APPENDIX B

TOXICOLOGICAL PROFILES

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TOXICOLOGICAL PROFILES

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B1: TOXICOLOGICAL PROFILE - DIOXINS

B1-1.0 BACKGROUND INFORMATION

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) constitute a group of highly persistent ubiquitous chlorinated organic chemicals (Haws *et al*., 2006; WHO, 2000a,b). They have been found to be persistent, bioaccumulative toxicants and have been found in fish, birds and animal tissue and in human adipose tissue and milk (Haws *et al*., 2006). They are generally unwanted contaminants that have no known industrial use but are by-products of industrial operations and combustion processes, including chlorine bleaching of paper and pulp, production of chlorinated phenols and their derivatives, burning of wastes and fuels and metal smelting (CEPA, 1990; ATSDR, 1998; Haws *et al*., 2006). The largest source of dioxins in Canada is the large-scale burning of Municipal and medical waste (Health Canada, 2005).

A number of dioxin or furan congeners, and co-planar, "dioxin-like" PCBs have demonstrated numerous toxic responses similar to the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 2,3,7,8-TCDD; or, dioxin). The effects include dermal toxicity, immunotoxicity, reproductive effects and teratogenicity, endocrine disruption and carcinogenicity (WHO, 2000b). Epidemiological studies of dioxin exposed workers have not consistently found effects beyond prolonged chloracne (CEPA, 1990).

Dioxins are found in humans with higher levels found in those inhabiting industrialized countries (Schecter and Gasiewicz, 2003). There is growing evidence that levels of dioxins in the U.S. and Europe are decreasing in the environment due to more stringent regulations on industrial practices throughout the industrialized world (Schecter *et al*., 2006). Dioxins are fat soluble, with human exposure almost exclusively through consumption of animal foods such as meat, fish and dairy products (Startin and Rose, 2003; U.S. EPA, 2004b). Because of the fat solubility, lactation may reduce levels in nursing women; however, this may lead to increased exposure to nursing infants (Schecter *et al*., 1996).

Structurally related compounds that bind to the aryl hydrocarbon receptor (AhR), the ligand-activated transcription factor, are commonly referred to as dioxin-like compounds (DLCs). Due to their persistence, tendency to biomagnify through the food chain and lipophilicity, once consumed they accumulate in humans potentially causing chronic lifetime human exposure. Since the mechanism of action is the same and because they are commonly found in the environment as a mixture, the Toxic Equivalency Factor (TEF) methodology has been developed. PCDDs, PCDFs and dioxin like PCBs have been assigned a TEF based on their relative potency as compared to 2,3,7,8-TCDD, which has been assigned a TEF of 1 (van den Berg *et al.,* 1998). The amount of congener present in a sample is multiplied by its TEF to give the "toxic equivalent" concentration (TEQ) for the congener expressed in terms of 2,3,7,8-TCDD. This procedure is followed for all AhR-active compounds in the sample. The TEQ for all compounds is summed, the result being "total TEQ" for the sample. This quantity represents the equivalent amount of 2,3,7,8-TCDD that would have to be present to be equally toxic as all the congeners present in the mixture. The evaluation of appropriate TEFs is ongoing and may change based on new data and recent reviews. The total dioxin toxic equivalency (TEQ) approach and current values have been accepted internationally as the most appropriate way to estimate the potential health risks of dioxin mixtures (Schecter *et al*., 2006). Table B1-1, adapted from Schecter *et al.*, (2006) presents the World Health Organization (WHO) TEFs.

Several TEF schemes have been developed (Table B1-1). Currently, the WHO_{98} scheme is the approach preferred by regulatory agencies internationally. Unless noted otherwise, all TEQs reported in this report utilize the WHO_{98} TEFs.

Table B1-1 Various Toxic Equivalency Factors (TEFs)

		EPA_{87}	$NATO_{89}$	WHO_{94}	WHO_{98}
Dioxins (PCDDs)	2,3,7,8-TCDD	1	1		1
	1,2,3,7,8-PCDD	0.5	0.5		1
	1,2,3,4,7,8-HCDD	0.04	0.1		0.1
	1,2,3,6,7,8-HCDD	0.04	0.1		0.1
	1,2,3,7,8,9-HCDD	0.04	0.1		0.1
	1,2,3,4,6,7,8-Hepta CDD	0.001	0.1		0.01
	OCDD	0	0.001		0.0001
Dibenzofurans (PCDFs)	2,3,7,8-TCDF	0.1	0.1		0.1
	1,2,3,7,8-PCDF	0.1	0.05		0.05
	2,3,4,7,8-PCDF	0.1	0.5		0.5
	1,2,3,4,7,8-Hexa CDF	0.01	0.1		0.1
	1,2,3,6,7,8-Hexa CDF	0.01	0.1		0.1
	1,2,3,7,8,9-Hexa CDF	0.01	0.1		0.1
	2,3,4,6,7,8-Hexa CDF	0.01	0.1		0.1
	1,2,3,4,6,7,8-Hepta CDF	0.001	0.01		0.01
	1,2,3,4,7,8,9-Hepta CDF	0.001	0.01		0.01
	OCDF	0	0.001		0.0001
PCBs	3,3',4,4'-TCB (#77)			0.0005	0.0001
	3,4,4',5-TCB (#81)			-	0.0001
	2,3,3',4,4'-PeCB (#105)			0.0001	0.0001
	2,3,4,4',5-PeCB (#114)			0.0005	0.0005
	2,3',4,4',5-PeCB (#118)			0.0001	0.0001
	2',3,4,4',5- PeCB (#123)			0.0001	0.0001
	3,3',4,4',5- PeCB (#126)			0.1	0.1
	2,3,3',4,4',5-HxCB (#156)			0.0005	0.0005
	2,3,3',4,4',5'-HxCB (#157)			0.0005	0.0005
	2,3',4,4',5,5'-HxCB(#167)			0.00001	0.00001
	3,3',4,4',5,5'- HxCB (#169)			0.01	0.01
	2,2',3,3',4,4',5-HpCB(#170)			0.0001	-
	2,2',3',4,4',5,5'- HpCB (#180)			0.00001	-
	2,3,3',4,4',5,5'-HpCB(#189)			0.0001	0.0001

Adapted from U.S. EPA (2003)

- U.S EPA_{87} – U.S EPA (1987)
- $NATO_{89}$ – NATO/CCMS (1988)
- WHO_{94} – Ahlborg *et al*. (1994)
- WHO_{98} – van den Berg (1998)

$NATO_{89}$ is commonly refered to as the NATO International TEF Scheme (I-TEQ)

For the evaluation of DLC, it is important to consider the contributions of the entire mixture. However, for the benefit of this report the toxicological profile outlines the physical and chemical properties and toxicological data specifically focusing on TCDD, the most toxic and well studied dioxin. Where it is necessary this document refers to relevant data related to other DLCs that are essential in the development of appropriate guidelines and limits.

This document is divided into various sections: physical and chemical properties, pivotal toxicological data, human health effects including a review of the non-cancer and cancer effects, populations at risk, toxicokinetics, review of international basis for dioxin exposure limits and environmental fate. For a complete toxicological profile for PCDDs and PCDFs please refer to ATSDR, 1998.

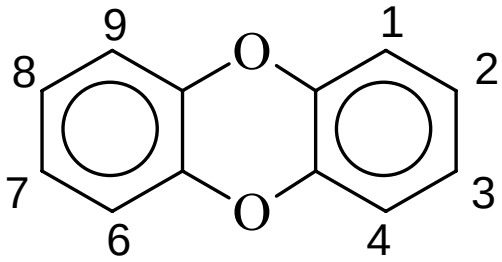
B1-2.0 CHEMICAL AND PHYSICAL PROPERTIES

Dioxins (polychlorinated dibenzo-p-dioxins) consist of two benzene rings connected by a two oxygen atoms and contain four to eight chlorines (Figure B1-1). Similarly, furans (polychlorinated dibenzo-p-furans) consist of two benzene rings connected at the 1,9 positions and by an oxygen at the 6,4 positions, and may contain four to eight chlorines (Figure B1-2).

Dioxins specifically are a group of chemicals of chlorinated hydrocarbons whose basic structural formula is a dibenzo-p-dioxin (DD). There are 8 homologues of CDDs, monochlorinated through octachlorinated. There are 75 congeners, consisting of 2 monochlorodibenzo-p-dioxins (MCDDs), 10 dichlorodibenzo-p-dioxins (DCDDs), 14 trichlorodibenzo-p-dioxins (TrCDDs), 22 tetrachlorodibenzo-p-dioxins (TCDDs), 14 pentachlorodibenzo-p-dioxins (PeCDD), 10 hexachlorodibenzo-p-dioxins (HxCDDs), 2 hepta-chlorodibenzo-p-dioxins (HpCDDs) and 1 octachlorodibenzo-p-dioxin (OCDD) (ATSDR, 1998). The numbers in Figure B1-1 indicate the positions for chlorine substitutions.

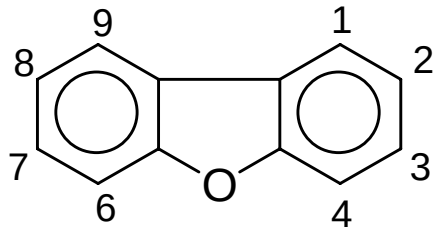
The most hazardous and well studied dioxin is 2,3,7,8-tetrachlorodibenzodioxin, which has chlorine atoms attached at positions 2, 3, 7 and 8.

Structures:



CDDs

Figure B1-1 Chemical Structure of CDDs



CDFs

Figure B1-2 Chemical Structure of CDFs

Chemical and Physical Properties:

The chemical and physical properties of CDD are outlined in Table B1-2. Information specifically on **2,3,7,8- TCDD are in bold**. Please refer to ATSDR (1998) for a thorough physical and chemical profile of all PCDDs and PCDFs.

Table B1-2 Chemical and Physical Properties of Tetrachlorodibenzo-p-dioxins (TCDD)

Chemical/Physical Property	Result	Reference
CAS #	30746-58-8 (1,2,3,4-) 53555-02-5 (1,2,3,8-) 34816-53-0 (1,2,7,8-) 33425-92-6 (1,3,6,8-) 50585-46-1 (1,3,7,8-) **1746-01-6 (2,3,7,8-)**	ATSDR, 1998
Synonym/ Trade names	1,2,3,4- or 1,2,3,8- or 1,3,6,8- or 1,2,7,8- or 2,3,7,8- Tetrachlorodibenzo-p-dioxin; 1,2,3,4- or 1,2,3,8- or 1,3,6,8- or 1,2,7,8- or 2,3,7,8- Tetrachloro-dibenzodioxin; 1,2,3,4- or 1,2,3,8- or 1,3,6,8- or 1,2,7,8- or 2,3,7,8- Tetrachlorodibenzo[b,e](1,4)dioxin; 1,2,7,8- or 2,3,7,8- Tetrachlorodibenzo-1,4-dioxin; 2,3,6,7-Tetrachloro-dibenzodioxin; 1,2,7,8-Tetrachlorodibenzo-p-dioxin; Dioxin; TCDBD; TCDD	ATSDR, 1998
Number of possible isomers	22	ATSDR, 1998
Empirical formula	$C_{12}H_4Cl_4O_2$	
Molecular weight	322	ATSDR, 1998; JW, 2006
Physical state	Crystalline solid (2,3,7,8-)	ATSDR, 1998
Boiling point ^oC (760 mm Hg)	446.5 ^oC (2,3,7,8-)	
Melting point	190 ^oC (1,2,3,4-) 175 ^oC (1,2,3,7-) 219-219.5 ^oC (1,3,6,8-) 193.5-195 ^oC (1,3,7,8-) **305-306 ^oC (2,3,7,8-)**	ATSDR, 1998
Density	1.827 g/mL	
Odour	No data	

Table B1-2 Chemical and Physical Properties of Tetrachlorodibenzo-p-dioxins (TCDD)

Chemical/Physical Property	Result	Reference
Solubility in water (at 25 °C)	$4.7x10^{-4}$ -$6.3x10^{-4}$ mg/L (1,2,3,4-) $4.2x10^{-4}$ mg/L (20 °C) (1,2,3,7-) $3.2x10^{-4}$ mg/L (20 °C) (1,3,6,8-) $2.0x10^{-4}$ mg/L (2,3,7,8) $1.9x10^{-5}$ mg/L (2,3,7,8) **$7.9x10^{-6}$-$3.2x10^{-4}$ mg/L (2,3,7,8-)**	ATSDR, 1998; JW, 2006
Solubility in organic solvents	o-dichlorobenzene, chloro-benzene, benzene, chloroform, n-octanol	ATSDR, 1998
Henry's Law Constant	**$16.1x10^{-6}$-$101.7x10^{-6}$ atm-m^3/mol (2,3,7,8)**; $7.01x10^{-6}$ -$101.7x10^{-6}$ atm-m^3/mol; $5.0x10^{-5}$ atm-m^3/mol (25°C)	ATSDR, 1998; JW, 2006
Vapour Pressure (at 25 °C)	$7.5x10^{-9}$ mmHg ; 4.8 x 10^{-8} mmHg ; **$1.5x10^{-9}$-$3.4x10^{-5}$ mmHg (2,3,7,8-)**; $5.3x10^{-9}$-$4.0x10^{-3}$ mmHg (1,3,6,8-); **$7.4x10^{-10}$ mmHg (2,3,7,8)**	ATSDR, 1998 ; JW, 2006
Log octonol/water partition coefficient (K_{ow})	7.02-8.7(1,2,3,4-) 7.02 (2,3,7,8-) 7.39-7.58 (2,3,7,8-) **6.8 (2,3,7,8-)** 6.6 (1,2,3,4-)	Mackay *et al.,* 1992 ATSDR, 1998; JW, 2006
Log K_{oc}	5.16	JW, 2006
Half life (environmental) for **2,3,7,8-TCDD**	Air: Range: 100-300h; Mean: 170h (~1 week) Water: Range: 300-1,000h; Mean: 550h (~3 weeks) Soil: Range: 1-3 years; Mean: 17,000 h (~2 years) Sediment water: Range: >30,000 h; Mean: 55,888 (~6 years)	Mackay *et al*., 1992; JW, 2006

B1-3.0 TOXICOLOGY SUMMARY

B1-3.1 Pivotal Toxicological Data

Numerous effects have been reported in multiple animal studies following exposure to PCDDs, PCDFs and PCBs. Because of the plethora of data available for 2,3,7,8-TCDD and the limited available data on other dioxin-like compounds, the focus of this review will be on 2,3,7,8-TCDD.

The most sensitive toxic and biochemical endpoints on a body burden basis are: endometriosis, developmental neurobehavioural (cognitive) effects, developmental reproductive (sperm counts, female urogenital malformations) effects, and adult and developmental immunotoxic effects (WHO, 2000b).

Table B1-3 Most Sensitive Effects of 2,3,7,8-TCDD in Animals

Effect	Species	Exposure (LOEL or LOAEL) (pg/kg bw/day)	Body Burden (increment to background) (ng/kg)
Adverse effects			
Developmental effects Neurotoxicity (object learning)	Rhesus monkey	~160	42
Reproductive toxicity Decreased sperm count Vaginal threads	Rat	 64 000 pg/kg bw 200 000 pg/kg bw	 28 73
Immunotoxicity	Rat	100 000 pg/kg bw	50
Immunological (viral sensitivity)	Mouse	10 000 pg/kg bw	10
Hormonal (endometriosis)	Rhesus monkey	~160	69
Effects that may or may not lead to adverse effects			
Biochemical effects			
CYP1A1	Mouse	150	3
	Rat	100	3
CYP1A2	Mouse	450	10
EGFR	Rat	100	3
IL1beta	Mouse	300	10
Functional effects			
Oxidative stress	Mouse	450	~10
Lymphocyte subsets	Marmoset monkey	~200	6-8

Adapted from WHO, 2000a

Of these endpoints, development of reproductive system in rats was identified by JECFA (2001) to be the most sensitive endpoints in male rat offspring of treated females. The most sensitive reproductive endpoint in male rat offspring were effects on sperm counts and ventral prostate weight (JECFA, 2001). In female rat offspring studies, whose effects included vaginal thread abnormalities the doses were somewhat higher than those that induced effects in male rat offspring. Table B1-4 summarizes developmental effects in rat offspring.

Table B1-4 Most Sensitive Effects of 2,3,7,8-TCDD in Rat Offspring

Effects	Dose (µg/kg)	Reference
Suppressed DTH response	0.1(males); 0.3 (females)	Gehrs and Smialowicz 1998
Diminished reproductive success	0.01	Murray *et al.*,1979
Toxic to reproductive capability	0.1	
Delayed testicular decent and Reproductive development	0.064 -0.4	Mably *et al.*, 1992b
Reproductive functions	0.064 -1	Mably *et al.*, 1992c
Reproductive success	0.064 -1	Mably *et al*., 1992d
Reproductive function	0.05 - 0.8	Gray *et al*., 1997a
Reproductive development	0.05 - 0.8	Gray *et al*., 1997b
Reproductive function (males)	0.025 µg/kg bw	Faqi *et al*., 1998
Reproductive function (females)	0.025, 0.3 µg/kg bw	Faqi *et al.*, 1998
Reproductive function (females)	0.010	Ostby *et al*., 1999
Reproductive development	0.0125 - 0.8	Ohsako *et al*., 2001

Adapted from JEFCA (2001)

A wide range of dose-dependent health effects have been documented in laboratory animals exposed to TCDD, but with large differences in sensitivity between species and even strains of animals (Paustenbach *et al*., 2006). Health effects documented include wasting syndrome, dermal toxicity, immunotoxicity, reproductive effects and teratogenicity, endocrine disruption and carcinogencity (WHO, 2000a). Developmental and reproductive toxicity, immunotoxicity and neurotoxicity have been found in rats, mice and nonhuman primates (Schecter *et al*., 2006). Most, if not all, of the effects of dioxins are mediated through interaction with the Ah receptor (AhR). The Ah-receptor is part of a naturally occurring system involved in the control and regulation of the actions of various hormones on the cells of the body. When dioxins/furans or dioxin-like chemicals bind to the AhR, a myriad of physiological response can be triggered, beginning with alterations in various enzyme activities in the cells of the body. Because of their importance in the normal functioning of biological systems, changes that alter the activity of these enzyme systems can result in several secondary effects, including changes in hormonal homeostasis and associated potential for consequences on reproduction, growth and development, as well as general maintenance of body functions.

The most sensitive indicators of TCDD toxicity appear to be the effects on the developing reproductive systems of male rat fetuses exposed *in utero* (UK COT, 2001). Several studies have reported these effects at various doses but the key study on which the evaluations by JECFA (2001), ECSCF (2001) and UK COT (2001) were based was a study reporting developmental effects in male rats following repeated subcutaneous exposure to the dams (Faqi *et al*., 1998). This study had not been published when WHO conducted its consultation regarding the reevaluation of the TDI for dioxin in 1998.

Faqi *et al*., (1998) studied the effects of low doses of 2,3,7,8-TCDD on male offspring rats (Wistar) exposed through pregnancy and lactation. The dams received an initial loading dose of 25, 60 or 300 ng/kg body weight 2 weeks prior to mating *via* subcutaneous injection. Subsequently they received weekly maintenance doses of 5, 12 or 60 ng/kg body weight throughout mating, pregnancy and lactation. Tissue concentrations were determined in a sub-group of dams killed on day 21 of gestation and in the offspring at weaning. In all exposed male offspring, caudal epididymis sperm counts were reduced and the sperm transit rate was decreased, relative to control rats. An increased number of abnormal sperm was also observed. In the highest dosed group, testosterone concentrations were decreased in adulthood.

This study represents an advance in that exposure covered both gestation and lactational periods to provide TCDD at a more steady state level, compared to previous studies that used only single-dose exposures (Faqi *et al*., 1998). In addition, target tissue concentrations were measured allowing LOAELs to be calculated on a body burden basis. It might be noted that several studies have reported effects on the developing male reproductive system due to TCDD exposure *in utero*, but the findings are inconsistent, particularly with regard to the doses at which effects on sperm counts are observed (Ohsako *et al*., 2001; Gray *et al*., 1995; 1997a; Mably *et al*., 1992a). For example, Ohsako *et al*., (2001) observed no changes in daily sperm production or sperm reserve, nor testosterone levels, in rats exposed to single oral doses up to 800 ng TCDD/kg administered on gestation day 15. Their study suggested that a decrease in ventral prostate weight in male offspring was the most sensitive effect of dioxin. The LOAEL for this effect was 200 ng TCDD/kg body weight (administered dose) with a NOAEL of 50 ng/kg body weight (Ohsako *et al*., 2001).

B1-3.2 Human Health Effects

Human health effects from exposure to dioxins are primarily from occupational, epidemiological studies and only a few intentional dioxin poisonings. Populations exposed to the highest levels of dioxin include occupationally exposed workers, for example herbicide producers. Accidentally exposed populations may be exposed *via* contamination of the environment or food as was the case for the local population in Seveso, Italy was exposed to substantial quantities of dioxin in 1976 following a chemical plant explosion (Bertazzi *et al*., 2001). Studies of highly exposed populations suggest various non-cancer health effects are associated with dioxin exposure; for example, chloracne (a skin condition), increases in liver enzymes, increased cardiovascular disease and developmental effects. However, most of these effects, such as chloracne, appeared only at doses several orders of magnitude greater than the general public receives from background contamination in food (JECFA, 2001). The pattern of exposure in these studies does not reflect long-term dietary exposure (UK COT, 2001). The human health clinical manifestations are summarized in Table B1-5.

Table B1-5 Clinical Manifestations Resulting from Exposure to 2,3,7,8-TCDD (unless otherwise indicated)

Clinical Manifestation	Source
Cancer	Fingerhut *et al.* (1991), Steenland *et al*. (1999)
Developmental abnormalities (TCDD)	Guo *et al*. (2003)
Endocrine pathology Diabetes Thyroid	Henrickson *et al*. (1997) Pavuk *et al*. (2003)
Elevated serum cholesterol and triglycerides	Kimbrough *et al*. (1977)
Liver damage	Kimbrough *et al*. (1977)
Skin rashes	Kimbrough *et al*. (1977)
Hypertrichosis	Kimbrough *et al*. (1977)
Enamel hpomineralization of permanent	Kimbrough *et al*. (1977)
Gum pigmentation	Kimbrough *et al*. (1977)
Eyelid pathology	Kimbrough *et al*. (1977)
Nausea	Kimbrough *et al*. (1977)
Vomiting	Kimbrough *et al*. (1977)
Loss of appetite	Kimbrough *et al*. (1977)
Change in serum testosterone	Egeland *et al.* (1994)

Adapted from Schecter *et al.,* 2006

Table B1-6 summarizes the ATSDR (1998) evaluation of systemic effects, which include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, and body weight. Neurological, reproductive and developmental effects are also discussed. A discussion of cancer is completed in the following sections.

