed its review and published an extensive critique in 2006 (NAS, 2006).

The NAS agreed that TCDD is at least "likely to be carcinogenic to humans" under the U.S. EPA's 2005 cancer guidelines. They recommended that U.S. EPA revise the risk assessment to provide

- Justification of approaches to dose-response modeling for cancer and noncancer end points.
- Transparency and clarity in selection of key data sets for analysis.
- Transparency, thoroughness, and clarity in quantitative uncertainty analysis, including providing ranges of plausible values and central estimates.

The committee also recommended that U.S. EPA consider the dose-response obtained in the most recent animal cancer bioassay (NTP, 2004) and encouraged U.S. EPA to develop an RfD for TCDD and its congeners to improve risk assessments for other than lifetime exposure scenarios. In addition, they recommended that U.S. EPA continue to use body burden as the preferred dose metric, but consider use of physiologically-based pharmacokinetic (PBPK) modeling for the rodent to human extrapolation.

The OEHHA analysis is generally consistent with the recommendations of both the SAB panel and the NAS committee. However a more detailed uncertainty analysis and PBPK modeling was beyond the scope of our risk assessment. OEHHA agrees that there is considerable uncertainty associated with extrapolation to low environmental exposures and low risk levels for this (or any other) chemical, and believes that 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). 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, 2003).

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, with the rationale that any RfD the Agency would recommend under the traditional approach for setting an RfD would likely be two to three 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 "no significant risk level" for TCDD calculated for California's Proposition 65 is 5 pg/day (OEHHA, 2004). This calculation uses a TCDD cancer potency factor of $1.3 \times 10^{5}$ $(mg/kg\text{-}day)^{-1}$ derived by the Air Toxics group in 1986 (DHS, 1986; OEHHA, 2009a). This potency factor was based on the incidence of liver tumors in a gavage study in male mice (NTP, 1982a). The higher potency factor derived in the current PHG document, $7.7 \times 10^{5}$ $(mg/kg\text{-}day)^{-1}$, or 0.77 $(ng/kg\text{-}day)^{-1}$, is based on the latest NTP study (NTP, 2004) in female rats. This new multisite cancer potency factor calculation, derived using updated methodology, is considered to represent a more accurate estimate of potential human cancer risk.

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# Responses to Major Comments on Technical Support Document

# 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**

**September 2010**

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## INTRODUCTION

The following are the combined responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for 2,3,7,8-tetrachlordibenzodioxin, commonly known as TCDD. The comments from the University of California reviewers and the Chlorine Council are based on the pre-release review draft, completed in 2005, while the last two reviewers were commenting on the second posted version (June 2007). Changes in response to these comments have been incorporated into the final version posted on the OEHHA website; no comments were received on the third posting. For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA web site at www.oehha.ca.gov. OEHHA may also be contacted at:

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# RESPONSES TO MAJOR COMMENTS RECEIVED

### Comments from Christopher Vogel, University of California, Davis

Comment 1: "The authors might be not correct finding that there is no report of human exposure only to TCDD. The accident of Seveso in 1976, which is cited later in the PHG document, describes a scenario where humans were exposed to high levels of almost pure 2,3,7,8-TCDD. Besides the non-cancer effects listed in the document, the authors should consider the results of the Seveso accident cohort showing evidence of excesses of several specific types of cancers (Bertazzi *et al*., 2001) which is in line with findings of epidemiologic studies from the U.S., the Netherlands, or Russia."

Response 1: Indeed, the 1976 Seveso, Italy accident differs in several important ways from other epidemiological studies involving exposure to dioxin. The Seveso accident exposed potentially large numbers of individuals in the local population to almost pure TCDD (the contents of a TCP reactor in a chemical plant were vented directly into the atmosphere). This is in contrast to most of the occupational studies, which entailed concomitant exposure to other chemicals in addition to dioxin, and were comprised mostly of male workers. A summary of the Bertazzi *et al.* (2001) study, including both cancer and nonmalignant results, has been included in the PHG document.

Comment 2: "With respect to developmental effects, the authors might consider a recent report showing that the AhR is not only important to mediate the toxicological response of TCDD but is also required for the developmental closure of a hepatic vascular shunt known as the ductus venosus (Walisser *et al.*, 2004)."

Response 2: This study has been reviewed and added to the PHG document.

Comment 3: "Regarding the dose-response assessment of noncarcinogenic effects another subchronic study could give some more support to the existing data: Vogel *et al.,* 1997."