Table B1-6 Summary of Human Health Effects of 2,3,7,8-TCDD Exposure

Effect	Suggested toxicity	Comment
Systemic Effects		
Respiratory	✖	Numerous workers were exposed to 2,3,7,8-TCDD following an industrial accident in Germany. Respiratory effects of acute exposure included bronchitis and laryngitis a few days after exposure, and hemorrhagic pleuritis 11 months after exposure (ATSDR, 1998). As summarized by ATSDR (1998), other occupational studies, Vietnam Air Force veterans involved in Operation Ranch Hand and follow-up cohort studies of those involved in Seveso, Italy suggest that acute exposure to high levels of CDDs may cause respiratory effects due to irritation of upper respiratory tract, however there is no indication that the respiratory system is a target for 2,3,7,8-TCDD toxicity.
Cardiovascular	✖	There is inconclusive evidence of adverse cardiovascular effects in humans exposed to high concentrations of PCDDs. Chronic heart disease was observed among the Seveso cohort; however confounding psychosocial factors impacted these results (Bertazzi *et al*., 1989, Pesatori *et al.,* 1998). In German workers exposed to PCDDs Flesch-Janys *et al*. (1995) found that there was an increased risk of mortality was associated with high levels of occupational exposure to dioxins with acute ischemic cardiovascular events. No clear evidence of cardiovascular effects have been found in other reports, including Ranch Hand exposed groups and other occupational incidences.
Gastrointestinal	✖	Unlike previous studies that indicated elevations in self-reported ulcers, the results of a thorough study on the U.S. Vietnam veterans suggested that there is no association between occupational exposure to 2,3,7,8-TCDD and gastrointestinal disease. The health study of Vietnam veterans involved in Operation Ranch Hand indicated an association between high 2,3,7,8-TCDD levels and increased erythrocyte sedimentation (Wolfe *et al*., 1995) and increased corpuscular volume (Wolfe *et al*., 1985) however these were minor changes not found in the follow-up study (USAF, 1991).
Hematological	✖	Other studies reviewed in ATSDR (1998) did not indicate that CDD caused adverse hematological effects.
Musculoskeletal	✖	No musculoskeletal effects have been found to be associated with exposure to TCDD.
Hepatic	✖	Hepatic effects such as increases in liver enzymes, alterations in liver function and a vast spectrum of altered liver parameters have been noted in occupational exposures, laboratory exposed groups, Seveso population studies and U.S. Vietnam veteran studies. ATSDR (1998) concluded that hepatotoxic effects, such as elevated gamma-glutamyltransferase (GGT) and changes in lipid profiles have been frequently observed in humans following exposure to high 2,3,7,8-TCDD levels; however, there is no definitive evidence that these effects are severe and it is unlikely that 2,3,7,8-TCDD causes hepatic toxicity.
Renal	?	One incident was identified of a child that developed hemorrhagic cystitis and focal pyelonephritis after playing in a sand box contaminated with waste oils containing 2,3,7,8-TCDD (Kimbrough *et al*., 1977). Similar to other studies there were inconclusive evidence demonstrating a correlation to 2,3,7,8-TCDD contamination and renal effects (ATSDR, 1998).
Endocrine	✓	The endocrine effects associated with exposure to dioxins has been well supported. A 35-year follow-up study provided evidence of thyroid dysfunction and disease for workers exposed during an accident to 2,3,7,8-TCDD at BASF (Zober *et al*., 1994). Vietnam veterans involved

Table B1-6 Summary of Human Health Effects of 2,3,7,8-TCDD Exposure

Effect	Suggested toxicity	Comment
		in Operation Ranch Hand and individuals in Seveso demonstrated increased risk of diabetes, subclinical effects in thyroid function correlated to 2,3,7,8-TCDD exposure (USAF, 1991). The follow-up evaluation found a significant increase in deaths from diabetes only among women (Pesatori *et al*., 1998); they explained that only women were effected because the systematically higher 2,3,7,8-TCDD concentrations in females than in males. It is evident from the available data that high concentrations of CDD may cause long-term effects in glucose metabolism and alter thyroid function.
Dermal	✓	Dermal effects are commonly encountered following high exposure to dioxins. Chloracne, characterized by follicular hyperkeratosis (comedones) occurring with or without pustules, and skin lesions are most commonly reported. As summarized by ATSDR (1998), studies of a chemical laboratory accident, a 2,3,7,8-TCDD accidental exposure due to an explosion in a trichlorophenol plant in Nitro, West Virginia, and in Seveso, identified acute lesions immediately along with hundreds of cases of chloracne. Operation Ranch Hand veterans examination did not identify any cases of chloracne. Burton *et al*. (1998) suggested that the exposure levels were insufficient to produce chloracne or that it quickly resolved itself. Other studies report hypertrichosis, hyperpigmentation and solar elastosis. There is significant evidence of dermal effects, especially chloracne with exposure to CDDs; however, a lack of chloracne does not mean that exposure has not occurred, as individual susceptibility also plays a significant role in the severity of the dermatological effects.
Ocular	?	Ocular effects and weight loss have been correlated with severe chloracne reported among workers employed at a 2,4,5-T factory (Oliver, 1975). The direct association with 2,3,7,8-TCDD and ocular effects is inconclusive.
Body Weight	✗	No conclusive association to alterations in body weight.
Immunological Effects	?	Limited studies have evaluated the immunotoxicity of 2,3,7,8-TCDD in humans. The available studies suggest alterations in lymphocyte populations (*e.g*., T cells, B cells), cell surface markers (*e.g*., CD4RO+, CD8+), or lymphoproliferative responses; however, no consistent exposure related immunological effects have been observed following exposure to elevated to high levels of CDDs. ATSDR (1998) suggest this may be due to the limitations in available functional assays.
Neurological Effects	✓ (short-term)	Data from specific cases and epidemiological studies indicates that exposure to CDDs may cause central and peripheral nervous system defects shortly after exposure. Symptoms of CDD intoxication include lassitude, weakness of the lower limbs, muscular pains, sleepiness or sleeplessness, increased perspiration, loss of appetite, headaches, mental and sexual disorders, increased pain sensation in lower extremities, abnormal vibration sensation and abnormal reflexes (Moses *et al*., 1984; Suskind, 1985; Webb *et al*., 1989). The primary neurological effect is reduced nerve conduction velocity, which causes both central and peripheral deficits. In some cases the effects lasted several years; however long-lasting abnormalities were not common.

Table B1-6 Summary of Human Health Effects of 2,3,7,8-TCDD Exposure

Effect	Suggested toxicity	Comment
Reproductive Effects	?	There is no conclusive evidence that exposure to dioxins causes reproductive toxicity. Small alterations in hormone levels (*i.e.*, testosterone and gonadotropins) have been identified, but the changes were small and may not adversely effect reproduction (Egeland *et al.*, 1994). The role of 2,3,7,8-TCDD and level of exposure is inconclusive in studies evaluating the incidence of spontaneous abortions, stillbirths and risk of adverse pregnancy outcomes.
Developmental Effects	?	Similar to reproductive toxicity data, many of the human studies evaluating developmental effects have poorly characterized exposure (ATSDR, 1998). Residents of Seveso, Italy, reported a significant rise in incidence of birth defects prior to exposure; however, these results may not be representative, and may be relative to increased incidence reporting rather than birth defects. The overall risk of having a child with birth defects was not significant in Vietnam veterans exposed to 2,3,7,8-TCDD. No statistically significant reproductive effects were identified in Operation Ranch Hand veterans, although a trend of increased risk of spina bifida in Ranch Hand offspring has been noted (Wolfe *et al.*, 1995). Vietnamese families exposed to 2,3,7,8-TCDD demonstrated increase incidence of unspecified congenital anomalies; however exposure data was unreliable. Based on the available developmental studies an association between 2,3,7,8-TCDD exposure and toxicity is inconclusive.

✓ Possibility of toxicity

✖ No evidence of toxic effects

? Inconclusive association between dioxin exposure and toxicity

Table summarized from ATSDR, 1998

Table B1-7 Health Effects in Humans Associated with Estimated 2,3,7,8-TCDD Body Burdens following *Acute* Exposure

Exposure Duration (days)	Effect	Current Serum levels (pg/g lipid)		Estimated levels at the time of exposure termination		Elimination half-life (years)	Reference
		Mean	Range	Serum level (pg/g lipid)	Body Burden (ng/kg bw		
<1	Chloracne in Children	19411	828-56,000	NA	2876	NA	Mocarelli *et al.*, 1991
<1	No increased risk of spontaneous abortion	NR	>10	>110	>24	7.1	Wolfe *et al.*, 1995
NR	Chloracne in 5/7 subjects	185	36-291	1900	493	NR	Schecter *et al.*,1994
11	Chloracne	604	163-1935	2935	646	7	Jansing and Korff, 1994
6.5	Immunosuppression	330	43-874	942-1108	207-244	8.5	Tonn *et al.*, 1996

Table B1-7 Health Effects in Humans Associated with Estimated 2,3,7,8-TCDD Body Burdens following *Acute* Exposure

Exposure Duration (days)	Effect	Current Serum levels (pg/g lipid)		Estimated levels at the time of exposure termination		Elimination half-life (years)	Reference
		Mean	Range	Serum level (pg/g lipid)	Body Burden (ng/kg bw		
<1	Change in sex ratio of children	540-mother 791-father	126-1650 104-2340	NA	119 174	NA	Mocarelli *et al.*, 1996
≥ 1	Increased cancer mortality rate	418	NR	1408-8444	310-1858	8.5	Fingerhut *et al.*, 1991

Adapted from ATSDR, 1998
NR – Not Reported
NA – Not Applicable

B1-3.2.1 Receptor-Based Toxicity of TCDD

2,3,7,8-TCDD has been shown to cause cancer in several chronic studies at multiple sites in multiple species (WHO, 2003). There is good evidence to indicate that this is the result of an initial binding to the Ah Receptor (Fischer *et al*., 1998; UK COC, 2001). Activation of the AhR is considered a necessary but not sufficient requirement for the toxicity of dioxins (EFSA Scientific Colloquium, 2004). Through a complex sequence of events from binding to the AhR to gene transcription, the consensus is that sufficient interaction with the AhR leads to specific cellular and tissue toxicity, the repair and regeneration of which lead secondarily to tumour formation (U.S. EPA, 2003; van Leeuwen *et al*., 2000; WHO, 1991). The greater the amount of TCDD available to bind the AhR, the greater the number of receptor-ligand complexes. As with all other receptor-mediated biological responses, a minimum number of these complexes is needed to elicit an adverse effect, implying that there is a threshold below which adverse effects do not result (Popp *et al*., 2006).

Current understanding is that Ah receptor binding is not likely to be a threshold related event, nor are early associated biochemical effects resulting from that binding; however, more complex biological responses of dioxin seem to have a threshold (EFSA Scientific Colloquium, 2004). In their review of mechanism data, the U.K. Committee on Carcinogenicity concluded that overall the data were consistent with a complex multi-step process involving receptor binding and thus a threshold interpretation of TCDD-induced carcinogenicity (UK COC, 2001). Receptor-based toxicity has been recognized for decades as having non-linear dose response relationships with biological thresholds (Popp *et al*., 2006).

There are many naturally occurring substances in the human diet (*e.g.,* indole carbinols, heterocyclic aromatic amines, flavanoids, carotinoids) that also bind to the AhR. These have been termed "endodioxin" and mass and potency estimates for human dietary intake suggest that some effects of these endodioxins may be greater than those of chemical dioxins (Safe, 1998; Connor *et al*., 2004; Popp *et al*., 2006). This strongly suggests that the human body contains homeostatic defence mechanisms that TCDD and other dioxins must overcome before causing toxicity, including cancer (Popp *et al*., 2006). The nature and role of these postulated endodioxins are unknown (EFSA Scientifi Colloquium, 2004).

B1-3.2.2 Low Dose Cancer Effects of TCDD in Animals

Dose-response curves derived from studies in laboratory animals provide strong evidence that cancer is a highly non-linear endpoint; *i.e.*, there is a threshold dose below which no tumorigenic response is observed. The lowest observed adverse effect of TCDD in the Kociba *et al*. (1978) study was the development of hepatic adenomas in rats at a daily dose level of 10 ng/kg body weight. No elevations in tumour rates were seen in rats exposed to 1 ng/kg body weight per day, equivalent to a body burden of 60 ng TCDD/kg body weight at steady state (Kociba *et al*., 1978; WHO, 2000a). More recently, the National Toxicology Program has conducted 2-year carcinogenicity bioassays of TCDD as well as 4-PeCDF, PCB-126 and a mixture of the three (NTP, 2004a,b,c,d). In the study of TCDD, no significant tumour response was seen for doses as high as 22 ng/kg body weight (5 days/week). The first significant response was observed at 46 ng/kg body weight (5 days/week) again providing evidence that the dose-response for TCDD carcinogenesis is non-linear with a threshold region.

Slope factors and cancer potency factors have been derived based on animal data by linear extrapolation of tumour data to low doses. However, the U.K. Committee on Carcinogenicity concluded that it was inappropriate to undertake quantitative risk assessment for cancer by modeling the dose-response for tumour data in animals fed diets containing TCDD in view of the assumptions needed for extrapolation from high doses used in such studies to background environmental exposures and the uncertainties involved in inter-species extrapolation (UK COC, 2001).

B1-3.2.3 Human Evidence of Carcinogenicity

Epidemiological studies of populations occupationally or accidentally exposed to dioxins present a mixed picture with respect to dioxins and cancer. Its classification as a carcinogen by the International Agency for Research on Cancer (IARC) was based on limited human data, sufficient animal data, and mechanistic considerations (IARC, 1997). The U.K. Committee on Carcinogenicity (UK COC, 2001) considered that updates of cancer mortality studies in key cohorts of herbicide producers in Germany (Flesch-Janys *et al*., 1998), the U.S. NIOSH cohort (Steenland *et al*., 1999) and the Netherlands (Hooiveld *et al*., 1998) provided evidence for an excess total cancer mortality in exposed individuals of 13 to 50%. These workers were exposed to a mixture of dioxins. Dose-response analyses showed significant results for total cancer mortality in all three studies, in that risks tended to be higher for workers with heaviest exposure. JECFA (2001) noted that increased risks with time since first exposure were observed in those studies in which latency was evaluated (Kogevinas *et al*., 1997; Steenland *et al*., 1999). By contrast Bodner *et al*., (2003) found no excess cancer risk in chemical workers exposed to sufficient dioxin to produce chloracne, which is characteristic of high exposures to TCDD.

A 20-year mortality follow-up of the population exposed to dioxin in the Seveso explosion was published by Bertazzi *et al*., (2001). A 10% increase in risk of total cancer mortality was observed for males but not for females. Among males, there was a 30% increase in lung cancer mortality and significant increases in the risk of mortality from lymphohematopoietic cancers was reported for both sexes (Bertazzi *et al*., 2001).

IARC concluded that the strongest overall evidence for the carcinogenicity of TCDD is for all cancers combined, rather than for any specific site (IARC, 1997). Similarly, JECFA (2001) concluded that low excess risks of the order of 40% were found for all neoplasms combined in

all the studies of industrial cohorts in which the exposure assessment was adequate. They noted, as have others, that the results were not consistent between studies and no single cancer site seemed to predominate. These excess risks were highly statistically significant, and any effect of chance can be excluded. The risk tended to be higher for workers with the heaviest exposure. Several reviews have pointed out that there are few precedents of carcinogens that increase the risk for cancer at all sites combined, with no excess risk for any specific tumour predominating (JECFA, 2001; WHO, 2000a; UK COT, 2001). They have also pointed out that while the relative risk is not likely to be explained by confounding, this possibility cannot be excluded since the overall excess risk observed is small (WHO, 2000a; JECFA, 2001; UK COC, 2001).

Important recent reports provide data on elimination rates for 2,3,7,8-TCDD in persons with moderate to very high exposures and suggest that at substantially elevated body burdens, elimination rates are much higher than previously estimated (Abraham *et al*., 2002; Geusau *et al*., 2002; Michalek *et al*., 2002; Aylward *et al*., 2005). This is important because it indicates that the difference in TCDD exposure levels experienced by the occupational cohorts compared to the general population is probably greater than previously thought (Aylward *et al*., 2005). A dependence of TCDD elimination rate on body burden has also been reported in rodents (reviewed by Carrier *et al*., 1995a,b).

Aylward *et al*., (2005) modeled serial measurements of serum lipid TCDD concentrations in 36 adults from Seveso, Italy and 3 patients from Vienna, Austria with initial serum lipid TCDD concentrations ranging from 130 to 144,000 ppt and confirmed earlier observations that the elimination rate for TCDD varies with body concentration, with substantially faster elimination at elevated body concentrations compared to lower body concentrations. The model also confirmed that elimination is slower in females than males on average and decreases with age; *i.e*., younger people appear to metabolize TCDD more rapidly than older persons, on average (Aylward *et al*., 2005). Significant inter-individual variability was also observed.

Previously estimates of exposures and cancer dose-response by individual scientists and regulatory/scientific bodies have all relied on cumulative exposure estimates based on back-calculations of measure levels over decades, assuming constant first-order elimination rates ranging from 7 to 9 years (Flesch-Janys *et al*., 1998; Starr, 2001; Crump *et al.,* 2003; U.S. EPA, 2003). Application of the dose-dependent elimination model to the U.S. NIOSH cohort of herbicide workers indicated that these previous dose estimates may have underestimated the maximum concentrations in these workers by several-fold to an order of magnitude or greater (Aylward *et al*., 2005). Accordingly, dose estimates for occupational cohorts should be re-evaluated in light of the demonstration of concentration-dependent elimination kinetics for TCDD (Aylward *et al*., 2005). In addition, the large degree of uncertainty in back-calculated dose estimates should be explicitly incorporated in quantitative estimates of TCDD's carcinogenic potency; for example, the cancer potency factor derived by the U.S. EPA (U.S. EPA, 2003; 2004b).

The work by Aylward *et al*. (2005) is important because regulatory agencies have used the previously back-calculated blood concentrations in occupationally-exposed workers to determine the relative sensitivity of humans *versus* laboratory animals. For example, WHO (2000a) noted that the estimated blood concentrations of TCDD in occupational cohorts overlap with the blood concentrations determined in rats of the highest dose group (100 ng/kg bw/day) of the Kociba study, which they conclude suggested that humans might be as sensitive as other animals to the

adverse effects of dioxin and related compounds. The relative sensitivity of humans compared to laboratory animals is discussed further in section 5.0 below.

In the U.S. EPA's draft dioxin assessment, researchers have derived a cancer slope or potency factor based on occupationally exposed cohorts of workers (U.S. EPA, 2003). They used a traditional default linear extrapolation tool that by design does not have the ability to find or estimate the presence of a threshold (Popp *et al*., 2006). The U.K. Committee on Carcinogenicity reviewed the U.S. EPA approach (UK COC, 2001) and concluded that modeling of dose-response data from the industrial cohort epidemiology studies was limited by the variable quality of the exposure estimates and the uncertainties associated with back-extrapolating estimates of body burden. They considered the linear extrapolation to estimate risks at background body burdens not to be acceptable in that the predicted kinetic profile of TCDD and other dioxins following occupational exposure predominantly *via* the skin over several decades was considerably different to that of background exposure *via* the diet (UK COC, 2001). They concluded that the review of cancer epidemiology studies and risk characterization of cancer undertaken by U.S. EPA was a detailed and valuable scientific assessment but the derivation of the cancer slope factor and risk at background exposure levels were not appropriate for risk assessment.

Popp *et al*. (2006) concluded from their weight-of-evidence analysis that based on all considerations, a non-linear, threshold dose-response relationship is best scientifically supported to estimate human cancer risk from TCDD exposures. They note that the observed threshold in rats lies somewhere between 22 and 28 ng/kg body weight (5 days/week) nominal dose rate. Although proof of a threshold can never be absolute, the level of certainty in the case of dioxin is high because of the concordance of many lines of evidence (Popp *et al*., 2006).

Cancer responses to single acute exposures to TCDD

Ott and Zober (1996) reported on the analysis of a cohort that received an exposure to TCDD over a very short time after an uncontrolled release form a reactor in a chemical plant (1953). The temporal pattern of exposure was considered to be essentially a pulsatile spike followed by an extensive elimination phase. Cancer incidence and cause-specific mortality were examined in a group of 243 men. Model based estimates of TCDD dose were based on detailed accounts of each employee's work activities and analyses of TCDD in blood lipid of 138 employees. The dose of TCDD for a low-dose group of 135 men was estimated as 0.1 µg/kg body weight; while the high-dose group of 69 men was estimated to have received doses 11.0 µg/kg body weight. Increased cancer risk ratios were found with higher doses of TCDD and longer interval since first exposure for all sites combined and digestive and respiratory cancers in particular. Within the high-dose group, total cancer mortality was increased 20 years after first exposure SMR = 1.97, [95% CI: 1.05-3.36] as was respiratory cancer (SMR = 3.06 [95% CI 1.12-6.66]). Regression analyses based on the Cox's proportional hazards model showed a positive relation between cumulative dose of TCDD and occurrence of both overall and digestive cancer. No evidence of an effect of TCDD on overall mortality or deaths due to circulatory disease was found and no cases of non-Hodgkin's lymphoma or soft tissue sarcoma. Because of the fact that the results were based on a small cohort, the risk estimates were considered not very stable and potentially affected by selection bias and confounding (Ott and Zober, 1996).

B1-3.2.4 Mode of Carcinogenic Action

Several studies have demonstrated that TCDD does not cause direct damage to the DNA, nor does it induce DNA adducts, which indicates that TCDD does not act as an *initiator* of carcinogenesis (Lucier *et al*., 1991; Pitot *et al*., 1980; Poland *et al*., 1982; Turtletub *et al*., 1990; WHO, 2000a). By contrast, TCDD has been repeatedly demonstrated to promote tumours in the skin and liver of laboratory animals, indicating that the carcinogenicity results from promotion of independently initiated cells subsequent to tissue damage (Pitot *et al*., 1987; Popp *et al*., 2006). Pathological evidence from rodent studies indicates that there is a correlation between the presence of overt hepatotoxicity and the development of liver neoplasms (Kociba *et al*., 1978; Goodman and Sauer, 1992). After binding to the Ah receptor, enzyme induction (*e.g.*, CYP1A1 induction) has been shown to occur very rapidly after TCDD administration, but increased cell proliferation is not observed for many weeks, suggesting it is not immediately receptor mediated but rather mediated by a set of events likely resulting from chronic injury and cell death. This supports the importance of hepatotoxicity as a requisite step in the development of tumours and suggests the dose of TCDD must be of a magnitude to cause substantial liver toxicity to a degree that leads to cell death, repair, regeneration and then tumours (Popp *et al*., 2006).

Low doses of TCDD have been shown to actually *suppress* hepatocellular proliferation (Teeguarden *et al*., 1999; Maronpot *et al*., 1993). This strongly argues that the dose-response for TCDD effects are not linear through low doses, supporting a threshold for carcinogenicity (Popp *et al*., 2006).

The carcinogenicity of TCDD in laboratory animals (NTP, 2004a,d) points toward a promotional mechanism of action by which previously initiated cells (either by spontaneous events or by exposure to direct-acting carcinogens) are conferred a selective growth advantage by subsequent exposure to the "promoting" agent, thereby inducing a rapid and sustained clonal expansion of the initiated cell population. Substances classified as "promoters" generally do not support DNA reactivity and mutagenicity in short term *in vitro* test systems. This absence of mutagenic activity and association with a non-genotoxic mode of action are typical of responses to exposures of TCDD (U.S. EPA 2000; Popp *et al.*, 2006).

A relationship between heaptocarcinogenicity and hepatotoxicity is strongly supported by multiple lines of evidence provided by animal studies (Popp *et al.*, 2006). The severity of liver toxicity induced by exposure to TCDD was linked to evidence of a carcinogenic response in female rats (Kociba *et al.*, 1978).

In the NTP study dealing with TCDD, completed in 2003 (NTP, 2004a), liver weights were significantly increased in all groups of female rats (Harlan Sprague-Dawley) that received chronic exposures to TCDDs; at 53 weeks, relative liver weights of rats administered 10 ng/kg (ppt) or greater were significantly increased as was the absolute liver weight of 100 ng/kg (ppt) rats. At 14, 31, and 53 weeks, increased incidences of hepatocyte hypertrophy correlated with increased liver weight. The incidences of hepatocyte hypertrophy were significantly increased in groups administered 22 ng/kg (ppt) or greater at 14 and 31 weeks and in all dosed groups except the 3 ng/kg (ppt) group at 53 weeks. The severities of this lesion generally increased with increasing dose. Toxic hepatopathy was significantly increased in the 100 ng/kg (ppt) group at 31 weeks and in the 46 and 100 ng/kg (ppt) groups at 53 weeks.

At 2 years, the incidence of hepatocellular adenoma was significantly increased at exposures of 100 ng/kg (ppt) in female rats (NTP, 2004 a). Dose-related increased incidences of cholangiocarcinoma were seen in rats administered 22 ng/kg (ppt) or greater with some rats in the 100 ng/kg (ppt) group displaying a multiple cholangiocarcinomas.

Hepatotoxicity related to TCDD parallels the dose-response relationship for tumour formation (Popp *et al.*, 2006). On the other hand, the relationship for other TCCD-induced responses such as enhanced gene expression are differentiated from tumour formation (Maronpot *et al.*, 1993; Viluksela *et al.*, 2000).

Popp *et al.* (2006) have pointed out the difference in timing between liver enzyme induction (CYP1A1) and the evidence for increased cell proliferation. Enzyme induction occurs rapidly after TCDD administration while cell proliferation (an expression of a response to hepatotoxicity) may remain unaffected for many weeks. Popp *et al.* (2006) suggest that cell proliferation is not immediately receptor mediated (AhR) but likely to be linked with chronic injury and/or cell death in the liver.

It is important to consider the dual staged response to TCDD since single or short-term acute and higher level exposures could be rapidly managed by AhR-mediated enzyme induction reducing the potential of chronic or long-term exposure and its attendant hepatopathology. This has important implications for carcinogenic potential. Evidence of hepatotoxicity may be an important predictor of carcinogenic potential of an exposure to TCDD (Popp *et al.*, 2006).

Low doses of TCDD in animal studies suggest a different mode of the action of TCDD from that observed at high levels of exposure. Low doses of TCDD administered to animals that have been initiated by exposure to a known carcinogen (diethylnitosamine) results in suppression of the development of preneoplastic tumours in the liver of treated rats. Such preneoplastic lesions have long been considered a surrogate for tumour development. Thus, low doses of TCDD suppresses hepatocellular proliferation (Popp *et al.*, 2006).

At low doses of TCDD, the reduction of effects (foci of cellular alteration, cell proliferation) that are increased by high doses strongly argues that the dose-responses for TCDD effects are not linear through low doses. These differences in response from high to low dose strongly supports the concept of a threshold for hepatocarcinogenicity (Popp *et al.*, 2006).

Although IARC (1997) has designated TCDD as a multisite carcinogen, there is no existing evidence from human studies sufficient to designate TCDD as a pluripotent carcinogen for humans (Popp *et al.*, 2006; Cole *et al.*, 2003). According to IARC (1997), the strongest overall evidence for the carcinogenicity of TCDD is for all cancers combined (relative risk [RR] = 1.4) (*i.e.,* no specific site of action). This level of relative risk was established from data for the most highly exposed and longer-latency subcohorts gleaned from herbicide exposure and acute accidental exposure events (Popp *et al.,* 2006).

B1-3.3 Populations at Special Risk

Populations exposed to the highest levels of dioxin include occupationally exposed workers, for example herbicide producers, as well as accidentally exposed populations exposed *via* contamination of the environment or food. Studies of highly exposed populations suggest various non-cancer health effects are associated with dioxin exposure; for example, chloracne (a

skin condition), increases in liver enzymes, increased cardiovascular disease and developmental effects. However, most of these effects, such as chloracne, appeared only at doses several orders of magnitude greater than the general public receives from background contamination in food (JECFA, 2001). In addition, the pattern of exposure in these studies does not reflect long-term dietary exposure (UK COT, 2001). For example, many of the occupationally exposed workers had substantial dermal exposure to dioxins. Based on a review of human health effects of dioxin, the Joint FAO/WHO Expert Committee (JECFA, 2001) concluded that dioxins can have mild effects at TEQ doses of 1,000 to 10,000 ng/kg body weight, while larger doses have more severe and persistent effects; in the Yusho incident, all patients who received such doses and were examined, developed chloracne. Men appeared to be more sensitive to the effects of dioxins than women. Very large doses, in the range of 50,000 to 100,000 ng/kg body weight were associated with very severe illness where reports were available (JECFA, 2001).

Studies have shown that accidental exposure to high levels of dioxins either before or after birth have lead to a number of developmental deficits (Charnley and Kimbrough., 2006). Infants are primarily exposed to dioxins through human milk, therefore on a body-weight basis, children receive higher doses of dioxins. The half-life of dioxin in adults is estimated between 7 to 11 years in humans; however, elimination rates are dose-dependant and vary with body composition (*i.e.*, the higher the fat content the greater the half-life) (Emond *et al*., 2004; Aylward *et al*., 2005). It is for this reason that children eliminate them more rapidly than adults (Kreuzer *et al*., 1997).

In the Yu-Cheng poisoning episode involving contaminated rice oil, several developmental deficits were reported in children exposed before birth (Rogan *et al*., 1988; Guo *et al*., 1993; Lai *et al*., 2002). These include effects on growth, intellectual development and sex organ development (*e.g.*, reduced penis length and abnormal sperm morphology). Maternal body burdens at the time of exposure were estimated to be 2,000 to 3,000 ng TCDD TEQ/kg body weight. By contrast, no developmental deficits were observed in children exposed to high levels of TCDD from the Seveso explosion where maternal body burdens were estimated to be 110 ng/kg body weight in the zone closest to where the explosion occurred (zone A) and 28 to 30 ng/kg body weight in the next nearest zone (zone B) (Bertazzi *et al*., 1998). Current body burden levels have been estimated to average 0.75 ng/kg body weight for a 60 kg person with 25% body fat (Aylward and Hays, 2002).

Potential developmental effects due to PCDD/F exposure have also been studied in several cohorts of children exposed to background levels in utero or *via* breastfeeding (Pluim *et al*., 1993; Huisman *et al.,* 1995; Koopman-Esseboom *et al*., 1996). These tend to be primarily in Europe; similar studies have been conducted in North America but these tended to focus more on PCBs. Transient subtle changes in measures of neonatal neurological development and alterations in thyroid hormone concentrations showed some relationship with toxic equivalents but these were within the normal biological range and there were inconsistencies among studies (Charnley and Kimbrough, 2006). Interpretation is also complicated by the simultaneous exposure to non-dioxin-like PCBs and possibly several other persistent compounds (ECSCF, 2001).

Charnley and Kimbrough. (2006) argue that current estimates of effects in children are exaggerated because they are predominantly based on unrepresentative studies on rats. Charnley and Kimbrough (2006) suggests that as body burdens and environmental levels of dioxins

continue to decline, it is unlikely that children will experience exposure from dioxins at levels that may cause health effects.

B1-3.4 Acute Effects

The Agency for Toxic Substances and Disease Registry (ATSDR, 1998) has compiled a series of studies that have identified levels of significant exposure to 2,3,7,8-TCDD following acute one-time exposure. These studies are outlined below:

***Acute Dermal Exposure*:** ATSDR identified a single study that demonstrated adverse effects following one-time dermal exposure to 2,3,7,8-TCDD. A study by Schwetz *et al.* (1973) reported a dermal LD_{50} ("serious" effect) value of 275 µg/kg body weight for rabbits. The deaths occurred 12 to 22 days post-exposure. The study by Schwetz *et al.* (1973) also reported a LOAEL of 2,000 µg for transient inflammation of conjunctiva in rabbits. This biological endpoint was classified by ATSDR (1998) as a "less serious" effect.

***Acute Oral Exposure*:** ATSDR has identified a large number of studies that demonstrated adverse effects following a single oral exposure to 2,3,7,8-TCDD. The studies examined a variety of "serious" and "less serious" biological endpoints. A summary of these studies is presented in Table B1-8. The lowest exposure level associated with an adverse effect was reported to be 0.01µg/kg/day for immunological effects in the mouse (Burleson *et al.*,1996; White *et al*., 1986). White *et al*. (1986) noted the ability of TCDD to suppress serum total hemolytic complement activity in mice at a LOAEL of 0.01 µg/kg/day . In the Burleson *et al.* (1996) study, female mice were administered a single gavage dose of 2,3,7,8-TCDD in corn oil. Seven days post-exposure the mice were infected with influenza A at a dose that does not typically cause mortality. Statistically significant increases in mortality were observed for mice that had been previously exposed to 0.01 µg 2,3,7,8-TCDD/kg body weight. The NOAEL for impaired resistance to influenza A virus infection was determined to be 0.005 µg/kg body weight. This biological endpoint is reversible and was classified by ATSDR (1998) as a "less serious" effect. As presented in Table B1-8, the lowest one-time oral exposure associated with a "serious" effect was reported to be 0.064 µg/kg body weight. This LOAEL was reported by Mably *et al.* (1992) and considered effects of TCDD on spermatogenesis and reproductive capability in male rats.

Table B1-8 Significant Exposure to 2,3,7,8-TCDD: One-Time Oral Exposures

End Point	Species	NOAEL (µg/kg bw/day)	LOAEL (Less Serious) (µg/kg bw/day)	LOAEL (Serious) (µg/kg bw/day)
Death	Guinea Pig; Hamster			0.6[A] to 5,051[B]
Systemic:				
- Cardiovascular	Mink; Rat; Monkey	2.5[C] to 75[D]	5[C] to 70[E]	
- Gastrointestinal	Rat; Hamster; Mink	2.5[C] to 6,000[B]	19[F] to 100[G]	5[C]
- Hematological	Monkey; Mink; Mouse	7.5[C]	1[H] to 70[E]	
- Hepatic	Mouse	0.5[I] to 15[J]	0.1[K] to 1,000[L]	50[J] to 75[M]
- Renal	Mouse	2.5[C] to 1,500[N]	5[C] to 1,950[N]	
- Body Weight	Rat; Guinea Pig; Mink	3.2[O] to 600[B]	1.8[P] to 1,500[N]	5[C] to 164[Q]
- Dermal	Monkey; Hamster	600[B]	70[E] to 1,000[B]	

Table B1-8 Significant Exposure to 2,3,7,8-TCDD: One-Time Oral Exposures

End Point	Species	NOAEL (μg/kg bw/day)	LOAEL (Less Serious) (μg/kg bw/day)	LOAEL (Serious) (μg/kg bw/day)
- Endocrine	Mouse	0.03[N] to 10[N]	0.1[N] to 97.5[N]	
Immunological	Mouse; Rat; Guinea Pig	**0.005**[R] to 2.5[S]	**0.01**[R] to 50[T]	0.8[L] to 280[L]
Neurological	Mink; Rat	2.5[C]	5[C,U]	
Reproductive	Rat; Monkey	3[V]	4.5[W]	1.0[X] to 12.5[W]
Developmental	Rat; Mouse	0.05[Y] to 1[Z,1]	0.05[Y] to 3[Z]	**0.064**[2] to 150[3]

NOAEL: No-Observable-Adverse-Effect-Level
LOAEL: Lowest-Observable-Adverse-Effect-Level

[A] Schwetz *et a*l., 1973; Guinea Pig
[B] Henck *et al*., 1981; Hamster
[C] Hochstein *et al*., 1988; Mink
[D] Christian *et al*., 1968; Rat
[E] McConnell *et al*., 1978; Monkey
[F] Theobald *et al*., 1991; Rat
[G] Ball and Chabra, 1981; Rat
[H] Zinkl *et al*., 1973; Mouse
[I] Pegram *et al*., 1995; Mouse
[J] Smith *et al.,* 1981; Mouse
[K] Turner and Collins, 1983; Guinea Pig
[L] Hanberg *et al*., 1989; Mouse; Guinea Pig
[M] Greig 1984, 1979; Mouse
[N] Weber *et al*., 1995; Mouse
[O] Roth *et al*., 1988; Rat
[P] Hanberg *et al*., 1989; Guinea Pig
[Q] Walden and Schiller, 1985; Rat
[R] Burleson *et al*., 1996; Mouse
[S] De Krey and Kerkvliet, 1995; Mouse
[T] DeWall *et al*., 1992; Rat
[U] Seefeld *et al*., 1984; Rat
[V] Li *et al*., 1995; Rat
[W] Moore *et al*., 1985; Rat
[X] McNulty, 1984; Monkey
[Y] Gray *et al*., 1997b
[Z] Chaffin *et al*., 1997; Rat
[1] Gehrs *et al*., 1979; Rat
[2] Mably *et al*., 1992b; Rat
[3] Dasenbrock *et al*., 1992; Mouse

ATSDR (1998) has derived an acute MRL of 200 pg/kg bw/day based on immunological effects in female mice (Burleson *et al*., 1996). ATSDR (1998) classified this as a "less serious" effect. "Serious" effects were reported by Mably *et al.* (1992) at a dose of 0.064 μg/kg body weight.

B1-4.0 TOXICOKINETICS

B1-4.1 Absorption

Fraction Absorbed Via Ingestion

MOE (1985) reports 10 to 50% of the TCDD adsorbed to soil may be absorbed through the gastrointestinal tract when ingested; whereas 5% of the TCDD adsorbed to fly ash may be absorbed. The last factor may be considered due to contamination of soil from windblown fly ash. MOE also reports that 36 to 86% of pure TCDD-contaminated food or drinking water may be absorbed. Ninety-five percent of an average lifetime uptake of TCDD is from ingestion of contaminated soil as predicted by the Centre for Disease Control and Prevention (CDC) (Paustenbach *et al.,* 1986). On this basis, the value of 86% (*i.e.,* absorption of pure TCDD from the diet) may be a generous overestimate of absorption through the gastrointestinal tract. Several factors may be considered when estimating an ingestion absorption factor for TCDD. These include type of particulate matter (*e.g.,* soil, fly ash), aging (*i.e.,* length of time soil is contaminated), concentration, bolus size, and additional contaminants (*e.g.,* other organics) (Umbreit *et al.,* 1985, 1986a,b, 1987; Gallo, 1986) may effect the absorption of TCDD at various sites.

The U.S. EPA and the CDC report 85% as the bioavailability of dioxins in soil (Environmental Reporter, 1985; Chemical Regulation Reporter, 1986). Table B1-9 indicates gastrointestinal absorption rates of TCDD in various species. In animal feeding studies, Poiger and Schlatter (1980) observed 16 to 24% of the consumed dose in the liver which may represent 70% of the body burden (Fries and Marrow, 1975). The absorption of TCDD in acetone:corn oil administered by gavage to rats ranged from 70 to 83% (Rose *et al.,* 1976; Piper *et al.,* 1973). More recently, 88% absorption of TCDD in emulphur/ethanol/water was reported in male Fischer 344 rats following oral exposure to TCDD (Diliberto *et al*., 1996). The bioavailability of TCDD administered to rats in the diet (7 or 20 ppb for 42 days) was reported to have been 50 to 60% (Fries and Marrow, 1975).

Table B1-9 Gastrointestinal Absorption of TCDD

Chemical	Species (Sex)	Dose (µg/kg)	Vehicle	% Administered Dose Absorbed [Mean (Range)]	Reference
2,3,7,8-TCDD	Rat (Sprague-Dawley) (M)	50	Acetone: corn oil (1:7)	70	Piper *et al*., 1973
2,3,7,8-TCDD	Rat (Sprague-Dawley) (M/F)	1.0	Acetone: corn oil (1:25)	84 (66-93)	Rose *et al*., 1976
2,3,7,8-TCDD	Guinea Pig (Hartley) (F)	1.45	Acetone: corn oil (1:45)	50	Nolan *et al*., 1979
2,3,7,8-TCDD	Hamster (Golden Syrian) (M)	650	Olive oil	74	Olson *et al*., 1980
2,3,7,8-TCDD	Human (M)	0.001	Corn oil	87	Poiger and Schlatter, 1986
1,2,3,7,8-PeCDD	Rat (Sprague-Dawley) (M/F)	9.2	Corn oil	NR (19-71)	Wacker *et al*., 1986
TCDD	Rat (Sprague-Dawley) (M)	--	Emulphor: 95% ethanol: water (1:1:3)	88	Diliberto *et al*., 1996
OCDD	Rat (Fischer 344) (M)	50	o-dichlorobenzene: Emulphor (1:1)	12	Birnbaum and Couture, 1988
		500	o-dichlorobenzene: corn oil(1:1)	15	
		500	Corn oil suspension	2	
		5000	Corn oil suspension	5	

Modified from U.S. EPA (2003)

Rats fed TCDD-contaminated Times Beach soil were observed to have an absorption fraction mean of 43% (Shu *et al.,* 1988). Kimbrough *et al.* (1984) cite an absorption factor of 30% based on the findings of McConnell *et al.* (1984) who observed 30 to 50% absorption in rats and guinea pigs fed TCDD-contaminated soil from Missouri. Furthermore, an oral bioavailability of 0.5 and 21% were estimated in the rat for soil at a New Jersey manufacturing site and a Newark salvage yard, respectively (Umbreit *et al*., 1986a,b).

The U.S. EPA (2003) indicates that the absorption factor for soil ingestion of dioxins is approximately 30%. For the current exposure assessment a value of 50% (0.50) was chosen for calculation of oral bioavailability of dioxins/furans based on a weight-of-evidence assessment of the literature cited above. Assuming that dioxins/furans would be bound to soil-borne particles, it is considered unlikely that the 50% oral bioavailability value would underestimate the fraction of dioxins/furans absorbed. For food-borne exposures, as value of 90% was selected.

Fraction Absorbed Via Inhalation

Paustenbach *et al.* (1986) suggests that absorption of TCDD through the respiratory tract is directly associated with the concentration of airborne dust due to the low volatility of TCDD. The CDC assume that 100% of the 2,3,7,8-TCDD adsorbed to inhaled particles is absorbed (Paustenbach *et al.,* 1986); whereas Schaum (1983) assumes that 25% is absorbed in the lower respiratory tract. Nessel *et al*. (1992) also concluded that the relative pulmonary bioavailability of 2,3,7,8-TCDD on respirable soil particles was 100%. Diliberto *et al*. (1996) found that 95% of an intratracheal applied dose of 2,3,7,8-TCDD was absorbed by male Fischer 344 rats three days following the application. Dann (1989) has indicated that TCDD are largely bound to particulate matter in air (85%) with a smaller percent being present in the vapour phase (15%). The U.S. EPA (2003) states that 80% of a TCDD dose that comes into contact with the body through inhalation is absorbed.

Fraction Absorbed Via Dermal Exposure

Numerous factors may affect the dermal absorption rate of 2,3,7,8-TCDD. These include the condition of the skin itself (*i.e.,* age, location on the body and injury due to trauma or disease) and the conditions of exposure (*i.e.,* temperature, humidity, concentration and duration). The two main routes of entry are the epidermis itself and the hair follicles and sebaceous glands. The hair follicles and sebaceous glands are important in initial and short term exposures, especially in the case of dioxins which may impact the skin and cause chloracne at these sites (Suskind, 1985).

The dermal absorbtion of 2,3,7,8-TCDD in male Fischer 344 rats was examined by Brewster *et al.* (1989) (Table B1-10). Their results suggest that the majority of the compound remaining at the dermal exposure site did not penetrate through to the dermis, but was associated wit the starum corneum. Banks and Birnbaum (1991) determined the rate of absorption of TCDD over 120 hours after a dermal application to male rats to be first order and have an absorption rate constant of 0.005 hour^{-1}. These results indicate that at lower doses (<0.1 µmol/kg) a greater percentage of the administered dose is absorbed. However, even low-dose dermal exposures have a very slow rate of absorption (*ie.*, rate constant of 0.005 hour^{-1}).

Table B1-10 Dermal absorption of 2,3,7,8-TCDD in the Rat

Chemical	Dose (µg/kg)	% Administered Dose	
		Skin site[a]	Absorbed
2,3,7,8-TCDD	0.05	61.73 ± 4.37	38.27 ± 4.37
	0.32	59.71 ± 1.90	40.29 ± 1.89
	3.2	72.60 ± 0.41	27.40 ± 0.41
	32	82.21 ± 2.85	17.78 ± 2.85
	160	80.92 ± 2.74	19.08 ± 2.74
	321	82.68 ± 3.69	17.30 ± 3.67

[a] Brewster *et al*., 1989

The penetration of TCDD into human cadaver skin was examined by Weber *et al*. (1991). The study found that the stratum corneum acted as a protection barrier. Using acetone as a vehicle with intact skin the rate of TCDD penetration into the dermis ranged from 6 to 170 pg/hour/cm2, while penetration intot he dermis and epidermis ranged from 100 to 800 pg/hour/cm2. Using mineral oil as a vehicle reduced the rate of penetration between five and ten-fold (Weber *et al*., 1991).