Response 3: This study has been reviewed and added to the PHG document.

Comment 4: "The proposed PHG value for non-carcinogenic effects at 7 pg/L is based on a study with sensitive endpoints in mice finding a relatively low LOAEL of 1 ng/kg-day (Toth *et al*., 1979). This value might be too high and reconsidered for sensitive individuals and children since it is calculated for adults with a bodyweight of 70 kg."

Response 4: Studies in animals do suggest that females and children may be more or especially susceptible to the toxic effects of TCDD. Also, since some segments of the population consume many times the average level of fat per day, the principal exposure pathway for dioxins in the general population, they may be at higher risk. These factors should be accounted for with the revised calculation, with a health-protective concentration for non-carcinogenic effects of 2 pg/L.

U.S. EPA has not seen fit to determine an RfD (a non-cancer health protective value) to which our non-cancer value might be compared. Their stated reason is that human body burdens are already "at or near levels associated with adverse health effects" for both cancer and non-cancer (with the customary large uncertainty factors used for non-cancer risk assessment). U.S. EPA has indicated that the estimated safe level would likely be far below background environmental exposure levels (U.S. EPA, 2003). We agree, but think it is useful to provide a value.

As for health-protectiveness, our PHG is based on cancer findings, which results in a still lower level. We believe that this PHG provides an adequate margin of safety to protect potential sensitive subpopulations against all of the noncarcinogenic effects of TCDD, including adverse effects on the immune system, cardiovascular system, liver, and reproductive/developmental effects, as well as the carcinogenic effects.

**Comments from Daniel Chang, University of California, Davis**

Comment 1: "In the section dealing with "Environmental Occurrence and Human Exposure" it might be useful to provide a context for the public regarding exposure to 2,3,7,8-TCDD and biologically related compounds. It could be pointed out that environmental releases of "dioxins" are dominated by releases to air from combustion sources, and that ambient air levels of "dioxins" have declined since about the mid-1970's."

Response 1: Additional information on temporal trends in release of dioxins, expressed as TCDD/TEQs, has been added under the Environmental Occurrence and Human Exposure section.

Comment 2: "In the last sentence of the second paragraph the statement is made, "The U.S. EPA (2000) has estimated that the general human population is exposed to daily doses of ~0.3 pg/kg-day". Because of the location of that statement in the context of the discussion on "air", confusion may result over whether this is the "airborne" dose".

Response 2: This sentence, which included, "from all sources," has been moved to avoid any potential confusion.

Comment 3: *The developmental studies listed in Appendix A should be added to the PHG document.*

Response 3: The following *in vivo* and *in vitro* developmental studies, in primates and human trophoblasts, respectively, were added to the PHG document: Moran *et al.* (2001, 2004), Scott *et al.* (2001), Chen *et al.* (2003).

Comment 4: "The document states that differences as large as a factor of 1000 are observed for the same biological endpoint between the most sensitive and least sensitive species. A statement of where humans lie on that spectrum, in the case of TCDD might assist the public in assessing the conservatism that is built into the proposed PHG. These data might also be added to Table 5 so that the dose at which the observed effect occurred would be more readily seen"

Response 4: The discussion has been expanded, which should clarify that humans are one of the less sensitive species to dioxins with regard to acute effects. Table 5 would not, in our opinion, be useful for providing much perspective on this point because the observations on toxic effects in humans involve far different conditions as well as endpoints. With respect to cancer, comparisons of human and animal $ED_{01}$s for increased tumors, on a body burden basis, show approximately equal potency for TCDD. This also should be more clear in the present form of the discussion – although the data from the animal studies are quantitatively more precise.

Comment 5: "In the summary it is stated that the PHG is based on TCDD alone rather than all its congeners, and a reason is provided in the first complete paragraph on page 2…little sense is provided as to the relative proportion of TCDD's contribution to the TEQ compared with congeners typically present. Thus the public may have difficulty gauging the level selected for TCDD provides an adequate margin of safety…."

Response 5: 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). The relative proportion of TCDD to other congeners depends on many factors, including the source of environmental contamination and the physical environment, However, TCDD is the major contributor to dioxin toxicity equivalent (TEQ) in most environmental media, and many researchers have chosen to measure only TCDD. For drinking water, relatively little data on levels of TCDD or any of the PCDDs are available; that is, none of the PCDDs are detectable in finished water. Therefore we think that, for the intended purposes of this risk assessment, there should be no particular reason for confusion.

Comment 6: "State clearly whether the USEPA max allowable concentration for dioxin in drinking water is isomer specific or TEQ. The public may also be confused then when they see the USEPA MCL of 0.03 ng/L on pg. 1. Did the

authors of the draft document mean to clarify “surface water guideline” rather than drinking water on pg. 7?”

Response 6: The U.S. EPA Maximum Contaminant Level (MCL) of 0.03 ng/L refers specifically to TCDD. Page one of the PHG document has been changed to reflect this. The reference to “surface water guideline,” now on page 5, specifically refers to ITEQ, or International Toxicity Equivalent Quotient, which refers to a mixture of isomers.

Comment 7: “The relationship of the calculation of daily intake, D, in the equation provided and Table 15 is not clear for the lay reader. The assumed value of the absorbed fraction, A(0.5), of the dose, D, is not provided and it is not explained that D should be divided by bodyweight in order to compute the human equivalent doses in Table 15 from the rat adipose tissue dose”

Response 7: Additional information on calculation of the Human Equivalent Doses has been added to the document.

**Comments from Clifford Howlett, Executive Director, Chlorine Chemistry Council**

Comment 1: “OEHHA’s reliance on the USEPA Draft Reassessment document is inappropriate. The analyses should not be relied upon until they have undergone a rigorous, thorough and final review. OEHHA should await the release of the NAS report before finalizing the draft PHG for TCDD.”

Response 1: The PHGs are developed from scientific studies in the peer-reviewed literature. U.S. EPA documents serve as one important source of information, and reports by NAS are another. However, as a practical matter, we do not wait for promised updated evaluations, because updates are sometimes delayed for years. The Calderon-Sher Safe Drinking Water Act of 1996 requires OEHHA to review and update the risk assessments of water contaminants at least every five years. Although with the present staffing level this is not generally possible, our timely production of chemical reviews would be made even more difficult if we waited for other agencies to finalize each of their evaluations.

However, it should be noted that the NAS committee’s report has now been released (NAS, 2006), and its conclusions are now cited in the risk assessment. Both the U.S. EPA’s Dioxin Reassessment Review Subcommittee (DRSS) of the Science Advisory Board (SAB) (U.S. EPA, 2001) and the NAS committee basically concurred with the major observations and conclusions in the U.S. EPA’s draft dioxin risk assessment.

The major concerns were on cancer risk modeling methods, quantitation and acknowledgement of uncertainty, and points related to risk communication, i.e., better acknowledgment of the uncertainty. The U.S. EPA SAB DRRS panel acknowledged that the various issues are not resolvable with current data, and

recommended that U.S. EPA complete the risk assessment with available data. The NAS (2006) recommended that the approach be justified better and the uncertainty and variability be more explicitly stated, in the final draft.

OEHHA concurs with the SAB recommendation. 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 in this case. We see nothing in the recommendations of either the SAB or the NAS which would justify further delay in publication of a drinking water standard.

Comment 2: "The use of a benchmark dose of a 1 percent response as the basis of non-cancer risk assessment results in additional, unstated conservatism in the risk assessment process"

Response 2: A benchmark dose approach was not used in the calculation of the non-cancer public-health protective level for TCDD. For the final version of the document, a lowest-observed-adverse-effect-level (LOAEL) of 3 ng/kg-day has been selected for calculation of a public health-protective concentration for noncarcinogenic effects of TCDD in drinking water, based on the NTP (2004) toxicology/carcinogenesis gavage studies of TCDD in female Sprague-Dawley rats. At the LOAEL, there were significant increased incidences of cell proliferation, gingival squamous hyperplasia, cytochrome P450 induction, as well as significant increases in lung and liver weights. The health-protective level was calculated from the estimated human body burden comparable to the LOAEL in rats.

Comment 3: "The info on general population exposure levels is out of date. See pg. 8 of PHG. Look at Lorber 2002 and Aylward and Hays, 2002 studies. Patterson et al. 2004…The exp characterization should be updated to include the most current information…"

Response 3: A section on temporal trends in TCDD/TEQs has been added to the document and updated information on general TCDD exposure levels is included in it. However, 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, as discussed by Lorber (2002), Aylward and Hays (2002), and Patterson *et al*. (2004).

Comment 4: "OEHHA's cancer potency calc is incompletely documented…"

Response 4: The calculation has been revised and additional information has been added to the PHG document to clarify calculation of the TCDD cancer potency.