There have been several interpretations of the data provided by Poiger and Schlatter (1980) who looked at the dermal uptake of radiolabeled 2,3,7,8-TCDD by rats. Kimbrough *et al.* (1984) estimated a dermal absorption fraction of 1% based on this study, indicating that this value was likely an overestimate due to the fact that rodent skin has a 3 to 10 fold higher permeability than human skin. Schaum (1984) determined a dermal absorption range of 0.07 to 3% assuming that 70% of the total body burden is found in the liver. MOE (1985) reports 20% of pure 2,3,7,8-TCDD is dermally absorbed, 1 to 10% of 2,3,7,8-TCDD that is adsorbed to soil is dermally absorbed, and 1% of 2,3,7,8-TCDD that is adsorbed to fly ash is dermally absorbed. After 24 hours of contact with the skin, the amount of TCDD dermal uptake in rats from contaminated soil was ~1% of the administered dose (Shu *et al*., 1998). U.S EPA (1992) concluded that the absorption of dioxin through the skin ranges between 0.5 to 3.0%.

For dermal contact with 2,3,7,8-TCDD through soil a value of 1% (0.01) was selected to represent the fraction absorbed through the skin for human exposure (Shu *et al*., 1988). Other dioxin/furan congeners were assumed to act in a similar fashion to TCDD. For direct contact with pure 2,3,7,8-TCDD, dermal absorption rates have been derived and discussed elsewhere.

B1-4.2 Distribution

Data on the distribution, retention and excretion of dioxins have been obtained from experimental animals using both radioactively labelled compounds and by identification of products in excreta using chromatographic and other methods.

Sprague-Dawley rats that received a single dose of ^{14}C-labelled TCDD *via* gastric intubation excreted 56% *via* the alimentary route over a period of 21 days. The total amount of TCDD eliminated in urine was 4.5%. Most of the remaining dose of labelled TCDD that persisted in treated animals was localized to the liver (Allen *et al.*, 1975). Another study in rats administered a single oral dose of TCDD (50 µg) resulted in the determination of a half-life (t½) for TCDD of 17.4 ± 4.6 days (Piper *et al.*, 1973).

The fate of ^{14}C activity after a single oral dose of 1.0 µg of [^{14}C]TCDD per kg body weight was described by Rose *et al.* (1976). The body burden for each day after exposure was determined from the cumulative percentage of the dose excreted in urine, feces and respired air on each day. As was reported by Allen *et al.* (1975) fecal excretion accounted for most if not all of the elimination of TCDD and any metabolites (Rose *et al.*, 1976). Based on a total of six rats (three of each sex) the half-life or body burden of the TCDD in rats receiving a single dose was 31 ± 6 days (Rose *et al.*, 1976). The fraction of the dose absorbed (*f*) was generally higher in females than males and ranged from 0.66 to 0.91 with an average of 0.84 ± 0.11 (Rose *et al.*, 1976). The distribution of dose in rats receiving a single oral dose of TCDD is given in Table B1-11:

Table B1-11 The Concentration of ^{14}C Activity in Selected tissues of Rats 22 Days After a Single Oral Dose of 1.0 µg of [^{14}C]-TCDD/kg [a]

Tissue	Percentage of dose/g of tissue
Liver	1.26 ± 0.31
Kidney	0.06 ± 0.06
Fat	1.25 ± 1.14
Thymus	0.09 ± 0.05
Spleen	0.02 ± 0.01

[a] Data from Rose *et al.*, 1976. Mean ± SD of three male and three females S-D rats

Recent studies that measured TCDD blood concentrations shortly after high-level exposure indicate that the half-life is dose dependent (Geusau *et al.*, 2002). Similar findings have been reported for the Ranch Hand cohort (Michalek *et al.*, 2002). In the opinion of Emond *et al.* (2005) the use of first-order elimination of TCDD could significantly underestimate past exposures, especially those that occurred over a short duration and involved relatively large concentrations to TCDD.

Peak exposures to TCDD have been estimated after assuming a mono- or biphasic elimination rate for TCDD. Estimates for a biological half-life range from 5 to 12 years (Hooiveld *et al.*, 1998; Michalek *et al.*, 2002; Steenland *et al.*, 2001). Clinical studies suggest that the elimination rate of TCDD is dose dependent (Michalek *et al.*, 2002). In both animal and studies that involve humans, as exposure increases the apparent half-life of TCDD decreases. It has been postulated that elimination of TCDD from humans might be accelerated by the sequestration of TCDD in the liver and induction of CYP1A2 (Emond *et al.*, 2005).

Emond *et al.* (2005) reported the reduction in body burden of TCDD in a subpopulation of 343 Ranch Hand veterans over a period of years. Blood samples collected every 5 years from 1982 to 1998 and TCDD in blood determined. These data were used to optimize the human PBPK model for dioxin. The second set of data used to optimize the model was from Poiger and Schlatter (1986), in which a single volunteer received a single oral dose of 1.14 ng TCDD/kg body weight and was followed for 40 days. These data were used in the optimization of the absorption and distribution processes occurring during the initial phase of the exposure.

B1-4.3 Metabolism and Elimination

Through the monooxygenase system dioxins are slowly metabolized to polar metabolites that undergo conjugation with glucuronic acid and glutathione (ATSDR, 1998). Guinea pig study did not identified metabolites of 2,3,7,8-TCDD following radiolabeled exposure to 2,3,7,8-TCDD indicating that metabolism is very slow. In rats and hamsters, metabolites are not generally found in tissue indicating that following metabolism, the dioxins are quickly excreted (Olsen *et al.*, 1980).

Following metabolism, CDDs are primarily excreted through bile and feces; smaller amounts are excreted through urine (ATSDR, 1998). In mammals, lactation is an effective means of eliminating dioxins from the liver and extra-hepatic tissues. Recent studies indicate a concentration-dependence of TCDD elimination rates in humans, with higher concentrations resulting in higher elimination rates (Aylward *et al.*, 2005).

Body Burden of TCDD Expressed as TEQ

Concentrations of PCDD/Fs (converted into equivalents of TCDD (TEQs)) determined in tissues or accumulated through the diet are expressed as picograms (10^{-12} g or pg), and nanograms (10^{-9} g or ng). The available information derived from numerous studies indicates a daily intake of PCDDs and PCDFs in the order of 50 to 200 pg TEQ/person/day, or 1 to 3 pg TEQ/kg bw/day for a 60 kg adult. This results in average human background levels in the range of 10 to 30 pg TEQ/g lipid, also equivalent to a *body burden* of 2 to 6 ng TEQ/kg bw. Studies that have assessed cumulative body burden based on TEQs have shown intake of PCDDs/ PCDFs increases during childhood and stabilizes in adults of about 20 years of age. The intake on a per kilogram basis decreases in this period due to the increasing body weight. Despite differences in

the absolute levels of PCDDs/PCDFs the congener profiles that result from exposure to background levels of environmental contamination and diet are usually very similar (WHO, 2000a; van Birgelen and van den Berg, 2000).

Body burdens that result from accidental or occupational exposure to PCDD/Fs show congener patterns different from background (dietary and other incidental environmental) exposure. Results of serum or tissue analysis following acute exposure to PCDD/Fs present as contaminants in an herbicide or other chemical mixture are normally dominated by only a few congeners. This observation is consistent with the fact that indirect exposure through the food supply may modify congener patterns because of bioaccumulation in various food sources (WHO, 2000a).

Cumulative Serum Lipid TCDD Concentration: Choice of Half-llife

The toxicokinetic determinants of TCDD and related PCDDs and PCDFs depend on three major properties: lipophilicity, metabolism, and binding to CYP1A2 in the liver. Greater levels of chlorination of PCDD/Fs increases their lipophilicity (the attraction to deposit in, or affinity for, fatty tissues). Metabolism of TCDD is the rate-limiting step for its elimination. Induction of the cytochrome P450 CYP1A2 in the liver leads to hepatic sequestration of TCDD. Binding of TCDD to the AhR stimulates the production of CYP1A2 as well as other proteins. The structure-activity relationships for induction are different from that for binding to CYP1A2. Binding to this inducible hepatic protein results in non-linear dose-dependent tissue distribution: as the dose of TCDD increases, the relative concentration in liver increases with a concomitant decrease in deposition to other tissues. The induction of CYP1A2 occurs in both animals and humans and results in an increase in the liver to fat ratio of PCDD/Fs. This effect has a minor impact on free TCDD and serum TCDD at the range of environmental exposure (WHO, 2000a). But at larger acute exposures the sequestration of TCDD in the liver becomes significant (van Birgelen and van den Berg 2000).

The basic determinants of pharmacokinetic behaviour of TCDD are similar in animals and humans (WHO, 2000a). Several robust classical and physiologically based models have been used to describe the kinetic behaviour. The development of such models has contributed to the recognition that the apparent half-life (t½) of TCDD is not absolute, but may vary with dose, body composition, age, and sex (WHO, 2000a; van Birgelen and van den Berg, 2000).

There appear to be real differences in the rate of elimination of TCDD (and therefore the toxicity and potency of TCDD) that depend on the level of exposure and the absorbed dose in humans. The data base of evidence of variation in the rate of elimination of acutely administered exposures high level exposure to TCDD in humans is small. Geusau *et al.* (2002) have described empirical data resulting from exposure to two women with severe 2,3,7,8-tetrachlorodibenzo-*p*-dioxin intoxication. Initial blood concentrations of TCDD first measured in spring 1998 were 144,000 pg/g blood fat in one patient and 26,000 pg/g in a second patient. Three years later the concentrations in blood fat were 35,900 and 9,500 pg/g, corresponding to overall elimination half-lives of 560 days (1.5 years) in one patient and 1,050 days (2.9 years) in the other patient. The half-lives based on these empirical observations were considerably shorter than median values of 7 to 9 years described above. It was hypothesized by Geusau *et al.* (2002) that 78 and 62% of the overall elimination in these patients was attributable to an induced hepatic metabolism caused by the high TCDD exposure.

The discrepancies between exposure to TCDD and concentrations determined in tissues some years after an exposure event or period has been the subject of study in persons who received occupational exposures. Aylward *et al.* (2005) examined an occupational cohort (n = 193) workers to reconstruct exposures and to compute an average serum lipid TCDD concentrations for a larger occupational cohort. The sampled workers had received their last occupational exposures to TCDD from 15 to 37 years before the sampling date necessitating a back-extrapolation from the measured concentration on the sampling date to presumptive earlier values assuming either an 8.7 year t½ for PCDD or a 7.1 year t½ for TCDD. Aylward *et al.* (2005) assumed an average daily intake of 0.33 and 0.40 pg TCDD/kg/day independent of occupational exposure. They then modeled the median area under the curve (AUC) (ppt-years) for workers according to seven exposure ranges (septile). The exposure reconstruction produced only moderate agreement between measured and modeled serum TCDD levels regardless of model assumptions used. The AUC estimates derived using the first-order model elimination with an 8.7 year half-life produced the lowest of all the estimated cumulative serum concentrations. The use of a non-linear approach, through the application of a concentration- and age-dependent model of elimination of TCDD (Carrier *et al.*, 1995 a,b) increased the AUC estimates by several fold. This finding suggests that assessments that employ a back calculation over decades and that failed to incorporate concentration dependence for the elimination rate likely underestimated exposures by a significant amount (Aylward *et al.*, 2005). In the case of more highly exposed individuals, the revised model approaches increased the cumulative serum exposure levels by five-fold (Aylward *et al.*, 2005).

The wide variation in predicted AUC (ppt-years for TCDD) that has been described by Aylward *et al.* (2005) for an occupational cohort would affect substantially the cancer potency estimates derived from mortality data for this group.

The choice of elimination half-life is important. Steenland *et al.* (2001) used the 8.7-year elimination half-life for TCDD in contrast to the 7.1-year half-life employed by Aylward *et al.* (2005), or the 7.2-year value estimated by Flesch-Janys *et al.* (1996) (the median of individual decay rates for TCDD was 0.097, corresponding to a half-life of 7.2 years. For the other PCDDs, median decay rates ranged between 0.187 (1,2,3,4,6,7,8-HpCDD) and 0.044 (1,2,3,7,8-PCDD), corresponding to half-lives of 3.7 years to 15.7 years, respectively.). In another cohort of BASF workers a 7.0-year value was employed by Ott *et al.* (1993), and in a fifteen year follow-up of Opration Ranch Hand military personnel a 7.6-year half-life (CI = 7.0 to 8.7 years) was used (Michalek and Tripathi, 1999). A similar type of analysis produces a half-life estimate of 8.2 years in 27 victims of the accident in Seveso, Italy (Needham *et al.*, 1998, 1999). The Seveso cohort had a greater initial exposure, resulting in serum levels of 130 to 3,830 ppt TCDD. This study also included the early and later portions of the TCDD decay curve, as the initial blood sampling began immediately following exposure and continued for 15.9 years (Needham *et al.*, 1998). Based on results from the Ranch Hand, the German, and the Seveso studies, the estimated half-life of TCDD in humans is from 7.1 to 8.7 years. Population studies have shown that dioxin levels, measured in plasma or tissues, are higher in female subjects (Papke *et al.* 1996; Schuhmacher *et al.* 1999). For instance, plasma TCDD levels measured approximately 20 years after the Seveso accident exhibited large differences across the contamination zones, but levels measured in female subjects were consistently higher than in men (Landi *et al.*, 1997). Michalek *et al.* (2002) observed that mean TCDD half-life in Seveso was 9.6 years among women and 6.5 years among men (Giaconini *et al.*, 2006). The mean half-life for the Ranch Hand cohort (all males) measured between 9 and 33 years after exposure was 6.9 years (Michalek *et al.*, 2002). Data provided by U.S. EPA (2003), including studies by Pirke *et al*.

(1989) and Wolfe *et al*. (1994), indicated that TCDD half-lives range between 5.8 and 11.3 years.

The use of back extrapolation is especially sensitive to the elimination half-life used to compare exposure for the purpose of risk assessment. Using Aylward *et al.* (1996) back-extrapolated values the maximum TCDD concentration would equal $2^{(40/7.5)} = 40.2$ times larger than serum levels that would be measured today. Using another half-life in the back-extrapolation (Steenland *et al.*, 2001) would generate a back-extrapolated value of $2^{(40/8.7)} = 24.25$ times larger, that is, about 39% smaller than the value employed by Aylward *et al.* (1996). By using the longer half-life, this approach produces a corresponding inflation of the apparent carcinogenic potency of TCDD (Starr, 2003).

PBPK Modeling to Describe Elimination Rates of TCDD

The elimination of TCDD in mammals is dependent upon diffusion into and out of adipose tissue, metabolism, hepatic sequestration, and hepatic elimination rate (Emond *et al.*, 2006). Recent animal studies support the participation of CYP1A2 and other CYPs in the metabolism and elimination of TCDD (Emond *et al.*, 2006).

The most recent pharmacokinetic models for TCDD describe the distribution of TCDD as diffusion limited (Andersen *et al.* 1997; Carrier *et al.,* 1995a,b; Kohn *et al.,* 1996; Wang *et al.,* 1997, 2000; Maruyama *et al.,* 2002; Emond *et al.,* 2004; Aylward *et al.,* 2005; Emond *et al.*, 2006). Some of these models also include an inducible cytochrome P450-TCDD binding protein regulated by the Ah receptor (AhR) in hepatic tissue (Diliberto *et al.,* 1996; Emond *et al.*, 2006). Empirical models developed from epidemiological data have described the rate of elimination of TCDD on the basis of first order kinetics with half-lives varying from 7 to 8.7 years (Aylward *et al.,* 1996; Crump *et al.,* 2003; Flesch-Janys *et al.,* 1996; Steenland *et al.,* 2001). Clearance rates from the hepatic compartment in proposed PBPK models treat the rate of elimination of TCDD in either of two ways (Emond *et al.*, 2006). The models described by Wang *et al.* (2000), Maruyama *et al.* (2002), and Emond *et al.* (2004) assume a constant hepatic clearance rate.

Andersen *et al.* (1997), Emond *et al.* (2005) and Kohn *et al.* (1996) assume a dose dependent rate of hepatic elimination of TCDD that increases with dose. In the toxicokinetic model of van der Molen *et al.* (1998; 2000) the t½ of TCDD is varied by body composition, but not dose. Aylward *et al.* (2005) extended the Carrier *et al.* (1995a, b) model by incorporating elimination due to lipid partitioning of TCDD from the blood into the large intestine based on published human data (Moser and McLachlan 2002). In the Carrier model (Carrier *et al.,* 1995a,b), hepatic concentrations increase with dose in a non-linear manner due to hepatic sequestration. As the fraction of TCDD in the liver increases from 15 to 70%, there is a 5-fold maximum induction of the elimination rate in rats. For humans, the model estimated that the fraction of TCDD in the liver ranged from 1 to 70%, resulting in approximately a 70-fold induction of TCDD elimination at high exposures (Carrier *et al.,* 1995a,b; Emond *et al*., 2006).

Despite these mechanistic differences, most models provide reasonable fits to the experimental data. Dioxins are highly lipophilic,and concentrate in adipose tissue. Recent studies suggest that body fat mass influences the elimination of TCDD (van der Molen *et al.,* 1998; 2000). Michalek and Tripathi (1999) found that the TCDD t½ increases with body mass index (BMI) in humans. Increasing body mass index (BMI) alters the pharmacokinetics of lipophilic chemicals. The

consequence of increased distribution of lipophilic compounds into the adipose compartment is the alteration of concentration of xenobiotic metabolizing enzymes (Emond *et al.*, 2006).

Emond *et al.* (2006) have recently described a PBPK model that is optimized for the induction of CYP1A2 that occurs at elevated exposures (doses) of TCDD.. The model assumes a maximum forty-fold induction of CYP1A2 resulting in estimates of the inducible elimination rate (hour^{-1}) that range from 0.06 hr^{-1} for 10^{-3} µg/kg to 2.46 hr^{-1} at exposures up to 10^{3} µg/kg. This results in terminal elimination t½ estimates that range from approximately 75 days to approximately ten days at the higher exposures (Emond *et al.*, 2006) (see Figure B1-3).

This use of a variable elimination rate that corresponds to enzyme induction and receptor binding provides better fits to the experimental data of Santostefano *et al.* (1998) when compared to models that use a fixed rate of elimination (Emond *et al.*, 2006). At conditions of maximal induction (10 µg/kg) Emond *et al.* (2006) reported that the inclusion of the CYP1A2 hepatic sequestration in the PBPK model resulted in higher TCDD concentrations in the blood, and a good fit with experimental observations. Alternatively, removal of the CYP1A2 sequestration decreased the concentration of TCDD in the blood and resulted in an underestimate (compared to observed values) by over an order of magnitude (Emond *et al.*, 2006).

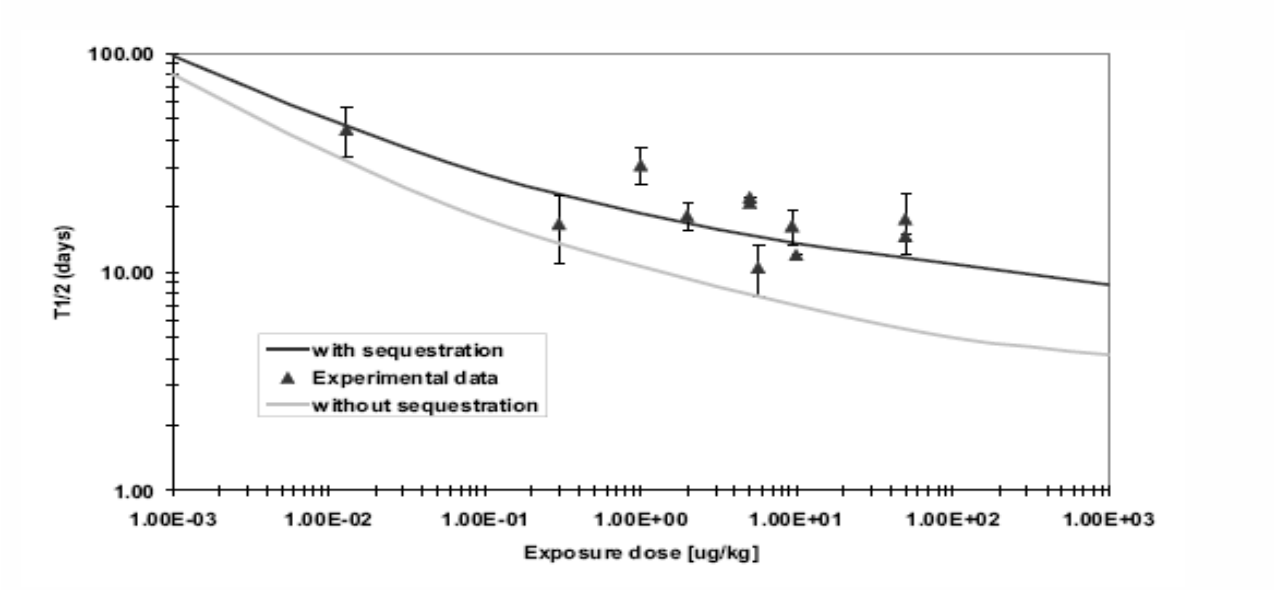


Figure B1-3 Taken from Emond *et al.*, 2006. Figure 2.2 from Emond *et al.* shows the relationship between elimination half-life (t½) and dose using PBPK modelling of TCDD

Selected Half-Life ($t_{½}$)

A half-life of 7.1 years has been assumed for this study, as it represents a conservative mid-point of TCDD specific half-lives noted in the literature, and is consistent with those reported by Pirkle *et al*. (1989), Wolfe *et al*. (1988) and Aylward *et al.* (2005). There exist a range of half-lives reported in the literature of up to 11.3 years. Essentially all exposures for herbicides that contained 2,4,5-T (Agents Orange and Purple as well as other formulations that were prepared for some plots) were exclusively to TCDD as virtually no significant levels of other dioxin congeners were present. The response to acute exposure to TCDD in humans is sequestration in the liver and an initial phase of rapid elimination; this suggests that the assumption of a constant

half-life for large exposures to TCDD is overly conservative. The constant half-life is utilized since current exposure and risk estimation methods do not accommodate a variable elimination rate.

B1-5.0 REVIEW OF REGULATORY EXPOSURE LIMITS

B1-5.1 Introduction

In deriving a tolerable intake of a particular chemical, regulatory agencies typically rely on both toxicological data from laboratory studies in animals and epidemiological data from exposed human populations to determine the most sensitive adverse effect observed after exposure. The WHO defines a tolerable daily intake (TDI) as: *"an estimate of the amount of a substance in food or drinking water, expressed on a body weight basis.. that can be ingested on a daily basis over a lifetime without appreciable risk"* (WHO, 2003). A TDI for a particular chemical is generally derived from either a no-observed-adverse-effect-level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL) that has been identified in animal toxicity studies demonstrating the most sensitive effect; *i.e.*, the adverse health effect occurring at the lowest dose of chemical tested. This NOAEL or LOAEL is then adjusted downwards by dividing by uncertainty factors to account for things like inter-species differences (between the test species and humans) and intra-species differences (among individuals within the population). Accordingly, exposure at or below the TDI is expected to pose no health risks, even in sensitive people who may be more susceptible. Although TDIs are typically derived from laboratory animal studies, careful consideration is also given to available studies of human exposure (ECSCF, 2001). The sections that follow for PCDD/Fs describe the basis of the various TDIs recommended by regulatory agencies are described.