Comment 5: "In the NTP (2004) carcinogenesis study, the predominant responding tumors are hepatic tumors, responding both at the lowest doses and to the greatest degree. Bioassay reports provide both adipose and liver tissue concentration data. Given the availability of hepatic tissue concentration data, use of adipose tissue concentrations as the dose metric for assessing dose-response in the NTP (2004) bioassay for hepatic tumors may be problematic."

Response 5: The choice of dose metric is dependent upon the data available. Liver and adipose tissues showed the highest levels of TCDD. No measurable concentrations of TCDD were observed in blood from treated rats at any of the study time points; thus metabolic rates for TCDD could not be calculated. Because of the liver toxicity, changes in physiological parameters (e.g., tissue volumes, organ perfusion rates) due to growth and toxicity (cell death) would have to be accounted for if one were attempting to use the liver concentration data in estimating steady-state tissue concentrations. Also, the liver/fat concentration ratio changes with TCDD dose because of an increase in the amount of microsomal TCDD-binding protein, CYP1A2, in the liver (Anderson *et al.,* 1993; Diliberto *et al.*, 2001). For high doses in chronic exposure studies, this leads to nonlinearity in the concentration of TCDD in the liver whereas, at low doses, TCDD concentration of liver as a function of dose is more or less linear. Therefore, we judged that applying estimated body burden (from adipose tissue concentrations) to cancer response data would provide the best approach.

Comment 6: "The data supporting a threshold should be acknowledged and discussed – even if OEHHA chooses to use a non-threshold approach to derive the PHG."

Response 6: We acknowledge in the Dose Response and Risk Characterization sections of the PHG document the varied opinions on the cancer dose-response extrapolation, as well as the quantitative uncertainty with regard to extrapolation to low doses and cancer risk levels for TCDD. OEHHA has utilized the approach used by the U.S. EPA (2003) and recommended in the current U.S. EPA cancer risk guidelines (U.S. EPA, 2005).

Comment 7: "[The PHG document]…does not contain key recent studies by Cole *et al.* (2003) and Bodner *et al.* (2003)…. [and] Aylward *et al*. 2005"

Response 7: The Cole *et al.* (2003) review, sponsored by the Chlorine Chemistry Council, concludes that, "The long-term accumulation of negative, weak, and inconsistent findings suggests that TCDD eventually will be recognized as not carcinogenic for humans." This is simply not supported by the weight of the scientific evidence, either in humans or experimental animals. The 1976 Seveso, Italy industrial accident was one in which several thousand people were potentially exposed to relatively pure TCDD. Bertazzi *et al.* (2001) conducted an extended follow-up of this population 20 years later. An excess of lymphohemopoietic neoplasms was found in both genders. In previous

experimental studies, a dose-related increase of lymphoma was found in both male and female mice (NTP, 1982, 2004; Della Porta *et al.*, 1987). In the Bertazzi *et al.* (2001) study, all-cancer deaths were *significantly* in excess after 15 years amongst males living in the high-exposure zones. The magnitude of the excess was similar to that estimated in previous long-term studies of high-exposure, male occupational cohorts (Saracci *et al.,* 1991; Flesch-Janys *et al.*, 1995; Kogevinas *et al.,* 1997). Mortality from rectal cancer and lung cancer was also elevated among males. The lung is one of the organs targeted by the carcinogenic action of TCDD in rats and mice (Kociba *et al.*, 1978; NTP, 1982, 2004). Also, at least one other occupational cohort study found an increase in rectal cancer (Flesch-Janys *et al.,* 1998).

Cole *et al.* (2003) state that, "The epidemiologic studies of occupational exposures, pesticide applicators, and community exposures following industrial accidents, notably Seveso, have generated overall risks of all cancer of about 1.0." In fact, in the Seveso population, the relative risks of Hodgkin's disease, non-Hodgkin's lymphoma, myeloid leukemia, and rectal cancer were 4.9, 2.8, 3.8, and 2.4, respectively (Bertazzi *et al.,* 2001). Although we reject the conclusions of Cole *et al.* (2003), it is now cited in the PHG document.

The epidemiological investigation of Bodner *et al*. (2003), which reports no significant increase in cancer mortality in a cohort of chemical workers, has been added to the cancer section.

Aylward *et al.* (2005) argue that current PBPK models need to be modified to account for elimination of unchanged TCDD via lipid partitioning from the circulation into the large intestine. Their study is based on published human data from 39 persons, in which the hepatic elimination rate parameter for each person was varied to optimize model fit to the data. According to the authors, the data and model results indicate that, for males, the mean apparent half-life of TCDD ranges from less than 3 years at serum lipid levels above 10,000 ppt to over 10 years at serum lipid levels below 50 ppt. Aylward *et al.* (2005) state that "specific values of the individual parameters used in this modeling should be interpreted with caution." We agree; this is not a model that has been rigorously tested or scientifically validated.

A number of other investigators have proposed that the elimination kinetics for TCDD are concentration-dependent, which is at least partly related to AhR-mediated induction of cytochrome P450 1A2 (CYP1A2). In both the human and animal data, as the dose increases the apparent half-life decreases, indicating an inducible elimination of TCDD. These studies are discussed in the PHG document. At present, human data are insufficient to determine the shape and parameters of the dose-response curve for the liver fraction due to induction of CYP1A2 in the liver. Increased elimination rates have typically been observed in instances where body burdens are substantially elevated, compared to exposures at environmental levels, although the data are too limited to validate a PBPK model that incorporates an inducible elimination of TCDD. Therefore the decision has been made to use the human half-life for TCDD of 7.1 years, which has been accepted by U.S. EPA (2003), for the PHG cancer calculation. (A

number of studies entailing TCDD exposure in both occupationally and non-occupationally-exposed cohorts have reported that the half-life for TCDD ranges from about 7 to 9 years (Flesch-Janys *et al.*, 1996, Michalek and Tripathi 1999; Needham *et al.,* 1994, 1997; Rohde *et al.*, 1999)).

Comment 8: "Finally, the benchmark dose modeling methodology used by the USEPA results in comparison of 1% responses across a wide range of biochemical, tissue, and adverse response endpoints, all with differing biological significance and control animal variability. A 1% percent change in enzyme activity is biologically trivial (and undectable); a 1% incidence of cleft palate is not trivial but is still undetectable in most experimental protocols. The USEPA (2000) analysis incorporates factors of unstated additional conservatism (several to more than 10-fold) compared to traditional risk assessments."

Response 8: We agree with the U.S. EPA that changes in biochemical indices can be linked to toxic responses, and that applicable data are certainly available for this purpose for TCDD. However, our analysis is not based on the benchmark approach used by U.S. EPA.

**Comments from Minnesota Department of Health**

Comment 1: "Why is the Goodman and Sauer (1982) re-evaluation of the Kociba data not discussed or included in Tables 10-12?"

Response 1: A discussion of the Goodman and Sauer (1982) paper was inadvertently omitted from the PHG document. This has now been corrected. The Goodman and Sauer (1982) tumor incidence data have also been added to Table 11 of the PHG document. Table 10 presents only male rat tumor incidence data, and Goodman and Sauer (1982) only re-evaluated liver sections; liver tumors were not found in male animals in this study. Similarly, Table 12 compares tumor incidences between the Kociba *et al*. (1978) and Squire (1980) reports, which include more than just liver tumor incidence data.

Comment 2: "Table 14 should include NTP 2004 data/calculations for comparison."

Response 2: We agree. These are now included.

Comment 3: "Did you attempt to account for the stop-dosage group in your analyses of the NTP 2004 data? The data seem to suggest that timing (of dosing and evaluation) may be very important – and may be more important than some of the human equivalent dose (HED) adjustments."

Response 3: For development of the PHG cancer-based number, the issue of concern is chronic exposure, so the cancer analysis focused on the most

relevant data for that endpoint. The NTP (2004) stop exposure data comprised an exposure duration of only 30 weeks (and at only a single dose level), so we decided not to attempt to incorporate these data into the analyses.

Comment 4: "The document specifies an absorbed dose of 0.5 – NTP cites 66-93% (84%). Typically, GI absorption is not corrected in the calculation of risk, especially when the difference from 100% is minimal. Adjustments are often incorporated into exposure equations."

Response 4: For this calculation, we are assuming that 100 percent of the TCDD present in drinking water would be absorbed, but that a lesser fraction would be absorbed under the conditions of the NTP study. The 0.5 estimate is more health-protective (in effect, doubling the potency per mg dioxin administered), but not excessive, in our opinion.

Comment 5: "How was the Monte Carlo used? It is not clear what the independent and dependent variables were in the Monte Carlo, nor is it clear what the uncertainty is for the data used. Why weren't deterministic calculations used?"