B1-5.2 Discussion and Comparison of Exposure Limits

Occupationally exposed workers and populations accidentally exposed *via* contamination of the environment and food are primary subjects to determine effects of dioxins. As an example, the local population in Seveso, Italy was exposed to substantial quantities of dioxin in 1976, following a chemical plant explosion (Bertazzi *et al*., 2001). In two other populations, the ingestion of edible oils accidentally contaminated with high levels of PCBs and PCDFs was associated with toxicity in Yusho, Japan and Yu-Cheng, Taiwan.

Small increases in overall cancer mortality and in some cases lung cancer mortality have been reported in some studies of occupationally or accidentally exposed populations and on this basis, together with animal data, 2,3,8,7-TCDD has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC) and by the U.S. EPA. This is discussed in more detail below but it is worth noting that exposure to TCDD in these populations was at least 2-3 orders of magnitude higher than that in the general population (*i.e.*, 100 to 1,000 times higher) (WHO, 2000a) and likely much higher even than that. Recent evidence on elimination rates of dioxin at elevated body burdens indicates that previously back-calculated blood levels of occupationally exposed workers likely substantially underestimated their exposure (Aylward *et al*., 2005).

Based on available evidence from human, animal and mechanistic studies, most regulatory agencies consider dioxin a threshold carcinogen; *i.e*., they believe there is a level of exposure below which cancer risk is not or is negligibly increased above background incidence (WHO, 2000a; JECFA, 2001; ECSCF, 2001).

With the exception of the U.S. EPA, who have proposed cancer potency factors derived from occupational studies by which low dose cancer risks can be predicted (U.S. EPA, 2003), regulatory agencies have proposed or established tolerable daily or weekly intakes for dioxin based on animal data rather than human data. Reasons cited include: a) most available epidemiological studies do not reflect the most sensitive population identified by animal studies (*i.e.*, exposure during development, particularly *in utero*); b) the considerable uncertainty in exposure assessments and inadequate allowance for confounding factors; and, c) patterns of exposure which do not reflect exposures in the general population, which are mainly from the diet (UK COT, 2001). The Scientific Committee on Food in Europe noted that a number of effects produced in occupational or accidentally exposed humans are clearly high dose effects, including cancer (ECSCF, 2001).

Up until the last several years, most regulatory agencies recommended a tolerable intake level for dioxin based on two key chronic (*i.e.*, long-term) studies in rats. One study was a multigeneration reproductive study that identified reductions in fertility in the second generation of rats exposed to 10 ng TCDD/kg body weight per day (Murray *et al.*, 1979). The other study was a 2-year carcinogenicity bioassay that identified elevated tumour rates at doses of 10 ng/kg body weight and above (Kociba *et al*., 1978). In both studies, a dose level of 1 ng/kg bw/day was identified where no adverse effects were observed (NOAEL). Health Canada applied an uncertainty factor of 100 to this NOAEL to account for potential interspecies and intraspecies variability, resulting in a tolerable daily intake (TDI) of 10 pg/kg bw/day, considered to be protective of both adverse effects on reproduction and development as well as cancer (Feeley and Grant, 1993). This remains the "official" Canadian TDI for dioxin to this date. Several other countries or agencies recommended the same TDI, including the WHO (1991), the U.K., the Netherlands and the Nordic Group.

The U.S. Agency for Toxic Substance and Disease Registry (ATSDR) has recommended a chronic reference dose or MRL (minimum risk level) of 1 pg/kg bw/day based on a developmental neurotoxicity study in monkeys (Schantz *et al.*, 1992) and a 120-fold safety factor (ATSDR, 1998). They also recommended an intermediate MRL of 20 pg/kg bw/day based on a 90 day immunotoxicity study in guinea pigs, with a 30-fold safety factor (DeCaprio *et al*., 1986).

More recently, reevaluations of the health risks posed by dioxin-like compounds have been undertaken by the WHO (1998; 2000a) and other regulatory bodies (*e.g.*, the European Commission Scientific Committee on Food and the U.K. Committee on Toxicity in Chemicals in Food) as well as the U.S. EPA (2003). The U.S. EPA approach and conclusions with respect to safe levels are described below but some key characteristics of the WHO and related evaluations have been summarized as follows:

- Body burdens were used as dose metrics rather than daily doses in order to compare across species with very different rates of metabolism and excretion for dioxin-like compounds and 2,3,7,8-TCDD half-lives across species range from 20 to 30 days in rats, to approximately 400 days in monkeys and between 5 and 11 years in humans. Thus, rodents require considerably higher doses (100 to 200-fold) to reach the same equivalent body burdens at steady state as humans do and humans have a much higher potential for accumulation;

- The evaluation deviated from the earlier evaluations of TCDD by not using the long-term rat studies to identify critical endpoints for the derivation of the TDI. Most of the studies identifying the most sensitive responses in the recent evaluations involved acute gavage (bolus) exposure at a critical time during pregnancy;

- Although studies in humans were carefully evaluated, the evaluation was ultimately based on sensitive endpoints in experimental animals; and,

- Developmental, reproductive and hormonal effects following 2,3,7,8-TCDD exposure of female rats and monkeys were identified as the most sensitive adverse effects reported; *i.e*., the adverse effects occurring at the lowest body burdens (ECSCF, 2001).

As noted by Aylward *et al*., (2005), all of the recent assessments focus on a body of data that indicates that the most sensitive adverse responses to TCDD in experimental animals appear to be effects on the development of offspring following *in utero* exposure to TCDD, in particular alterations in male rat reproductive parameters. The WHO consultation recommended a TDI of 1 to 4 pg TEQ/kg body weight based on body burdens in experimental animals associated with the most sensitive adverse responses that have been reported, namely developmental and reproductive effects in rats and monkeys (WHO, 1998; 2000). They presented a range of reported animal lowest observed adverse effect levels (LOAELs) that occurred at a body burdens measured in the range of 10 to 73 ng/kg (see Table B1-6). Using pharmacokinetic calculations, these body burdens were transformed into estimated daily human intakes that on a chronic basis would be expected to lead to similar body burdens in humans under steady state conditions. The equation is as follows:

Body burden at steady state (ng/kg bw) = f * intake (ng/kg bw/day) * half-life in days/ln(2)

where f is the fraction of dose absorbed (assumed to be 50% for absorption from food for humans) and an estimated half-life for 2,3,7,8-TCDD of 2740 days (7.5 years)

The estimated daily intakes associated with the animal LOAELs were calculated as 14 to 37 pg/kg body weight per day (WHO, 2000a; Table B1-12). A 10-fold uncertainty factor was applied to this range of intakes to account for the use of LOAELs instead of NOAELs, as well as potential differences in susceptibility. Normally, a factor of 10 would be applied for each of these considerations but the WHO assessment concluded that evidence suggests: a) only a small uncertainty factor is necessary for differences in susceptibility amongindividuals (*i.e.*, 3.3-fold rather than 10-fold) and b) the LOAELs identified (Table B1-12) were considered to be within a factor of 2-3 to the NOAELs, as opposed to 10. A composite uncertainty factor of 10 was therefore considered to be sufficient. An interspecies uncertainty factor was unnecessary because a) the use of body burdens accounts for differences in toxicokinetics and b) evidence suggests that humans in general are less sensitive than rats, but the possibility exists that the most

sensitive humans may be as sensitive as rats. The resulting TDI was expressed as 1 to 4 pg TEQ/kg body weight per day (WHO, 2000a).

Table B1-12 LOAELs identified by WHO (2000a) with Measured or Derived Body Burdens and the Related Human Estimated Daily Intake (EDI)

Study	Response (LOAELs)	Dose Given (pg/kg bw)	Body Burden (ng/kg bw)	Related human EDI (pg/kg bw/day)
Rats				
Gray *et al*., (1997a) Mably *et al*., 1992a)	Decreased sperm counts in offspring	64,000	28	14
Gehrs *et al*., (1997) Gehrs and Smailowicz (1998)	Immune suppression in offspring	100,000	50	25
Gray *et al*., (1997b)	Increased genital malformations in offspring	200,000	73	37
Monkeys				
Schantz and Bowman (1989)	Neurobehavioral (object learning) effects in offspring	160	42	21
Rier *et al*., (1993)	Endometriosis	160	69	35

Adapted from WHO (2000a)

The ECSCF (2001), JECFA (2001) and the U.K. Committee on Toxicity and Food (UK COT, 2001) re-evaluated dioxins based on similar studies but they relied on newly published data allowing calculation of the total amount of dioxin in the fetus (fetal body burden) associated with maternal exposure at steady state. They also focused in on the studies reporting effects on the male reproductive system in offspring of dams exposed during pregnancy. Effects in female offspring have also been studied extensively but in most studies, these are observed at higher doses than those inducing effects in male offspring (JECFA, 2001). Noteworthy comments were made by the committees regarding the monkey studies summarized in Table B1-12. With respect to the neurobehavioral study, the Scientific Committee on Food in Europe noted that chronic dietary exposure of female rhesus monkeys to 5 ppt TCDD in the diet produced a subtle change in only one, among several parameters related to cognitive recognition (object learning) in offspring (Schantz and Bowman, 1989). They concluded that this non-persistent change was likely of doubtful significance for humans (ECSCF, 2001). With respect to the endometriosis study, the U.K. Committee on Toxicity and Food noted that a follow-up study of the same monkeys reported that the incidence of endometriosis was correlated with serum levels of certain PCB congeners, but not TCDD (Rier *et al*., 2001; UK COT, 2001). They also noted that a number of aspects of this observational study undermined confidence in the results and in the earlier findings (UK COT, 2001).

Although there were some differences in assumptions and calculations, ECSCF (2001), JECFA (2001), and the U.K. COT (2001) all came up with similar recommendations for a tolerable intake based on Faqi *et al*. (1998) study. Similar to WHO, they relied on body burden and half-life data to transform the body burden LOAELs into an estimated human daily intake. A 9.6-fold safety factor was applied to the LOAELs to account for uncertainty. The ECSCF (2001) recommended a tolerable *weekly* intake of 14 pg/kg body weight for 2,3,7,8-TCDD, based on the LOAEL identified by Faqi *et al*. (1998) (equivalent to 2 pg/kg/day). The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001) proposed a provisional tolerable *monthly* intake of 70 pg TEQ/kg body weight, based upon the lowest LOAEL (Faqi *et al.*, 1998; sperm

counts) and a NOAEL (Ohsako *et al.*, 2001; decreased ventral prostate weight) for developmental effects in male rat offspring (equivalent to 2.3 pg/kg bw/day). For the NOAEL, an uncertainty factor of 3.2-fold was applied rather than 9.6-fold. The provisional tolerable monthly intake of 70 pg/kg bw/day represents the mid-range of TDI estimates based on the LOAEL *versus* the NOAEL (JECFA, 2001). Health Canada participated in this evaluation and they have provisionally adopted this TDI for risk assessment purposes but they are in the midst of a comprehensive reassessment of the risks posed by dioxins (Health Canada, 2005). Assuming 20 days per month, Health Canada (2005) has endorsed 2.3 pg TEQ/kg bw/day as the tolerable level (70 pg TEQ/kg bw/month ÷ 30 days/month). The U.K. Committee on Toxicity in Food (UK COT, 2001) derived a TDI of 2 pg/kg bw/day based upon the lowest LOAEL for male reproductive effects in offspring (Faqi *et al*., 1998).

ECSCF (2001) and JECFA (2001) expressed their tolerable intakes on a weekly or monthly basis, respectively, to reflect the very long half-lives in the human body of TCDD and related compounds. They noted that a daily intake at the TDI would need to occur for a prolonged time period (20 to 30 years) in order to achieve the body burden levels of concern at steady state (ECSCF, 2001). Similarly, UK COT (2001) point out that short-term variation in intake dose does not significantly alter the body burden and occasional exceedance of the TDI would not be expected to result in harmful effects, provided that intake averaged over a prolonged period is within the TDI.

Table B1-13 presents a summary of the recent tolerable intake levels recommended by various agencies, including the study/studies on which they were based and the safety factors applied.

Table B1-13 Acceptable Daily Intakes Established by Regulatory Agencies or Scientific Bodies After Recent Re-evaluations of Fioxin and Fioxin-like Compounds

Regulatory agency/scientific body	Basis for TDI/RfD	Tolerable intake
ATSDR (1998)	Chronic MRL: Reproductive toxicity in monkeys with 120-fold safety factor applied to LOAEL (Schantz *et al*., 1992; Intermediate MRL: 90-day immunotoxicity study in guinea pigs with 30-fold safety factor (DeCaprio *et al*., 1986); Acute MRL: immunological effects in female mice (Burleson *et al*., 1996).	Chronic MRL= 1 pg/kg bw/day Intermediate MRL = 20 pg/kg bw/day Acute MRL = 200 pg/kg bw/day
Health Canada (2004; 2005)	Official TDI: Chronic and reproductive toxicity studies in rats with 100-fold safety factor applied to NOAEL (Murry *et al*., 1979; Kociba *et al*., 1978) Provisional TDI: based on JECFA TDI of 70 pg/kg bw/month	Official TDI= 10 pg/kg bw/day Provisional TDI = 2.3 pg/kg bw/day

Table B1-13 Acceptable Daily Intakes Established by Regulatory Agencies or Scientific Bodies After Recent Re-evaluations of Fioxin and Fioxin-like Compounds

Regulatory agency/scientific body	Basis for TDI/RfD	Tolerable intake
WHO (2000a)	Reproductive toxicity in rats with 10-fold safety factor applied to LOAELs (Gray *et al*., 1997a, 1997b; Gehrs and Smialowicz, 1998; Gehrs *et al*., 1997) calculated from maternal body burden with half-life of 8.5 years	Tolerable daily intake = 1-4 pg/kg bw/day
European Commission (ECSCF, 2001)	Reproductive toxicity in rats with 9.6 fold safety factor applied to LOAEL for male rat offspring (Faqi *et al*.,1998; Ohsako *et al*., 2001) calculated from maternal body burden with half-life of 7.6 years	Tolerable weekly intake: 14 pg/kg bw/wk (equivalent to 2 pg/kg/day)
United Kingdom (UK COT, 2001)	Reproductive toxicity in rats with 9.6-fold safety factor applied to a LOAEL and NOAEL for male rat offspring (Faqi *et al*., 1998) calculated from maternal body burden with half life of 7.5 years	Tolerable daily intake: 2 pg/kg bw/day
Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001)	Reproductive toxicity in rats with 3.2-fold to 9.6-fold safety factor applied to NOAEL and LOAEL, respectively for male rat offspring (Ohsako *et al*., 2001; Faqi *et al*., 1998) calculated from maternal body burden with 7.6 years half-life.	Provisional tolerable monthly intake: 70 pg/kg bw/month (equivalent to 2.3 pg/kg bw/day)
U.S. EPA (2003, 2004a)	Cancer potentcy factor of 1,000,000 (mg/kg bw/day$^{)-1}$ deived from analyses of occupationally exposed workers (Becher *et al.,* 1998; Steenland *et al*., 2001; Crump *et al*., 2003).	Risk-specific dose (RsD) = 0.01 – 0.1 pg/kg bw/day for incremental cancer risks of 1 in a million or 1 in 100,000, respectively.
U.S. EPA (2003, 2004a)	ED01 (estimated dose where 1% affected) of 0.025 ng/kg bw in rats based on Mably *et al*. (1992a) sperm effects, converted to human daily dose of 0.13 pg/kg bw/day assuming 50% bioavailability and 7.6 year half-life.	0.13 pg/kg bw/day. Margin of exposure (MOE) approach: <0.1 pg/kg bw/day relative to background intake

Adapted from Paustenbach *et al*., 2006

The U.S. EPA began a re-assessment of dioxin exposure and human health effects in 1991. In its most recent draft (U.S. EPA, 2004a), they chose not to establish a TDI but rather relied on a margin-of-exposure (MOE) approach to evaluate potential health risks from PCDD/Fs. An MOE is calculated by dividing a dose or exposure level affecting a given percentage of humans or test animals (*i.e.*, a benchmark dose) by the corresponding actual or estimated human dose or exposure level from background or a particular exposure source. The larger the margin, the more confidence there is that adverse effects are not occurring in humans. U.S. EPA considers MOEs in the range of 100 to 1,000 are adequate to rule out the likelihood of significant effects in humans based on sensitive animal responses. For PCDD/Fs, they have concluded based on laboratory animal data that the MOEs at current background levels are less than 10 or less for

both cancer and non-cancer effects. Consequently, they assert that a reference dose (*i.e.*, TDI) for TCDD would be of no practical benefit since this "safe" dose would fall below background exposure levels (U.S. EPA, 2000). However, as noted by Paustenbach *et al.* (2006), several reviewers have pointed to the inconclusive nature of the studies the U.S. EPA relied on as evidence for human effects of dioxin at doses/body burdens near background levels (Bertazzi *et al*., 1998; Cole *et al*., 2003; De Rosa *et al*., 1999a,b; Feeley and Brouer, 2000; Green *et al*., 2003; Kogevinas, 2001; Pohl *et al*., 2002; Starr, 2001; Sweeney and Mocarelli, 2000).

Charnley and Kimbrough (2006) note that evaluating the significance of the U.S. EPA's estimated MOEs in terms of human health requires taking into account the following:

- The likelihood that the effects observed in laboratory animals are relevant to humans;
- The likelihood that humans are more or less sensitive to PCDD/F related toxicity than laboratory animals;
- The fact that the MOEs were estimated on the basis of average intakes or body burdens for the population as a whole and not for children, who have lower body burdens but higher intakes than adults; and,
- Data indicating that current intakes and body burdens are lower than those assumed by U.S. EPA.

With respect to the last point, the U.S. EPAs reassessment of dioxins include dietary intake estimates that exceed those determined recently by the U.S. Food and Drug Administration as part of its Total Diet Study (U.S. FDA, 2003), as well as intake estimates reported by Charnley and Doull (2005) (U.S. EPA, 2003). The over-estimation of background exposures through the use of old consumption and contaminant level data resulted in lower MOEs than would otherwise have been calculated.

U.S. EPA (2004b) has also recommended a cancer potency factor (CPF) derived from occupational cohort studies of dioxin-exposed workers (Becher *et al*., 1998; Steenland *et al*., 2001; Crump *et al*., 2003). The recommended CPF is 1,000,000 $(mg/kg\ bw/day)^{-1}$. Assuming linear extrapolation to low doses, this translates to an "acceptable" risk specific dose for TCDD of 0.01 pg kg/day for a 1 in 100,000 cancer risk or 0.001 pg/kg bw/day for a 1 in a million cancer risk. As previously discussed, the appropriateness of their assumption that dioxin can increase cancer risk at low doses is controversial. A recent paper by Pavuk *et al*. (2005) suggests that dioxin exposure is associated with an increase in all-sites cancer risk although the issue of a low-dose mechanism remains unclear. The risk-specific dose associated with the U.S. EPAs cancer potency factor is one to three orders of magnitude lower than the tolerable intakes developed by international scientific panels that have evaluated PCDD/Fs (*i.e.*, WHO, JECFA and ECSCF).

B1-5.3 Conclusions of Various Regulatory Agencies

TCDD does not affect genetic material and there is a level of exposure below which cancer risk would be negligible (WHO, 1999).

The COC agreed that TCDD should be regarded as a probable human carcinogen on the basis of all the available data. The Committee agreed that, although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (i.e., negative genotoxicity in standard assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate. In this respect Members commented that any increased risk of cancer at background levels of exposure is likely to be extremely small and not detectable by current epidemiological methods. (U.K. Committee on Carcinogenicity of Chemicals in Food and Consumer Products (UK COC, 2001).

The consultation recognized that the epidemiological evidence for the most highly TCDDD-exposed cohorts studied produces the strongest evidence of increased risks for all cancers combined, along with less strong evidence of increased risks for cancers of particular sites. The relative risk for all cancers combined in the most highly exposed and longer latency sub-cohorts was 1.4. While the relative risk is not likely to be explained by confounding, this possibility cannot be excluded (WHO, 2000a).

U.S. EPA's reassessment of dioxin and related compounds may place too much confidence in the ability to accurately predict cancer risks at low doses. This approach dramatically increases cancer risk estimates that are not based on compelling new data but rather on the application of statistical models applied to results of occupationally exposed cohorts that have been associated with significant uncertainty regarding actual exposure. This is further confounded by the fact that these models are not yet fully validated and that we still have knowledge gaps with respect to the mechanism of action and interaction for the dioxin-like group of chemicals (Agency for Toxic Substances and Disease Registry scientists; Pohl et al., 2002).

In the Priority Substance List assessment, Health Canada concluded that there is no adequate demonstration that human populations exposed to dioxins and furans have suffered excess cancer. However, based on the results of studies in animals, it was assumed that chlorinated dioxins and furans are non-genotoxic carcinogens and reproductive toxicants with a threshold, and therefore a tolerable daily intake for human exposure was derived (Canadian Environmental Protection Agency (CEPA, 1997)). Based on JECFA (2001) Health Canada (2004; 2005) has adopted a tolerable level of 70 picograms per kilogram body weight per month or approximately 2.3 picograms per kilogram of body weight per day. This TDI of 2.3 pg/kg body weight/day has been adopted for the current study.

ATSDR (1998) has derived an acute MRL of 200 pg/kg bw/day based on immunological effects in female mice (Burleson et al., 1996). ATSDR (1998) classified this as a "less serious" effect. "Serious" effects were reported by Mably et al. (1992) at a dose of 0.064 μg/kg body weight. This value has been adopted for acute exposures for the current study.

B1-6.0 ENVIRONMENTAL FATE

The chemical properties of dioxins (low water solubility, high stability and semi-volatility) favour long distance transport in particulate form and are eventually deposited on soils, surface waters or plant vegetation through wet or dry deposition (ATSDR, 1998). In water, mono, di and tri chlorinated dibenzo-p-dioxins (CDDs) will slowly volatilize from the water column and the more highly chlorinated CDDs will be deposited into the sediment following adsorption to

suspended particulate (Fletcher and McKay, 1993; Muir *et al*., 1992). CDDs tend to adsorb to organic matter once on soil and may enter the atmosphere on soil dust particles or enter surface water during periods of runoff. CDDs tend to bioaccumulate in aquatic and terrestrial organisms due to their low water solubilities and high lipophilicity; however, due to their tendency to bind to organic matter the actual uptake by organisms is contentious.

B1-6.1 Air

If released to the air, 2,3,7,8-TCDD will exist primarily in the particulate or vapour phase (HSDB, 2004; ATSDR, 1998). Vapour-phase CDD will degrade by reaction with photo chemically produced hydroxyl radicals, with an estimated half-life for this reaction of 2 days (Atkinson, 1991). Wet or dry deposition is the primary removal pathway for particle bound CDDs and to a lesser extent, photolysis removes.