Response 5: The linear term ($q_1$) of the multistage model is first estimated based on dose-response data for each of the treatment-related tumor sites (tumor incidence data taken from Table 9). Statistical distributions, rather than point estimates, are generated at each site by tracing the profile likelihood of the linear term ($q_1$). The distributions of $q_1$ for each of the treatment-related sites are then statistically summed using a Monte Carlo approach and assuming independence. The sum is created by adding the linear term for each tumor site, according to its distribution, through random sampling with 100,000 trials. The upper 95 percent confidence bound on the summed distribution is taken as the multisite cancer potency estimate ($q_1$*). Deterministic calculations are less useful when summing results from multiple sites.

Comment 6: "HED conversions are not shown or explained. You appear to be normalizing to "rat adipose tissue concentrations" but your units in Table 15 are pg/g-day. Should this be pg/g? It appears that you are using the mean of the adipose tissue concentrations (pg/g) from the NTP study (Table 13). What is the adipose tissue equivalence $q_1$* in Table 16? Why is it calculated and how is it used in your risk calculations?"

Response 6: Yes, the correct units for Rat Adipose Tissue Concentrations in Table 15 are pg/g. This has now been corrected. For calculating body burden, we used each of the adipose tissue levels at four different time points. The trapezoid rule was then used to estimate the overall average. Then, using this data and U.S. EPA's steady-state, Human Equivalent Doses (HEDs) were calculated for the various dose levels. In the previous draft of the PHG, the applied dose $q_1$* combined site estimate was mistakenly used in the final cancer

calculation in place of the adipose equivalence $q_1$*, which no doubt caused considerable confusion. This has been corrected.

Comment 7: "How can a combined site estimate for $q_1$* be less than the $q_1$* at one site (see Table 16, third column, Lung and combined site estimates)?"

Response 7: The value for lung (applied dose $q_1$*) in Table 16 should have been entered as $2.66 \times 10^3$. This has been corrected in the final document.

Comment 8: "The potency estimates in Table 16, second column, appear to over-represent tumors that were not observed very often in the study (pancreas acinar adenomas, lung epitheliomas, liver hepatocholangiomas). Further – the estimates for the three tumors are identical. Again, more transparency in how these calculations were made is needed."

Response 8: The human cancer potency estimates for pancreas acinar adenomas, lung epitheliomas, and liver hepatocholangiomas (Table 16) are $0.268 \times 10^4$, $2.66 \times 10^3$ (corrected), and $0.265 \times 10^4$, respectively. The same equation is used to fit all three sites. Those are the upper confidence bound on the lowest estimates.

Comment 9: "You do not appear to have used the actual dose to the animals anywhere in your calculations. Instead you use the adipose tissue concentration as your metric. This should be stated in your document."

Response 9: Our use of body burden (i.e., adipose tissue concentration) as a dose metric for the PHG cancer calculation is stated in several key sections in the PHG document (in the introduction, in the dose response assessment section, etc.). This follows the approach of U.S. EPA for TCDD (U.S. EPA, 2003). According to U.S. EPA, body burden (estimated at steady-state conditions) provides for a more reasonable description of dose.

Comment 10: "'Because this study and cancer potency derivation appears to be superior to earlier approaches, OEHHA has chosen these for development of the proposed PHG for TCDD'. This is not a reason to use this method – but a summary evaluation. Why is this method superior?"

Response 10: Our cancer potency derivation utilizes body burden as a dose metric, as opposed to the more traditional method of using daily intake, for species extrapolation. Body burden takes into account the considerable difference in half-life of TCDD in rats vs. humans. Although the assumption of a single TCDD half-life is uncertain, because limited data exist to validate a PBPK model that incorporates an inducible elimination of TCDD, the decision was made to use the human half-life of 7.1 years recommended by the U.S. EPA (2000, 2003) for the PHG cancer calculation because it accounts for more

uncertain variables. The cancer PHG is derived from the NTP (2004) gavage study because this provides a superior data set compared to the study of Kociba *et al*. (1979). In the latter study, which U.S. EPA used to estimate human cancer risk, survival was poor in all groups of control and exposed rats; at 2 years, only 8-22 percent of males, and 8-32 percent of females were still alive. The early mortality reduced the sensitivity of this study for determining the actual number of neoplasms induced by two years of exposure to TCDD. We believe that the NTP (2004) study, given its careful design and conduct, as well as improved survival rate, provides a superior basis for risk assessment.

Comment 11: "Can you justify the use of new female rat data and the methods used to calculate the CSF rather than other CSFs that could be calculated using other species/sex and/or other methods? (e.g., CSF calculations from male mice data (NTP 1982) would likely be different."

Response 11: All long-term carcinogenicity studies on TCDD have produced positive results. TCDD is a carcinogen at multiple sites in both sexes of rats and mice (U.S. EPA, 1985; IARC, 1997; NTP, 2004). Several studies in animals have demonstrated that female rats are more susceptible to TCDD-induced liver neoplasms than males. Sex hormones appear to exert a profound influence on the carcinogenic action of TCDD. Higher tissue concentrations and longer half-lives have been reported in females vs. males (Li *et al.,* 1995). The study design, species, and dose range used in the NTP (2004) study was based on earlier animal carcinogenicity studies. That is, female Sprague-Dawley rats were chosen because of the high incidence of hepatocarcinogenicity in females in this species and strain compared to males of this strain, as well as other species of test animals. Use of the most sensitive species, strain, and sex is standard procedure for health-protective risk estimates. The combined-site CSF calculation is now our default cancer potency calculation method, where data allow.

Comment 12: "Page 48 – carcinogenic effects – the implication is that the EPA human-data derived CSF is not conservative enough. You never say why this EPA CSF shouldn't be used or why your CSF (which is consistently less than the EPA Human CSF) is better."

Response 12: We believe that use of the NTP (2004) animal study from which our CSF was derived (and including U.S. EPA's recommended use of body burden as a dose metric for species extrapolation) constitutes a superior approach to U.S. EPA's derivation of a human CSF using epidemiological data. On pages 44-45 of the PHG document, we discuss the considerable limitations and uncertainties associated with the TCDD epidemiological literature, and in particular the lack of good exposure information. In general, potency estimates from animal studies have been found to be similar to those derived from human data (U.S. EPA, 2000).

Comment 13: *Non-cancer Hazard Calculation:* "If amyloidosis and dermatitis are effects that would be expected after a short-term exposure (prior to reaching steady state), why are they used as the principal/most sensitive chronic non-cancer endpoints? … Assuming that the dose metric used for amyloidosis and dermatitis is correct (dose and not body burden), using other studies that rely on body burden in a HED calculation would undoubtedly result in a lower RfD (e.g. NTP 2004). Why wasn't this discussed?"

Response 13: The critical study for the non-cancer PHG value has been changed to the NTP (2004) chronic exposure of female rats. The health-protective value is based on the LOAEL for significantly increased incidences of cell proliferation, gingival squamous hyperplasia, and cytochrome P450 induction, as well as significant increases in lung and liver weights.

**Comments from Cambridge Environmental, Inc., Edmund Crouch**

Comment 1: "The first and second entries, for liver, hepatocellular carcinoma, and liver cholangiocarcinomas are inconsistent with the remaining entries. The remaining entries have been calculated using MSTAGE (or a similar program) using a total of 6 parameters. To obtain the values in the "Applied Dose", the confidence limit has been calculated using 5 parameters for liver, hepatocellular carcinoma and 4 parameters for liver cholangiomas. The values using 6 parameters are 5,345 kg-d/mg and 14,134 kg-d/mg, respectively."

Response 1: When calculating values for the applied dose column, OEHHA constrained the MSTAGE model to four parameters (for liver cholangiomas) because of instability in fitting. Use of the later version of the MSTAGE model results in a small percent change in the combined site estimate for TCDD (2.7 vs. 2.6), a change of ~ 4 $x10^{-2}$ (0.1/2.6).

Comment 2: "The entry for "lung" is a factor of 10 too high. The correct value is 2,661 kg-d/mg. This looks like a typo."

Response 2: Agreed; the value for lung has been corrected in the PHG document (stated in the equivalent form of 0.266 x $10^{4}$ $(mg/kg\text{-}day)^{-1}$).

Comment 3: "A substantial part of pages 42-47 is spent detailing the purported advantages of using body burden to extrapolate to humans. At page 48, we are told "OEHHA agrees with the U.S. EPA's use of body burden as dose metric.…" Despite this, at the top of page 49, we have, "The combined cancer potency for the seven tumor sites identified in the NTP (2004) study is 2.6 x $10^{-2}$ $(ng/kg\text{-}day)^{-1}$." But this is potency calculated using intake doses and extrapolating to humans in the OEHHA standard way (assuming 70 kg human, 0.35 kg rat, and an interspecies factor proportional to the 1/3 power of the body

weight ratio). See Table 16 where this value is quite clearly derived for the "applied Dose q1*" for the "Combined site estimate for TCDD". This value does NOT correspond to using a body burden metric for extrapolation".