B1-6.2 Water

Photolysis is the dominant transformation process in aqueous solutions (Hutzinger *et al*., 1985). Although this process is generally very slow, in the presence of organic hydrogen donors (*i.e*., natural organic films from soils or chlorophenol pesticides) and when exposed to ultraviolet light degradation may be rapid (Crosby *et al*., 1973). Mackay *et al*.,1992 estimated a half-life in water to be approximately 1 week. Volatilization from water surfaces may also be an important fate process; however, dioxins are primarily expected to be absorbed to suspended solids and deposited into the sediment. Arthur and Frea (1989) determined that the potential for biological degradation in water was low.

B1-6.3 Sediment and Soil

Soils and sediments are the primary "sink" for dioxins (CEPA, 1990). PCDD is very slow to degrade in soil and sediment with estimated half lives ranging from 2 to greater than 6 years. Soil studies have demonstrated that photo-degradation may occur on soil surfaces, with photolysis occurring between 0.06 and 0.13 mm below the surface (Miller *et al*., 1989). Specific species of fungus and bacteria have been found to degrade 2,3,7,8-TCDD; however, in general, biological degradation is not an important environmental fate process.

The National Academy of Sciences' (NAS) Institute of Medicine (IOM) issued various studies to determine the fate and degradation of Agent Orange Contamination and 2,3,7,8-TCDD potential to effect ground troops in Vietnam (Young *et al*., 2004; Young and Newton, 2004). Young and Newton (2004) reported that following test application of Agent Orange in 1969, 1970 soil concentrations of TCDD were below a detection limit of 1 ppb in all soil cores. Young *et al*. (2004) reports that only a very small portion of TCDD would reach the ground as the majority would be collected in the canopy. In soil, TCDD will be eliminated through photolysis, volatilization and re-adsorption (Young *et al*., 2004). At the Eglin spray testing, nearly all of the TCDD in the herbicides quickly degraded during and immediately after application (Young *et al*., 2004). Young *et al*., 2004 reports that once below the surface, typically confined to the top 15 cm, low residues of TCDD remained for at least 14 years. Crosby and Wong (1977) found that when Agent Orange was applied to loam soil and exposed to sunlight the degradation was "somewhat slower" than when applied to plant leaf surfaces (which had an estimated half-life of 6 hours).

JW (2006) has indicted a soil half-life of TCDD on surface soils may vary from less than 1 year to 3 years; but half-lives in deeper soil may be as long as 12 years. Mackay *et al*. (1992) provide a similar range as JW (2006) and provide a mean of approximately 2 years.

B1-6.4 Environmental Fate of Contaminants Applied to Vegetation

Photodegradation is a dominant environmental transformation process for polychlorinated dibenzo-p-dioxins (PCDDs) as it has been found to occur for chlorinated organics in surface waters, on soil and in the atmosphere (Niu *et al*., 2003). McPeters and Overcash (1993) reported 60 to 85 % photodegradation of total TCDD in contaminated soils. They also reported substantial reduction in concentration of TCDD in soil after 60 days. The primary factors controlling the fate of TCDD deposited on foliage is tissue absorption and adsorption or dissipation through evaporation and photodegradation (Young *et al*., 2004). Various photodegradation studies of dioxins on vegetation found a transfer of photo-oxidation products of PCDD/Fs from the atmosphere into the terrestrial food web (Crosby and Wong, 1997; McCrady and Maggard, 1993; Niu *et al*., 2003). Niu *et al.* (2003) exposed spruce needles to PCDD/Fs generated by combustion of chlorinated plastics. They found that photodegradation of PCDD/Fs followed pseudo first-order kinetics and that photodegradation played a major role in the fate of PCDD/Fs. Calculation of toxic equivalents in mixtures determined a significant reduction in the likely risk to humans after varying periods of direct exposure to sunlight. Photolysis half lives were greater for higher chlorinated PCDD/Fs (penta-, hexa-, and octa-) than for the tetra- CDD/Fs (*i.e*., 2,3,7,8-tetrachlorodibenzo-p-dioxin).

Following spray application of Agent Orange and associated TCDD, Young *et al*. (2004) found that TCDD was absorbed into the wax layer and remained lodged in the cuticle. Depending on the amount of sunlight levels the surface contaminant would be destroyed by light within a few hours, smaller portions may absorbed more deeply into the plant and become biologically unavailable (Young *et al*., 2004). When Agent Orange was applied to leaves and exposed to natural sunlight, Crosby and Wong (1977) estimated a half-life of 6 hours for TCDD. Norris (1996) determined a half-life of 7 to 10 hours even when ultraviolet light intensity was low.

Although no TCDD analyses appear to have been made during the period of heavy use of Agent Orange in Vietnam and Southeast Asia, data from Crosby and Wong (1977) strongly suggest that environmental residues of TCDD should be considerably below what might have been expected based on the half life and environmental fate attributed to pure preparations of TCDD (Crosby and Wong, 1977).

When Agent Orange was applied over the leaf surface of a rubber plant and subsequently exposed to direct sunlight, TCDD was rapidly degraded (Crosby and Wong, 1977). Sunlight was identified as the principal factor in dioxin disappearance from inert surfaces, plants and soil treated with herbicides (or other pesticides) containing TCDD. Light caused TCDD concentrations to decline sharply while dark controls were virtually unaffected (Crosby and Wong, 1977). Furthermore, it appeared that the presence of the formulation was important to the process, since pure TCDD appeared to be more resistant to the effects of sunlight. In comparison tests that used similar concentrations of TCDD applied to the leaf material or to soil samples as Agent Orange or as pure dioxin, the TCDD in the Agent Orange formulation was more rapidly photodegraded (Crosby and Wong, 1977).

A few studies have shown that photodegradation of dioxins on vegetation resulted in the transfer of photo-oxidation products of PCDD/Fs from the atmosphere into the terrestrial food web (Crosby and Wong, 1977; McCrady and Maggard, 1993; Niu *et al*., 2003). The hypothesis put forward by Schuler *et al*. (1998) and Crosby and Wong (1977) predicted that photolysis of PCDD/Fs on plants generally took place in the cuticle and especially the cuticular wax coating of the leaves (Crosby and Wong, 1977; McCrady and Maggard, 1993).

In one study PCDD/Fs generated by combustion of chlorinated plastics and that adhered to or were adsorbed to spruce needles were examined. Following exposure to exhaust gas containing PCDD/Fs a clear sensitivity for photodegradation upon exposure to sunlight was observed (Niu *et al*., 2003). The rate of photodegradation of PCDD/Fs was determined by plotting the natural logarithm of the concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and PCDFs against time. The disappearance of recoverable chlorinated dioxin and/or dibenzofurans from the spruce needles followed pseudo-first-order kinetics (Niu *et al*., 2003). It was concluded that under field conditions, photodegradation played a major role in the fate of PCDD/Fs adsorbed to spruce needles. Calculation of toxic equivalents (TEQ) in mixtures of PCDD/Fs recovered from spruce needles after varying periods of exposure to direct sunlight predicted a significant reduction in potential toxicity to humans. The photolysis half lives for higher chlorinated PCDD/Fs (*e.g*., penta-, hexa- and octa- chlorinated dibenzo-p-dioxins and dibenzofurans) were greater than for the tetra- chlorinated compounds (Niu *et al*., 2003).

In another study, Schuler *et al*., (1998) reported that photolysis rates for free (not particle-bound) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin dissolved in the cuticular waxes of laurel cherry were more rapid than rates for dioxins with higher levels of chlorination (Table B1-14). Dry deposition of PCDDs from particles has been reported as negligible (Simonich and Hites, 1995). The results of Schuler *et al.* (1998) demonstrated that even at low levels of sunlight irradiation intensity PCDD/Fs dissolved in cuticular wax were readily degraded by natural sunlight.

Table B1-14 Rate constants of photodegradation (k_{photo}) of selected PCDD/Fs Dissolved in Cuticular Wax from the Laurel Cherry[a]

Congener	Sunlight k_{photo} (hr^{-1})
2,3,7,8-TCDD	**0.035**
1,2,3,7,8-PeCDD	0.109
1,2,3,4,7,8-HxCDD	0.101
1,2,3,4,6,7,8-HpCDD	0.137
OCDD	0.161
2,3,7,8-TCDF	0.077
1,2,3,7,8-PeCDF	0.098
2,3,4,7,8-PeCDF	0.129
1,2,3,4,7,8-HxCDF	0.148
1,2,3,4,6,7,8-HpCDF	0.218

[a] Adapted from Schuler *et al*., 1998

Photodegradation can be assumed to be process that proceeds continuously from the time of application. An equilibrium concentration in the wax layer of leaved can be expressed by the equation below (from Schuler *et al*., 1998):

$$c_{wax} = c_{air} \bullet \frac{k_{uptake}}{k_{vol} + k_{photo}}$$

Where:

c_{wax} is the concentration of PCDD in the wax layer (pg/m^3)
c_{air} is the concentration of PCDD in the ambient air (pg/m^3)
k_{uptake} is the rate constant for uptake from the vapour phase (1.9 x 10^4 h^{-1} for 1,2,3,4-TCDD)
k_{vol} is the rate constant for volatilization from the leaf surface (2.1 x 10^{-4} h^{-1} for 1,2,3,4-TCDD)
k_{photo} is the rate constant of photodegradation (3.5 x 10^{-2} h^{-1} for 1,2,3,4-TCDD) (Table B1-14)

The equation accounts for some volatilization of TCDD from surfaces and soils, but as photodegradation is a much faster process than volatilization the equilibrium concentrations in the wax coating of leaves should be controlled largely by photodegradation. The rapid rate of loss of PCDDs from vegetation may explain the reportedly great variability in TCDD concentrations recovered from environmental samples including spruce needles and grasses (Simonich and Hites, 1995; Safe *et al*., 1992) as well as inconsistent recovery from bare soils in comparison to soils that have vegetative coverage (Hagenmaier and Krauss, 1993).

B1-7.0 REFERENCES

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B2: HEXACHLOROBENZENE

B2-1.0 BACKGROUND INFORMATION

IUPAC: HCB

CAS: HCB

CASRN: 118-74-1

Hexachlorobenzene (HCB) has been used as a wood-preserving agent, as a porosity-control agent in the manufacture of graphite anodes, as a peptizing agent in the production of nitroso and styrene rubber for tires, in the production of pyrotechnics and tracer bullets for the U.S. and Russian military (Shekhovtsov, 2002), as a fluxing agent in the manufacture of aluminum, and as a chemical intermediate in dye manufacturing (ATSDR, 2002; Barber *et al.*, 2005). There are no current commercial uses of HCB as an end product in North America (ATSDR, 2002). In addition, HCB is a known contaminant of the herbicide picloram.

In addition to these primary deliberate and accidental emissions, significant re-emission of old HCB from soils and sediments may be occurring. Indeed, a substantial portion of the HCB currently measured in the atmosphere is thought to come from volatilization of old HCB in the soil from past contamination (Bailey, 2001).

HCB is a chlorinated hydrocarbon which may contain some higher polychlorinated dibenzofurans and dioxins as impurities (Villanueva *et al.*, 1974). It has been used in the manufacture of industrial chemicals, including chlorinated pesticides, and as a fungicide and seed dressing in agriculture. Both production and use of HCB have decreased since the 1970s owing to bans and restrictions on its use in many countries, but it still occurs as a by-product of the production of a number of chlorinated solvents and other industrial chemicals (Barber *et al.*, 2005). HBC registration was withdrawn in Canada in 1976 and eventually banned in 2003 (Barber *et al.*, 2005). Although HBC production has ceased in most countries, it is still generated inadvertently as a by-product and/or impurity in several chemical processes (Barber *et al.*, 2005; WHO, 2005). Current emissions are estimated to be 70 to 95% lower than emissions in 1970 (Barber *et al.*, 2005). In North America, the highest concentrations of HCB have been reported in the region of the Great Lakes where industrial sources were greatest.

Occupational exposure to HCB has occurred during its production and use in industry and agriculture. HCB has been detected in many foodstuffs, but dietary intake has decreased in recent years. (Appendix C).

B2-2.0 CHEMICAL AND PHYSICAL PROPERTIES

Formula: C_6Cl_6

Activity: fungicide (organochlorine)

Notes: Withdrawn Canada: 1976; Banned Canada, 2003

Structure:

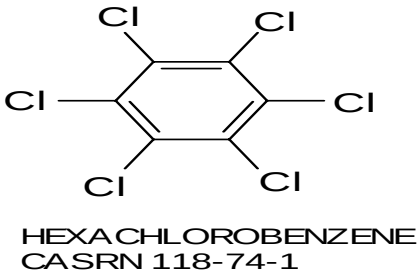

Figure B2-1 Structure Hexachlorobenzene Carsn 118-74-1

Table B2-1 Chemical and Physical Properties of HCB

Chemical/Physical Property	Result	Reference
Synonyms/Trade names	Perchlorobenzene; HCB; pentachlorophenyl chloride	Lide, 2005
Empirical formula	C_6Cl_6	
Molecular weight	284.78	Lide, 2005; JW, 2006
Physical state	White crystalline solid	Lide, 2005
Boiling point ^oC (760 mm Hg)	325 ^oC	Lide, 2005
Melting point	231 ^oC	Lide, 2005
Density	2.044 g/cm^{-3}	Lide, 2005
Odour	No data	
Solubility in water	Insoluble: 0.006 mg/L (25°C)	Lide, 2005; Verschueren, 1996; JW, 2006
Solubility in organic solvents	sl ethanol; s ether, chloroform; *vs*. benzene	Lide, 2005
Henry's Law Constant	131 pKa m^3mol^{-1}; 1.7 x 10^{-3} (25°C); 5.8 x 10^{-4} atm-m^3/mol	Lide, 2005; JW, 2006; Hulscher *et al.*, 1992 (Cited In: ATSDR, 2002)
Vapour Pressure	1.09 x 10^{-5} mmHg (20 ^oC) ; 1.8 x 10^{-5} mmHg (25°C)	O'Neil, 2001 ; JW, 2006
Log octonol/water partition coefficient (K_{ow})	5.74	Lide, 2005; Hansch *et al.*, 1995
Log K_{oc}	6.08 or 3.6-5.5	U.S. EPA, 1981 (Cited In: ATSDR, 2002); HSDB, 2005.
Log K_{SA} (soil/air partition coefficient)	5.0-7.3	Hippelein and McLachlan, 1998; Meijer *et al.*, 2003

Table B2-1 Chemical and Physical Properties of HCB

Chemical/Physical Property	Result	Reference
Half life (environmental)	Air: 0.4-4.3 years (photo-oxidation) Groundwater: 5.3-11.5 years (biodeg) Soil: 2.7-5.7 years Surface water: > 3.4 years; mean: ~ 6 years	JW, 2006; Mackay *et al.*, 1992
Air Concentration Conversion	1 ppm = 11.65 mg/m^3	

B2-3.0 TOXICOLOGY SUMMARY

B2-3.1 Human and Animal Health Effects

Table B2-2 Health Effects Resulting from Acute and Chronic Exposure to HCB

Exposure	Effects	Response
Inhalation Chronic	Vital Signs	No recorded effects
	Immunological	Decreased neutrophils chemotaxis and cytolytic activity. Increased serum imunoglobulins among workers receiving inhalation exposures. Reduction in interferon-γ. None of these symptoms were overtly toxic. Primarily indicators of exposure. In animals, decreased immunocompetence was reported after HCB inhalation followed by a bacterial challenge test. Humoral pulmonary defence altered
	Respiratory	None recorded
	Neurologic	Delayed or impaired motor development in newborns receiving exposures *via* mother's milk. No relation to Parkinson's disease. No relation to intellectual development.
	Hepatic	Liver damage; heme biosynthesis. In rats porphyria induced at high doses
	Hematologic	Heme biosynthesis interrupted. Photosensitivity
	Reproductive/Developmental	No reliable human data. No evidence of increased abortion, low birth weight or congenital malformation. In animals, developmental effects of acute exposure not reported.
	Cancer	Chronic exposure to HCB in air was associated with thyroid cancer, soft-tissue sarcoma. (Flix, Spain)
	Cardiovascular	No reliable human data. Animal studies do not suggest treatment related effects on cardiovascular tissue
	Respiratory	No reliable data for humans. Airway hyperresponsiveness in animals. Other pathological effects have been reported in rats. Hypertrophy and proliferation of the lining of pulmonary venules. Accumulation of lipid and foamy-looking macrophages. Intra-alveolar hemorrhage, inflammation in rats at high doses. No pulmonary lesions in monkeys, dogs or mice.
	Gastrointestinal	No reliable human data. No reliable data on effects in animals
	Hepatic	Oral exposure results in hepatopathology in humans. Porphyria, abnormal levels of precursors to porphyrin reported in urine. Changes in liver histopathology. In animals, induction of microsomal enzymes (CYP-P450) has been reported. Inhibition of hepatic uroporphyrinogen decarboxylase and δ-aminolevulinic acid synthestase in rats. Pattern of effects like human porphyria cutanea tarda.
	Genitournary	No reported effects on renal tissues in humans. In animals, the kidney is a target for HCB. Increased kidney weight;

Table B2-2 Health Effects Resulting from Acute and Chronic Exposure to HCB

Exposure	Effects	Response
		accumulation of porphyrins linked with inhibition of heme metabolism. Direct evidence for damage to renal cortex secondary to stimulation of lipid peroxidation (oxidative stress) in this tissue. Increased levels of alkaline phosphatase and renal microsomal enzymes. Renal tubule cells affected. Nephrotoxicity likely male rat-specific (α_2-microglobulin).
Ingestion Chronic	Hematologic	No reliable human data. Animal studies suggest anemia and leukocytosis. Haemoglobin reduction because of porphyria. No anemia in monkeys, pigs or rabbits exposed 12 -16 wks.
	Musculoskeletal	Swelling of joints, osteoporosis and severe shortening of digits. In animals reduced bone resorption. Secondary effects of hyperparathyroidism include interruption of calcium regulation.
	Endocrine	HCB adversely effects endocrine function. Target = thyroid. Thyromegaly detected in 60% of Turkish females exposed to HCB in bread. Hirsutism (abundant shaggy hair) and small stature have been reported. In animal studies hypothyroidism and hyperparathyroidism are induced by HCB. Serum T4 levels decreased in rats treated by gavage. Serum TSH was affected by fluctuations in T4 production. Hamsters treated with HCB demonstrated increased thyroid weights accompanied by changes in follicle histology. The adrenal gland is also a target in rats. Effects included adrenal hypertrophy but mainly in females.
	Reproductive	In animals HCB affects serum levels of estrogen and progesterone.
	Dermal	Photosensitivity due to porphyria produces dermal lesions in humans exposed to HCB in the diet. Tissue damage results from the production of reactive oxygen species (mainly hands and face). Areas of erythema were noted especially on skin exposed to light (head, neck, shoulders). Sclerodermatous skin thickening has also been reported among exposed humans. Among individuals who showed evidence of dermal effects of HCB exposure, most continued to show some symptoms for thirty years.

HCB undergoes limited metabolism, yielding pentachlorophenol, tetrachloro-hydroquinone and pentachlorothiophenol as the major metabolites in urine. The acute toxicity of HCB to experimental animals is low (HSDB, 2005). The acute lethal dose for HCB has been reported for a number of species. In the U.S. EPA (1985) review of the toxicology of chlorinated benzenes, the LD50 for HCB in mice, rabbits and cats was reported as 4,000, 2,600 and 1,700 mg/kg body weight, respectively. Furthermore, the LD50 for HCB in rats ranged between 3,500 and 10,000 mg/kg body weight. No data were available concerning the acute lethal dose of HCB for the inhalation or dermal routes of exposure. Death in experimental animal studies was usually associated with liver and central nervous system toxicity (Courtney, 1979).

The available data on the systemic toxicity of HCB indicate that the pathway for the biosynthesis of haeme is a major target of HCB toxicity. Elevated levels of porphyrin and/or porphyrin precursors have been found in the liver, other tissues and excreta of several species of laboratory mammals. Porphyria has been reported in a number of studies in rats with subchronic or chronic oral exposure. Less frequently reported organ effects of repeated exposure to HCB include effects on the liver, lungs, kidneys, thyroid, skin and nervous and immune systems.

HCB is a mixed-type cytochrome P-450-inducing compound, with phenobarbital-inducible and 3-methylcholanthrene-inducible properties. It is known to bind to the Ah receptor.
The carcinogenicity of HCB has been assessed in several adequate bioassays on rodents. Increased incidence of neoplasms have been reported in the liver (hepatoma), hemangioendotheliomas of the liver, adenomas of the thyroid, neoplastic liver nodules and adrenal pheochromocytomas. Also reported were parathyroid adenomas, renal cell adenomas, hepatocellular carcinomas, bile duct adenomas/carcinomas, and adrenal cortical adenomas.

HCB has been shown to cause death, systemic (*e.g.*, liver, skin, bone, and thyroid) effects, neurological, developmental, endocrine, and immunological toxicity in humans (ATSDR, 2002). Animal studies have demonstrated that HCB causes reproductive toxicity and increases the risk for cancer formation. The most sensitive target organs for HCB are the liver, ovary, and central nervous system (ATSDR, 2002).

Data for the inhalation effects of HCB in humans are represented by studies of workers in an organochlorobenzene factory and the residents of a nearby rural town (Flix, Spain). Analysis of blood HCB and urinary porphyrins in 604 residents of Flix, including 185 factory workers, showed that blood HCB levels were roughly 5-fold higher in factory workers than in other residents. No cases of clinical *porphyria cutanea tarda* were reported in either, nor was there any evidence that preclinical porphyria was more prevalent in the factory workers than in other residents (Herrero *et al.*, 1999; Sala *et al.*, 1999). Exposure to HCB (primarily airborne) pollution has been linked with elevated blood levels of HCB and hepatic effects (increased porphyrins and hepatic enzymes), thyroid effects (decreased thyroxine levels; weakly with hypothyroidism, goiter, and thyroid cancer), and impaired development of locomotor skills in infants (ATSDR, 2002).

In humans, effects of HCB exposure have been linked to accidental poisonings that took place in Turkey in 1955 to 1959. Widespread ingestion of bread made from grain that had been treated with HCB as a pesticide (fungicide) caused an epidemic of porphyria in this region. The ingested dose of HCB was estimated to be in the range of 0.05 to 0.2 g/day, equivalent to 0.7 to 2.9 mg/kg bw/day for an average person. Some 600 cases of *porphyria cutanea tarda* (PCT) were identified. PCT is the most common form of porphyria characterized by cutaneous photosensitivity that causes scarring and blisters (bullae), hyperpigmentation, excessive facial hair (facial hypertrichosis), and sometimes thickening of the skin (scleroderma) and balding (alopecia). This disease is frequently associated with alcohol abuse, liver disease, or hepatic siderosis. In the incident in Turkey disturbances in porphyrin metabolism were noted. In addition, dermatological lesions, hyper-pigmentation, hypertrichosis, enlarged liver, enlargement of the thyroid gland and lymph nodes, and (in roughly half the cases) osteoporosis or arthritis were observed, primarily in children. An extremely high (95%) rate of mortality occurred in infants under 2 years of age who had been breast fed by mothers who had ingested the contaminated bread. Breast-fed infants of mothers exposed to HCB in this incident developed a disorder that resulted in skin lesions called *pembe yara* (pink sore) (ATSDR, 2002). There has also been limited evidence that *porphyria cutanea tarda* occurs in humans with relatively high exposure to HCB in the workplace or in the general environment. (HSDB, 2005).

In a recent review (ATSDR, 2002) no studies were located regarding health effects in humans or animals following dermal exposure to HCB. However, an acute study in rats suggested that HCB can be absorbed across the skin (Koizumi, 1991). In the rat, the rate of absorption of HCB was 3.51 (SD 0.81) µg/h/4 cm^2 of skin surface and the absorption constant was 1.40 (SD 0.33) x

10^{-3}/h. Washing with soap at six hours after dosing removed 34% of the dose and decreased absorption by 50% in the next 66 hours (Koizumi, 1991).

Concentrations of HCB in human milk were three times higher in samples from northern Canada when compared to samples from southern Canada (Newsom and Ryan, 1999). Elevated levels were also reported for urbanized locations in southern Ontario (Frank *et al.*, 1988). In Canada, levels of HCB in milk decreased by 50 to 75% between 1975 and 1992 (Craan and Haines, 1998). Levels of HCB in umbilical cord blood collected from newborns in the St. Lawrence River Region of Canada fell at a rate of 12% per year between 1993 and 2000 (Dallaire *et al.*, 2002).

B2-3.2 Health Effects by Route of Exposure

B2-3.2.1 Oral Exposure

B2-3.2.1.2 Systemic Effects

The available data in humans and laboratory animals indicate that the liver, and specifically, the heme biosynthesis pathway, is the major systemic target of HCB toxicity. The most common effects include inhibition of heme biosynthesis in exposed people. Human data have also shown effects on other systemic targets, including the skin, bone, and thyroid. Animal data (but no human data) are available regarding respiratory, cardiovascular, gastrointestinal, hematological, renal, ocular, or body weight effects following oral exposure to HCB (ATSDR, 2002).

The highest NOAEL values and all reliable LOAEL values for systemic effects of HCB in each species and the associated duration of exposure have been extensively reviewed (ATSDR, 2002).

***Respiratory Effects*:** Respiratory effects of oral HCB exposure in humans have not been systematically investigated in humans.

Animal studies have shown that ingested HCB can produce pathological effects in the lungs. The most widely reported lesions were hypertrophy and proliferation of the lining endothelial cells of the pulmonary venules and intra-alveolar accumulation of foamy-looking macrophages. These lesions, typically occurring together, were found in six different strains of rats and both sexes, at doses as low as 3 mg/kg bw/day in intermediate-duration feeding studies ATSDR, 2002). More severe pulmonary effects have also been observed in rats at higher doses.

***Cardiovascular Effects*:** Cardiovascular effects of oral HCB exposure in humans have not been systematically investigated. in humans.

HCB exposure was associated with arteriopathy affecting multiple organs in dogs treated with 110 mg/kg bw/day of HCB for 1 year. Other studies that included pathological examination of cardiovascular tissues in dogs, rats, and monkeys did not find treatment-related lesions (ATSDR, 2002).

***Gastrointestinal Effects*:** Gastrointestinal effects of oral HCB exposure in humans have not been systematically investigated in humans.

Gastrointestinal effects have been uncommon in animal studies.

***Hematological Effects*:** Hematological effects of oral HCB exposure in humans have not been systematically investigated in humans.
Animal data suggest that HCB can produce anemia and leukocytosis (Leukocytosis is defined as a transient increase in the number of leukocytes in the blood that may occur with hemorrhages, fever, infection or inflammation). Several studies reported decreases in hemoglobin, hematocrit, and/or red blood cell count especially among female rats at doses ranging from 5 to 32 mg/kg bw/day in subchronic studies (ATSDR, 2002). Neither changes in leukocyte concentration or anemia were reported at lower dose rates in rats (Arnold *et al.*, 1985), or after exposures of much shorter duration (Lecavalier *et al.*, 1994).

***Hepatic Effects*:** Humans who received large oral exposures to HCB developed porphyria. In Turkey the consumption of bread prepared from HCB-contaminated grain from 1955 to 1959 resulted in significant poisoning (Cripps *et al.*, 1984; Peters *et al.*, 1982, 1987).

The appearance of abnormal levels of porphyrin precursors in the urine suggested that HCB exposure unbalanced the body's porphyrin metabolism in the liver. A secondary effect of the porphyrin imbalance was changes in liver histopathology (ATSDR, 2002; Cripps *et al.*, 1984; Peters *et al.*, 1982, 1987). No quantification of exposure (dose and duration) was presented that would permit the development of a clinical dose-response for HCB. An estimated dose of 0.05 to 0.2 g/day HCB (0.7 to 2.9 mg/kg bw/day for a 70-kg person) was identified as a reliable estimate of total exposure to HCB (ATSDR, 2002).

***Musculoskeletal Effects*:** HCB has been associated with painless arthritis (swelling of the joints distinct from rheumatoid arthritis), osteoporosis, and small distinctive hands in patients exposed to HCB through consumption of bread prepared from contaminated grain (Cripps *et al.*, 1984; Peters *et al.*, 1982, 1987).

***Renal Effects*:** Renal effects of oral HCB exposure have not been systematically investigated in humans.

In studies carried out in animals HCB targeted renal tissues (ATSDR, 2002). Renal effects included increased kidney weight, accumulation of porphyrins in association with disruption of heme metabolism (as in the liver), and direct and indirect evidence of renal tissue damage (ATSDR, 2002). Increased kidney weights in animal studies have usually been observed in studies of greater than or equal to 7 weeks of exposure duration. Studies of shorter than 7 weeks in duration failed to note increases in kidney weight (ATDSR, 2002). Multiple-dose feeding studies of 12 to 16 weeks identified LOAEL values of 19 to 32 mg/kg bw/day and NOAEL values of 5 to 9.5 mg/kg bw/day for increased kidney weight in male and female rats (den Besten *et al.*, 1994; Kimbrough and Linder 1974; Kuiper-Goodman *et al.*, 1977).
Histopathological examination of the kidneys of some rats included in studies that treated rats with HCB have provided direct evidence of damage to renal tubule cells. However, despite numerous data supporting an effect of HCB on the kidney, several well-conducted investigations of kidney histopathology failed to find any treatment-related lesions in either male or female rats, even with high exposures (up to 50 mg/kg bw/day for 4 months) (ATSDR, 2002). There is some evidence that lesions noted in male rats were species-specific ($\alpha_{2\mu}$-globulin-induced male rat-specific protein nephropathy) (ATSDR, 2002).

B2-3.2.1.3 No Observed Adverse Effect Levels for Oral Exposure to HCB

Table B2-3 NOAELs and LOAELs for Oral Exposure to HCB

Test Type Organism	Species Route)	Effect	Value (duration)	Endpoint [sex]	Reference
Acute Systemic					
Rat	Sprague-Dawley (gavage, oil)	Endocrine LOAEL	50 mg/kg bw/day (5 days)	Decreased serum thyroxine [females]	Foster *et al.*, 1993
	Wistar (gavage water)	Endocrine LOAEL	250 mg/kg bw/day (7 days)	Decreased serum T4) [females]	Kleiman de Pisarev *et al.*, 1990
	Wistar (gavage water)	Endocrine NOAEL	484 mg/kg bw/day (2 wk)	Decreased serum total and T4) [males]	Van Raaij *et al.*, 1993a
	Sprague-Dawley (gavage, oil)	Renal LOAEL	100mg/kg bw/day (2 wk)	Increased kidney weight [males] (microglobulin)	Bouthillier *et al.*, 1991
	CD (food)	Hepatic NOAEL	5 mg/kg bw/day (1 wk)	Increased hepatic enzymes (ALA-S activity) [females]	Goldstein *et al.*, 1978
	Wistar (food)	Hepatic LOAEL	50 mg/kg bw/day (1 day)	Increased liver porphyrins [females]	Kennedy and Wigfield, 1990
	Sprague-Dawley (gavage, water)	Hepatic NOAEL	700 mg/kg bw/day (2 day)	Increased hepatic enzymes (ornathine decarboxylase)	Kitchin and Brown, 1989
	Wistar (gavage water)	Hepatic NOAEL	250 mg/kg bw/day (7 days)	Decreased URO-D activity [females]	Kleiman de Pisarev *et al.*, 1990
	Sprague-Dawley (gavage, oil)	Hepatic LOAEL	25 mg/kg bw/day (2-16 day)	Incr urinary /hepatic porphyrins [females]	Krishman *et al.*, 1991
	Sprague-Dawley (gavage, oil)	Hepatic LOAEL	10 mg/kg bw/day (6 day)	Increased liver weight [males]	Mehendale *et al.*, 1975
	Brown Norway	Hepatic LOAEL	45 mg/kg bw/day (7-21 days)	Increased liver weight	Michielsen *et al.*, 2001
	CD	BWt LOAEL	10 mg/kg bw/day	[females]	Goldstein *et al.*, 1978
	Wistar	BWt NOAEL	40 mg/kg bw/day (during gestation)		Khera 1974
Acute Neurological					
Rat	Wistar (gavage, oil or water)	Neuro NOAEL	40 mg/kg bw/day (during gestation)	Hyperplasia, tremours, convulsions [female]	Khera, 1974
Acute Reproductive					
Rat	Sprague Dawley (gavage oil)	Reprod LOAEL	50 mg/kg bw/day (5 days)	Increased serum progesterone [females]	Foster *et al.*, 1993
Acute Developmental					
Rat	Sprague Dawley (gavage oil)	Develop LOAEL	2,5 mg/kg bw/day (4 days)	Hyperactivity in young	Goldey and Taylor, 1992
	Wistar (gavage water/oil)	Develop LOAEL	40 mg/kg bw/day (during gestation)	Increased skeletal variations	Khera, 1974

B2-3.2.2 Dermal Exposure

In the recent toxicological review for HCB, no studies were identified that suggested dermal exposure led to a variety of effects including systemic, immunological, neurological or reproductive effects. There was no evidence in animal studies or in humans that that dermal exposure to HCB could lead to cancer (ATSDR, 2002).

Evidence from rats suggested that HCB can be absorbed across the skin (ATSDR, 2002). Koizumi (1991) conducted a mass-balance study of dermal absorption of HCB in male Fisher 344 rats using radiolabelled HCB. Cumulative absorption of HCB (the sum recovered from the urine, feces, liver, carcass, skin not directly contaminated, and subcutaneous tissue) increased with duration of exposure from 1.05% of the applied dose after 6 hours to 2.67% after 24 hours and 9.71% after 72 hours. Calculations by Koizumi (1991) predicted an absorption constant of 1.40×10^{-3} per hour for HCB.

Studies that describe the distribution, metabolism or excretion of HCB in humans or animals are currently not available (ATSDR, 2002).

Given that the biological half-life of HCB is 100 to 730 days, HCB blood levels in a 70-kg man should increase with duration of exposure if the rate constant for dermal absorption of HCB determined in rats is accepted. Based on chronic dermal exposure (daily) to HCB at concentrations of 2.56 to 18.2 mg over a period of years (*i.e.*, occupational exposure), HCB levels in blood could eventually reach 200 ppb (ATSDR, 2002). In humans, a concentration of 200 ppb HCB in blood has been identified as the upper safe limit (Currier *et al.,* 1980).

B2-3.2.2.1 No Observed Adverse Effect Level

No data were available to establish a NOAEL or LOAEL for dermal exposure to HCB for any endpoint evaluated: systemic effects; immunological effects; neurological effects; reproductive effects; developmental effects or cancer (ATSDR, 2002).

B2-3.2.3 Inhalation Exposure

General assumptions with respect to absorption of HCB in humans following inhalation exposure have been based on observations of toxicity in animal studies (Grimalt *et al.,* 1994; Herrero *et al.,* 1999; Queiroz *et al.,* 1997, 1998a, 1998b; Richter *et al.,* 1994; Sala *et al.,* 1999; Selden *et al.,* 1997; To-Figueras *et al.,* 1997).

No data on distribution in humans or in animals following inhalation exposure were available, but limited information on the distribution of HCB following oral exposure was located. Available data suggest that HCB is preferentially and rapidly distributed to tissues with high lipid content (Cripps 1990; Ingebrigtsen and Nafstad 1983; Jarrell *et al.,* 1993; Mehendale *et al.,* 1975).

Evidence to show systemic effects in humans exposed to HCB *via* inhalation are limited. Inhalation of HCB has not been shown to produce porphyria in humans as reported for HCB ingestion. Most human data suggestive of effects include exposures to other chlorinated organics. Levels of urinary porphyrins among individuals who received occupational exposure to both HCB and octachlorostyrene were elevated. HCB levels among workers were 5 times

those reported for members of the neighbouring community (Flix, Spain) but no clinical symptoms of hepatic damage were detected (ATSDR, 2002). The NOAEL for these chronic exposures (40 years) was determined to be 0.000035 mg/m^3 (Herrero *et al.*, 1999; ATSDR, 2002).

In animal studies, inhalation exposure has not been shown to produce hepatic effects (ATSDR, 2002).

***Renal Effects*:** In humans chronically exposed to low concentrations of HCB resulting from industrial activity (Flix, Spain) were not reported. Chronic exposure to HCB in an occupational setting (Czechoslovakia) may have resulted in renal proteinuria and other alterations of kidney functions, but no additional studies to support this have been reported (ATSDR, 2002).

No studies were located regarding excretion of HCB in animals or humans following inhalation.

***Immunotoxicity*:** Studies that have examined occupational exposure to HCB have associated inhalation exposure to HCB with effects on immunological parameters (neutrophil chemotaxis and cytolytic activity, serum immunoglobulin and IFN-γ levels) (Daniel *et al.,* 2001; Queiroz *et al.,* 1997, 1998a, 1998b; Richter *et al.,* 1994). Case-control studies have associated increased body burdens of HCB (putatively resulting from consumption of contaminated food) with alterations in markers of immune function and susceptibility to infection (Belles-Isles *et al.,* 2000; Dewailly *et al.,* 2000).

***Neurotoxicity*:** No studies on the neurotoxicity of HCB in animals after inhalation or dermal exposure were located.

Biomarkers of Effect: Porphyria is the primary biomarker of effect from human acute, intermediate, and chronic exposure to HCB. Increased serum γ-glutamyl transferase, uroporphyrin and d-Alanine in the urine, and uroporphyrin and copro-porphyrin in the stool have been considered indicative of an effect of HCB (Booth and McDowell 1975; ATSDR, 2002).

B2-3.2.3.1 No Observed Adverse Effect Level for Inhalation Exposure in the Rat

Table B2-4 NOAEL and LOAEL Acute Exposure *via* Inhalation to HCB

Test Type	Test Organism (Species)	Effect	Value (duration)	Endpoint	Reference
Rat	Sprague-Dawley	Immunological/ lymphoreticular NOAEL	4.4 mg/m^3 1-4 days (4h/day)	Impairment (slight) of pulmonary immune response [males]	Sherwood *et al.*, 1989
	Sprague-Dawley	Immunological/ lymphoreticular LOAEL	33 mg/m^3 1-4 days (4h/day)	Impairment (slight) of pulmonary immune response	Sherwood *et al.*, 1989

B2-3.3 Carcinogenicity

Carcinogenicity in Humans

The risk for breast cancer has been investigated in relation to life-long, accumulated exposure to HCB in nine studies. Five small case-control studies that included fewer than 50 cases of breast cancer each showed no overall association with the concentration of HCB in contemporary samples of adipose breast tissue. A secondary subgroup analysis in one of the studies revealed a significant association in postmenopausal women with estrogen receptor-positive cancer, based, however, on a small number of cases (IARC, 1991).

One large case-control study of exposure to HCB has been reported from Canada and three studies from the USA. In three of these, the concentration of HCB was measured in biological samples (serum fat or breast fat) from the study subjects, obtained close to the time of breast cancer diagnosis. No consistent increase in the risk for breast cancer was found in women with elevated concentrations of HCB. In the fourth case-control study (from the USA), banked serum samples obtained before the breast cancer diagnosis were used to assess the body burden of HCB. The risk for breast cancer of women whose concentration of HCB was in the upper three quartiles was twice that of those whose samples were in the lower quartile. However, there was no evidence of a dose–response relationship, and the association was limited to women whose blood was collected close to the time of diagnosis of their breast cancer (IARC, 1991).

One case-control study each of endometrial cancer, pancreatic cancer and hairy-cell leukemia yielded no notable results with respect to exposure to HCB (IARC, 1991).

Animal Carcinogenicity Data

There was sufficient evidence for carcinogenicity of HCB in experimental animals (IRIS, 1991). The liver was the primary target organ for HCB-induced cancer, although neoplasms of the thyroid and kidney have also been observed.

Studies in Rats

Groups of 94 Sprague-Dawley rats/sex/dose were fed 0, 75, or 150 ppm HCB (purity >99.5%) in the diet for up to 2 years (Ertürk *et al.*, 1986). Treated animals of both sexes surviving past 12 months showed significant increases in liver and renal tumors. Hemangiohepatomas, hepatocellular carcinomas and bile duct tumors were significantly increased in treated females; males and females in both dose groups had increased incidences of renal cell adenomas and hemangiohepatomas. Females were far more susceptible to hepatocarcinogenicity while males were generally more sensitive to renal carcinogenicity. The time-to-tumor onset in each dose group was generally longer than 1 year (IRIS, 1991).

Smith and Cabral (1980) reported 100% incidence of liver tumors in a single dose study involving small groups of female Angus (14) and Wistar (6) rats. Rats received 100 ppm HCB in arachis oil in the diet for 90 weeks compared to 0% in small groups of controls (12 Agus and 4 Wistar rats) (IRIS, 1991).

In a 2-generation feeding study parental Sprague-Dawley rats were fed 0.32 to 40 ppm HCB in the diet for 3 months. Following mating, females were maintained on the diet through pregnancy and lactation. Pups received 0.32 to 40 ppm dietary HCB for 130 weeks. F_1 females in the high-dose group had significant elevation in the incidence of neoplastic liver nodules (10/49 *vs*. 0/49 for controls) and adrenal pheochromocytomas (17/49 *vs*. 0/49 for controls), and F_1 males showed increased parathyroid tumors (12/49 *vs*. 2/48 for controls) (Arnold *et al.*, 1985) (IRIS, 1991).

Studies in Mice

Hepatomas were produced in a dose-related fashion in both male and female Swiss mice exposed through the diet to 50, 100, or 200 ppm HCB for up to 120 weeks (Cabral *et al.*, 1979). The females in the high-dose group were observed to have a liver tumor incidence (14/41) significantly elevated over controls (0/49) (IRIS, 1991).

Short-term exposure to HCB did not significantly increase tumor incidence. Shorter exposures of Swiss mice (15 weeks) to 300 ppm HCB in the diet produced negligible incidences of liver tumors (1/26 female, 1/16 male) (Cabral *et al.*, 1979). Lower doses of HCB (10 or 50 ppm) administered in the diet for 24 weeks did not result in increased liver tumor formation in ICR mice. There was hypertrophy of the centrilobular region, however, and 50 ppm HCB was found to enhance tumor induction and nodular hyperplasia in combination with 250 ppm polychlorinated terphenyl (Shirai *et al.*, 1978) (IRIS, 1991).

Studies in Hamsters

Groups of 30 to 60 Syrian golden hamsters/sex/dose were fed 0, 50, 100, or 200 ppm HCB (>99.5% pure) in the diet over their lifetime (Cabral *et al.*, 1977). After 50 weeks, survival in treated groups was comparable to controls; however, there was reduced lifespan among high-dose male and female animals after 70 weeks of exposure. A significant dose-related increase in the incidence of hepatomas and liver hemangioendotheliomas was observed in males and in females. The incidence of hepatomas was statistically significantly increased in each treated group compared to controls while liver hemangioendothelioma incidence was statistically significantly elevated in the high-dose groups of both sexes and in middle-dose males. While thyroid alveolar adenomas were observed in all treated groups except low-dose males (none were observed in control groups), a significantly increased incidence was found only in high-dose males (IRIS, 1991).

Table B2-5 Animal Carcinogenicity Data

Test Subjects	Exposure	Dose	Response	Reference
Sprague-Dawley rats	F_1 progeny of exposed F_0 dams 0 to 40 mg/kg bw in diet	0 to 1.72 mg/kg bw-day	Parathyroid adenomas. +ive males; adrenal pheochromatocytomas. +ive males and females	Arnold *et al*., 1985
Syrian golden hamster	0-200 mg/kg (diet)	0-16 mg/kg bw-day	Hepatomas, hemangioendotheliomas, thyroid adenomas. + ive males and females. ≥ 4 mg/kg bw-day	Cabral *et al.*, 1977
Swiss mice	0-200 mg/kg (diet)		Liver tumors (not specified). + ive males and females >100 mg/kg bw	Cabral *et al.*, 1979

Table B2-5 Animal Carcinogenicity Data

Test Subjects	Exposure	Dose	Response	Reference
Sprague-Dawley rats	0-150 mg/kg (diet)		Hepatoma/hemangioma; hepatocarcinoma; bile duct adenoma/carcinoma; renal cell adenoma. + ive males and females	Lambrecht *et al*., 1983 Ertürk *et al*., 1986

B2-3.4 Genotoxicity

Despite the carcinogenicity of HCB which has been demonstrated in animal testing, there is limited information available regarding the genotoxic potential of this chemical. The majority of the published mutagenicity/genotoxicity test data indicate that HCB has very limited mutagenic and/or genotoxic potential. HCB was non-mutagenic, both with and without an exogenous source of metabolic activation, to *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537 at concentrations of up to 1,000 µg/plate (Haworth *et al.*, 1983). HCB also did not induce any revertant auxotrophs in *Salmonella typhimurium* (Lawlor *et al.*, 1979); however positive results for mutagenicity have been reported in *Saccharomyces cerevisiae* with HCB at a concentrations of 100 ppm (Guerzoni *et al.*, 1976). Negative results were obtained in two *in vivo* dominant lethal assays in which mice were administered either 0 to 221 mg/kg body weight *via* gavage for five consecutive days or treated *via* gavage with 0 to 60 mg HCB/kg body weight for 10 consecutive days (Khera, 1974; Simon *et al.*, 1979).