Response 3: The combined site estimate used in the initial PHG draft for the cancer calculation was incorrect. Instead, the adipose tissue equivalence combined site estimate $q_1$* should have been used. This has been corrected, and the cancer PHG value has been re-calculated.

Comment 4: *Inadequate description of methodology:* "The methodology described at page 46 for "Multi-Site Analysis" is too abbreviated to be adequate, although I believe I have reproduced what was done (see above). I believe that the 0.1 to 99.9 percentile points by steps of 0.1% were calculated (a total of 999 points), and these were sampled with equal frequency (but see item 7 below). The precise methodology should be specified. MSTAGE produces the percentiles one value at a time (there is a tabular facility, but it does not produce that particular table), and I understand some automated procedure was used to run MSTAGE. That procedure should be made publicly available (or I will modify MSTAGE if it is felt desirable to produce such tables; however, it is really unnecessary, see below). It is not clear whether 6, 5, or 4 parameters were used for some of the analyses (see items 1 and 3 above), and the basis for any such selection is not given. The spreadsheet that was used for the Monte Carlo procedure, and the data input to that spreadsheet, should also be provided, in order to allow an adequate technical evaluation. The approach of generating individual percentage points to approximate a distribution is cumbersome. A more elegant approach is to use the tables produced by MSTAGE that provide the change in log likelihood and various gradients as the parameter values are stepped. These tables can be used to fit the log likelihood very accurately with cubic splines, and these splines can then used for the distributions. However, this approach is unnecessary in this case (see item 5)."

Response 4: A combined response to comments 4 and 5 is provided at the end of comment 5, below.

Comment 5: "The Monte Carlo procedure described at page 46 ("Multi-Site Analysis") is unnecessary to sum across multiple end-points. An approach that simply extends the standard EPA style likelihood-based approach (as carried out for single end points in MSTAGE) is much easier, more in the spirit of the original (single-end-point) approach, and is readily implemented in a spreadsheet [indeed, all the calculations performed by MSTAGE are easy to carry out in a spreadsheet]. The standard approach to analysis of these bioassays simply calculates the loglikelihood for the observations, assuming binomial results and a linearized multistage dose-response. See Anderson *et al.* (1983), Quantitative Approaches in use to assess Cancer Risk, Risk Analysis 3(4)277–295. The upper confidence limit on the linear term is found by maximizing that linear term (treating all the parameters of the dose-response model, including the linear

term, as free to vary) subject to twice the decrease in loglikelihood from its maximum value being less than or equal to a critical value (approximately 2.70554). Extension to the sum of multiple end points is straightforward. The log likelihood in this case is formed as the sum of the log likelihoods for all the end points treated in exactly the same way as for evaluation of each end point individually (with individual dose-response curves for each). Then the sum of the linear terms is obtained, and its upper confidence limit is found in exactly the same way (maximizing this sum, treating all the parameters of all the dose-response models for the individual end points as free to vary, subject to twice the decrease in log likelihood being less than or equal to the critical value)."

"If my hypothesis as to the procedure adopted is correct [see item 4 above], the Monte Carlo procedure adopted is slightly incorrect. Sampling the 0.1% step 0.1% to 99.9% points effectively omits the two 0.05% regions at the top and bottom ends. A better approximation would be to generate the 0.05% step 0.1% to 99.95% points, and sample those (1000) points with equal probability. [It is possible that the 0.1% step 0.2% to 99.9% points were sampled with equal probability, which would be correct, but I cannot tell from the material presented]. The effect of this correction would be small (I have not bothered to evaluate it) compared with other approximations involved."

Response to comments 4 and 5: While using MSTAGE tables may be a more elegant methodological approach, it does not alter the overall result (i.e., accuracy). The Monte Carlo procedure has been peer-reviewed through the regulatory setting processes, and provides an acceptable degree of transparency. We believe that the Monte Carlo approach to approximating a distribution is more easily understood by the public than discussing alterations and likelihood functions. OEHHA has adopted several standards based on this approach.

Comment 6: *The introduction of $LED_{01}$ on page 49 is misleading.* "On page 49, following the first equation, the definition of CSF is given as $0.01/LED_{01}$. This is incorrect, however, since the CSF in this case is not so derived".

Response 6: Agreed. This mistake has been corrected.

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