Humans

Among a group of 85 workers exposed to a number of organochlorine compounds including carbon tetrachloride, perchloroethylene and HCB, an *in vitro* micronucleus test was carried out on lymphocyte obtained from 41 of 85 workers. The authors concluded that exposure to a mixture of organochlorines resulted in increased clastogenic activity in peripheral lymphocytes. Although serum concentrations of HCB increased with years worked, there was no direct evidence that micronuclei observed in lymphocytes resulted from HCB exposure (da Silva Augusto *et al*., 1997).

Animal Studies

In vivo studies in rats revealed the lack of significant genotoxic activity in mammals following oral exposures to HCB. Two dominant lethal mutation assays in rats at oral doses ranging from 60 to 221 mg/kg bw were negative (Khera 1974; Simon *et al.*, 1979).

HCB did not induce micronuclei in a standard mouse bone marrow micronucleus assay after single or double dosing regimes at concentrations up to 70% of the LD_{50} (Morita *et al.,* 1997). Following oral exposure to HCB, mouse DNA in specific target tissues showed no evidence of single or double strand breaks (Sasaki *et al.,* 1997). In this study no evidence of genotoxicity measured by Comet assay was observed in mouse liver, lung, kidney, spleen, or bone marrow after oral dosing (Sasaki *et al.*, 1997).

Oral exposure to HCB in Wistar rats provided equivocal evidence for direct reaction of HCB with DNA (Gopalaswamy and Nair 1992). Male rats pretreated with phenobarbital (0.1% sodium phenobarbital in drinking water for 2 weeks) or untreated for induction of liver

microsomes were administered 25 mg HCB /kg bw in 0.1 mL refined peanut oil for 24 hours. The animals were sacrificed and DNA extracted from livers. Upon analysis, HCB label was found bound to isolated DNA (2.23 ± 0.27 pmoles/mg DNA without phenobarbital treatment, and 3.56 ± 0.18 pmoles/mg DNA for phenobarbital pretreated rats). No HCB untreated control values were provided in the study report. These authors were unable to show evidence of direct binding to DNA in an *in vitro* system using a liver S9 preparation from phenobarbital treated rats. Gopalaswamy and Nair (1992) also failed to observe evidence of gene mutation or unscheduled DNA repair in microbial assays. A similar lack of mutagenicity in standard bacterial mutation reversion assays has been reported by others (Haworth *et al.*, 1983; Brusick, 1986; Siekel *et al.*, 1991).

HCB did not produce chromosomal aberrations in human peripheral lymphocytes *in vitro* (Siekel *et al.*, 1991). DNA fragmentation was not observed in assays of rat hepatocytes treated in vitro with HCB (0.1 to 0.56 mM) (Canonero *et al.*, 1997). In primary cultures of human hepatocytes treated *in vitro* with HCB, weak positive results were observed in assays for DNA fragmentation and micronuclei formation (Canonero *et al.*, 1997). According to the authors HCB may be metabolized in human liver to produce reactive oxygen species that could be responsible for observed effects on nuclear DNA (Canonero *et al.*, 1997; van Ommen *et al.,* 1985).

In a series of DNA adduct assays that established evidence of covalent DNA adduct formation with pentachlorophenol metabolites, treatment with HCB induced only minimal formation of DNA adducts in cultured human Hep G2 hepatoma cells (Dubois *et al.,* 1997). This suggests weak activity reported by Canonero *et al.* (1997) in human hepatocytes were likely due to interference with the cell division apparatus (aneugenic activity) and not direct DNA activity.

HCB was used in a non-standard assay to test *in vivo-in vitro* replicative DNA synthesis in the mouse. This report included results of forty-one mouse liver carcinogens previously identified as *non-genotoxic carcinogens* (Miyagawa *et al.*, 1995). When the incidence of replicative DNA synthesis (RDS) in cells was greater than 0.4% at 24, 38 or 48 hours after exposure a chemical was deemed to give a positive result in the assay. In this assay HCB was positive (0.66% RDS) at 1,000 mg/kg bw (the maximum tolerated dose) after 39 hours of incubation, but no dose-response was observed (Miyagawa *et al.*, 1995). This was characterized by the authors as a positive (without statistical significance) (Miyagawa *et al.*, 1995).

HCB tested negative or ambiguous in reverse mutation assays in *S. typhimurium* (Gopalaswamy and Aiyar 1986; Gopalaswamy and Nair 1992; Haworth *et al.*, 1983; Siekel *et al.*, 1991) and *E. coli* (Siekel *et al.*, 1991) with and without metabolic activation, although an assay for reverse mutation in the yeast *Saccharomyces cerevisiae* was positive (Guerzoni *et al.*, 1976). This single report of HCB-induced reverse mutation in yeast is suspect due to the failure to establish either dose-response effects or an acceptable level of statistical significance (Brusick, 1986). HCB also tested negative in a DNA repair assay in *E. coli* (Siekel *et al.*, 1991).

Conclusion Regarding Genotoxicity

Based on the weight-of-evidence it was concluded that HCB does not act as a genotoxic carcinogen, and is only a weak non-genotoxic carcinogen based on results of *in vitro* assays. The mode of action of HCB is unclear from the genotoxicity and metabolic data available. It has been suggested that further metabolism of pentachlorophenol (which is one metabolite of HCB) may lead to the production of reactive oxygen species (van Ommen *et al*., 1985). These were

responsible for binding of radiolabelled HCB metabolite to microsomal proteins. The results of van Ommen *et al.* (1985) could also suggest a mechanism for the reported micronucleus formation in *in vitro* assays with human hepatocytes (Canonero *et al.*, 1997).

B2-3.5 Human Populations at Special Risk

Infants and young children appeared to be especially sensitive to the effects of HCB in the Turkish grain poisoning epidemic. Mothers known to have ingested HCB through consumption of bread prepared from HCB contaminated grain put their babies at risk. Breast-fed infants of these mothers developed skin lesions known as the disease pembe yara or "pink sore." Other symptoms reported among these infants were weakness and convulsions. Many of the sickened infants died from this disease (ATSDR, 2002).

B2-3.6 Toxicokinetics

B2-3.6.1 Absorption

Fraction Absorbed Via Ingestion

The results of several studies indicate that the bioavailability of HCB (HCB) *via* the oral route of exposure is highly dependent upon the medium in which the HCB is dissolved (Albro and Thomas, 1974; Koss and Koransky, 1975). Schlummer *et al*. (1998) found that the absorption of HCB is relatively uniform 70 to 82% in young adults (one female and three males tested ranging in age from 24 to 36 years). Further analysis indicated that 85.4% of ingested HCB is absorbed when the blood contains no HCB; however, and this percentage will decreases by 0.2% for each ng of HCB per g lipid in blood. Animal data suggest that oral absorption of HCB is rapid if dissolved in a lipid, but absorption from aqueous solutions is slow (Ingebrigtsen and Nafstad 1983; Koss and Koransky 1975).

For the current exposure assessment a value of 85.4% (0.854) was chosen for calculation of oral bioavailability of HCB based on the study conducted by Schlummer *et al*. (1998).

Fraction Absorbed Via Inhalation

ATSDR (2002) reports that there is limited data showing that HCB can be absorbed through the respiratory tract in humans, although no information is available as to the rate and extent of respiratory tract absorption in either humans or animals. In air, HCB is found almost exclusively in the gas phase (>90 to 95%). Therefore, for the current assessment, it is conservatively assumed that 100% absorption occurs *via* inhalation.

Fraction Absorbed Via Dermal Contact

For dermal contact with HCB through soil a value of 10% (0.1) was selected to represent the fraction absorbed through the skin for human exposure (U.S. EPA, 2004). This value is also in accordance with the fraction of HCB absorbed *via* dermal contact of 13% provided by Health Canada (2004). Other dioxin/furan congeners were assumed to act in a similar fashion to TCDD. For direct contact with pure 2,3,7,8-TCDD, dermal absorption rates have been derived and discussed elsewhere.

B2-3.6.2 Distribution

Available data suggest that HCB is preferentially and rapidly distributed to tissues with high lipid content (Cripps 1990; Ingebrigtsen and Nafstad 1983; Jarrell *et al.,* 1993; Mehendale *et al.,* 1975).

B2-3.6.3 Metabolism

The metabolism of HCB has not been studied in humans. Studies in monkeys and rats indicated that chlorines on HCB are removed to yield chlorobenzenes, chlorinated phenols, other minor metabolites, and glucuronide and glutathione conjugates (Figure B2-2) (Ingebrigtsen and Nafstad 1983; Ingebrigtsen *et al.,* 1981, 1986; Jansson and Bergman 1978; Koss *et al.,* 1986; Rozman *et al.,* 1977).

Oral studies in animals regarding the excretion of HCB indicate that the parent compound HCB is excreted primarily in feces, while the metabolites were detected in urine (Albro and Thomas 1974; Ingebrigtsen *et al.,* 1981; Mehendale *et al.,* 1975; Rozman *et al.,* 1977;1981; To-Figueras *et al.,* 1991).

HCB is lipophilic, accumulates in humans and is excreted as a cysteine conjugate of pentachlorobenzene. In rats, HCB has been shown to follow several metabolic pathways, which include the formation of pentachlorobenzene, tetrachlorobenzene and tri- and tetrachlorophenol (ATSDR, 2002.)

In animals, HCB is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms) and reductively dechlorinated to form pentachlorobenzene (See Figure B2-2). Other metabolites include less chlorinated benzenes, chlorophenols. Other metabolites that appear in urine are conjugated with glutathione to yield pentachlorothiophenol, or other *S*-conjugated phenols, and benzenes. Pentachlorophenol is subsequently converted to tetrachlorohydroquinone (ATSDR, 2002).

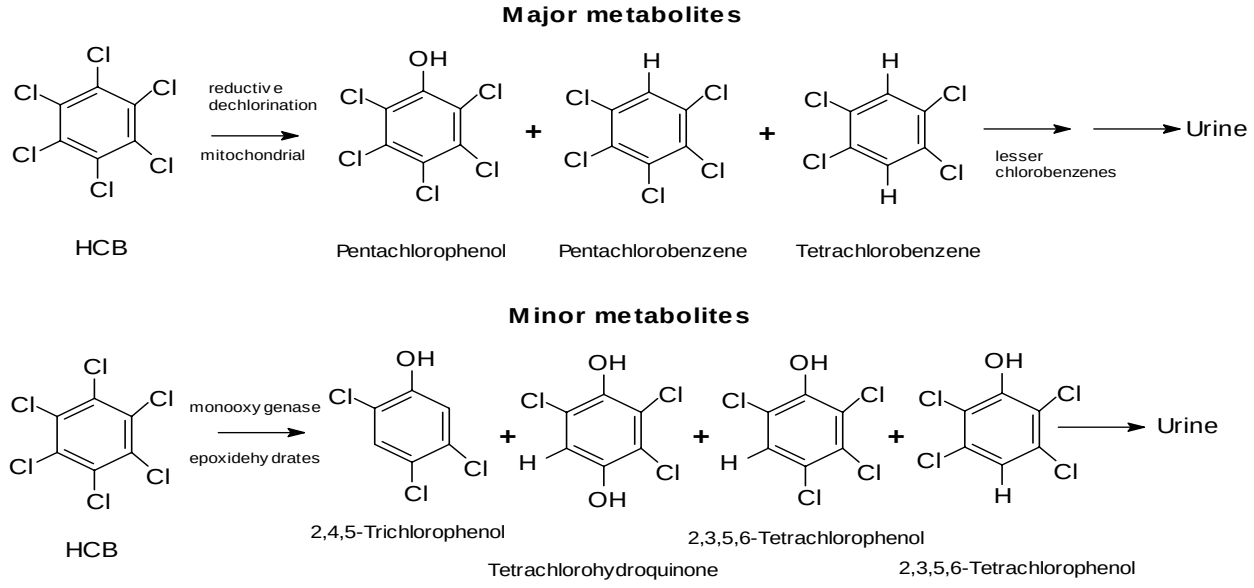

Figure B2-2 Metabolism and urinary metabolites of HCB (Adapted from ATSDR, 2002)

B2-3.6.4 Elimination and Excretion

Pentachlorobenzene and pentachlorophenol were identified as the major metabolites of ^{14}C-labeled HCB (0.03 mg/kg bw/day) administered in the diet to Rhesus monkeys for 15 months (Rozman *et al.*, 1977). In the urine, approximately 50% radioactive HCB was excreted as metabolites including pentachlorophenol, 25% as pentachlorobenzene, and the remaining 25% as unidentified metabolites and unchanged HCB (ATSDR, 2002).

Of the radioactivity excreted in the feces, 99% was unchanged HCB, with <1% pentachlorobenzene and trace amounts of pentachlorophenol. A subsequent report of a similar study in Rhesus monkeys found that fecal excretion consisted of 99% unchanged parent compound, about 1% pentachlorobenzene, and traces of pentachlorophenol (Rozman *et al.*, 1978). Urinary metabolites consisted of 50 to 75% pentachlorophenol. The remainder of radioactivity (25 to 50%) was composed of pentachlorobenzene, HCB, and tetrachlorobenzene. Only the unchanged parent compound was found in the plasma, and the red blood cells contained 95% unchanged parent compound and 5% pentachlorophenol (ATSDR, 2002).

B2-3.7 Review of Regulatory Exposure Limits

The U.S. EPA (IRIS, 1991) RfD for HCB is 0.0008 mg/kg bw/day. This RfD is based on the Arnold *et al*. (1985) 130-week feeding study in male and female rats that also included a 90-day exposure to offspring. The U.S. EPA judged the NOAEL for liver effects at a dose of 0.08 mg/kg bw/day with a LOAEL at 0.29 mg/kg bw/day. The LOAEL was characterized by U.S. EPA (1995) as "an increase ($p<0.05$) in hepatic centrilobular basophilic chromogenesis" in the offspring of the chronically exposed rats. The U.S. EPA used an uncertainty factor of 100 to derive the RfD of 0.0008 mg/kg bw/day. On the basis of evidence that when administered orally, HCB has been shown to induce tumors in the liver, thyroid and kidney, the U.S. EPA (IRIS, 1991) have also derived an oral slope factor based on the results of the Ertürk *et al*. (1986) study previously discussed. The oral slope factor, derived on the basis of hepatocellular carcinomas, is 1.6 $(mg/kg\ bw/day)^{-1}$.

Health Canada (2004) has reported both non-carcinogenic and carcinogenic toxicity reference values for HCB. The non-cancer based tolerable daily intake was reported as 0.0005 mg/kg bw/day (0.5 µg/kg bw/day). Likewise, the cancer based slope factor has been reported as 0.83 $(mg/kg\ bw/day)^{-1}$. The slope factor is based on the $TD_{0.05}$ value derived from the results of the Arnold *et al*. (1985) study. CEPA (1993) reports a $TD_{0.05}$ range from 0.06 mg/kg bw/day for hepatic neoplastic nodules in females to 0.17 mg/kg bw/day for parathyroid adenomas in males, which translates into slope factors ranging from 0.29 $(mg/kg\ bw/day)^{-1}$ to 0.83 $(mg/kg\ bw/day)^{-1}$ (Slope Factor = $0.05 / TD_{0.05}$).

ATSDR (2002) have derived an MRL of 0.008 mg/kg bw/day for *acute-duration* oral exposure (14 days or less) for HCB. This MRL is based on a critical evaluation of a developmental study (Goldey and Taylor 1992) that observed a lowest-observed-adverse-effect level (LOAEL) of 2.5 mg/kg bw/day for hyperactivity in offspring rats. An uncertainty factor of 300 was used (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL). A battery of tests to demonstrate evidence of developmental neurotoxicity of HCB was assessed in offspring (Goldey and Taylor, 1992). Evidence for hyperactivity was observed in pups treated with 2.5 or 25 mg/kg bw/day for 4 days, 2 weeks prior to mating with unexposed males. They reoriented themselves significantly more quickly in a negative geotaxis test (on postnatal days 6

and 8), required less time in an olfactory discrimination test, and demonstrated increased exploratory activity in a motor activity test (on postnatal days 9 to 11). No significant effects on learning (swim T-maze) or motor activity (measured in older offspring on postnatal days 40 and 50, respectively) were detected. HCB exposed offspring at the 25 mg/kg bw/day dose level exhibited significantly altered acoustic startle responses (decreased at 23 days of age and increased at 90 days of age compared to controls). Thus, the study identified a LOAEL of 2.5 mg/kg bw/day for hyperactivity in the offspring rats (ATSDR, 2002).

ATSDR (2002) has derived an MRL of 0.0001 mg/kg bw/day for *intermediate-duration* oral exposure (15-364 days) for HCB. This MRL is based on a LOAEL of 0.01 mg/kg bw/day for minimal ovarian effects in monkeys (Babineau *et al.,* 1991). An uncertainty factor of 90 was used (3 for extrapolation from monkeys to humans, 10 for human variability, and 3 for use of a minimal LOAEL) (ATSDR, 2002). Ultrastructural studies of ovaries collected from monkeys (Babineau *et al.,* 1991; Bourque *et al.,* 1995; Jarrell *et al.,* 1993) fed doses of 0.01 to 10 mg/kg bw/day of HCB for 90 days provided the most appropriate data for development of an intermediate-duration oral MRL (ATSDR, 2002).

An MRL of 0.00005 mg/kg bw/day has been derived for *chronic-duration* oral exposure (365 days or more) for HCB (ATSDR, 2002). The chronic oral exposure MRL was based on a critical evaluation of a multigenerational study (Arnold *et al.,* 1985), which observed a LOAEL of 0.016 mg/kg bw/day for hepatic effects in F_1 male rats. An uncertainty factor of 300 was used (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

B2-3.7.1 Other Regulatory Information

IARC (2001) Group 2B (possibly carcinogenic to humans)

ACGIH (2001) A3 (confirmed animal carcinogen; relevance to humans unknown)

U.S. EPA (2001) B2 (probable human carcinogen)

WHO (1996) Drinking water guideline: 1µg/L

WHO (1997) TDI (total daily intake)
Non-cancer effects 0.17 µg/kg bw body weight/day
Neoplastic effects 0.16 µg/kg bw body weight/day

AIR (inhalation exposure)

ACGIH (2001) TLV (8-hour TWA) 2 µg/m^3

WATER

U.S.EPA (2001) Drinking water standard: 0.001 ppm [40CFR141.32(e)(68)]
MCL 1µg/L [40CFR141.61(c)]

B2-3.7.2 Selected Exposure Limits

The non-carcinogenic and carcinogenic toxicity reference values derived by Health Canada (2004) have been selected for the evaluation of long-term (chronic) health effects of HCB. The non-cancer based tolerable daily intake was reported as 0.0005 mg/kg bw/day (0.5 µg/kg bw/day), and the cancer based slope factor has been reported as 0.83 $(mg/kg\ bw/day)^{-1}$.

For short-term (acute) effects, the ATSDR (2002) derived MRL of 0.008 mg/kg bw/day for acute-duration oral exposure (14 days or less) for HCB was selected.

B2-4.0 ENVIRONMENTAL FATE AND EXPOSURE

The average half-life of HCB estimated from results of a number of studies is ~ 9 years (Barber *et al.*, 2005). Atmospheric degradation is extremely slow. In air, HCB is found almost exclusively in the gas phase (>90 to 95%). It has been suggested that HCB can be transported great distances before deposition or ultimate degradation (Barber *et al.*, 2005). Thus, HCB is considered ubiquitous nationally and globally so some exposure is unavoidable (Barber *et al.*, 2005; USDA, 2003).

The hydrophobic nature of HBC results in preferential partitioning into sediment, soil and plant surfaces from water or air. As a result of the air/soil partition coefficient (Table B2-1) soil is expected to contain a much greater mass of HBC than air (Barber *et al.*, 2005). Despite the preferential adsorption to organic material in sediments, chemical degradation is not considered an important removal process from sediments or water. HBC has a high volatility and only moderate partition coefficients compared with other POPs (Table B2-2). This means that HCB can move around in the environment in multiple hops in a manner that has been described as the "grasshopper effect" (Barber *et al.*, 2005; Wania and Mackay, 1996).

B2-4.1 Air

HCB may be removed from the atmosphere by photolysis, with a suggested half-life of about 80 days (Mill and Haag, 1986), and by chemical reaction with hydroxyl (OH) radicals, with a half-life of 1.7 years (Brubaker and Hites, 1998), or ranging from 156 days to 4.2 years (Howard, 1991; Kwok and Atkinson, 1995). The reported photolysis rate has been characterized as unaccountably fast, because of the likely steric hindrances to this reaction caused by the presence of chlorine on each carbon atom (Barber *et al.*, 2005).

B2-4.2 Water

HCB may be removed from water by photolysis, but at a very slow rate, with a half-life of about 70 days (Mill and Haag, 1986). HCB in the water column rapidly sorbs to particulate matter, making it unavailable for photolysis (Schauerte *et al.*, 1982). In the event of sorption to suspended matter in the water column, hydrolysis is not expected to be an important fate process (Barber *et al.*, 2005). A half-life ranging from 2.7 to 5.7 years in surface water and 5.3 to 11.4 years in groundwater has been suggested (Howard, 1991)

B2-4.3 Sediment and Soil

The half-life for residence of HCB in soil has been estimated to be 970 to 2,100 days (Griffin and Chou, 1981), with volatilization from the soil surface as the major loss process. Aerobic and anaerobic biodegradation are the major means of HCB removal at lower soil depths, with half-lives of 2.7 to 5.7 years (Beck and Hansen, 1974) and 10.6-22.9 years (Howard, 1991), respectively. Meijer *et al.* (2001) reported a half-life of 11.7 years for sewage-sludge treated soils between 1968 and 1990. On the other hand, the data after 1980 supports a longer half-life of 4 to 6 years for HCB in sediment. A major problem with these studies is that measured disappearance from soils does not discriminate between degradation and volatilization (Barber *et al.*, 2005).

JW (2006) has indicted a soil half-life of HCB on surface soils may vary from 2.7 year to 7.5 years. Mackay *et al*. (1992) provides a mean of approximately 6 years.

Beurskens *et al.* (1993) investigated microbial degradation of HCB in a sedimentation area of the Rhine River, and calculated a maximum half-life of HCB in sediment to be 7 years. This rate included desorption to water over a period when other inputs to the water have declined (Barber *et al.*, 2005). Zhao *et al.* (2003) investigated the anaerobic degradation of HCB in sediments and observed a half-life of 1.7 years in un-amended sediments. A wide range of other degradation rates have been reported, dependent on experimental set-up, with values of 0.021 day^{-1} (Jackson and Pardue, 1998), 0.026 day^{-1} (Masunaga *et al.*, 1996), 0.11 day^{-1} (Susarla *et al.*, 1997), and 0.0022 day^{-1} (Prytula and Pavlostathis, 1996) found by different authors. These correspond to half-lives of 33 days, 27 days, 6.3 days and 315 days, respectively (Barber *et al.*, 2005).

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