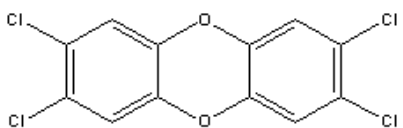# Polychlorinated Dibenzo-*p*-dioxins (PCDDs), Dibenzofurans (PCDFs) and Biphenyls (PCBs)
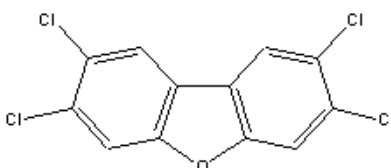## Including: all 2,3,7,8-chlorinated PCDDs and PCDFs, and PCBs
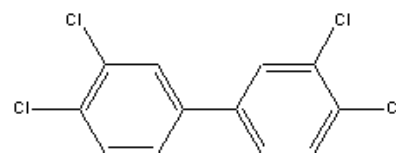
I.   Selected structures:

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	2,3,7,8-Tetrachlorodibenzofuran (TCDF)	3,3′,4,4′-tetrachlorobiphenyl (PCB #77)

Selected Physical and Chemical Properties:

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

Description	White crystalline powder at 25°C
Molecular formula	$C_{12}H_4Cl_4O_2$
Molecular weight	321.97 g
Water solubility	1.93 ng/100 ml at 22°C
Log P (octanol-water)	6.80
Air concentration conversion	Not available

2,3,7,8-Tetrachlorodibenzofuran (TCDF)

Molecular formula	$C_{12}H_4Cl_4O$
Molecular weight	305.975 g
Water solubility	69.2 ng/100 ml at 26°C
Log P (octanol-water)	6.53
Air concentration conversion	Not available

3,3',4,4'-tetrachlorobiphenyl (PCB #77)

Molecular formula	$C_{12}H_6Cl_4$
Molecular weight	291.99 g
Water solubility	56.9 ng/100 ml at 25°C
Log P (octanol-water)	6.63
Air concentration conversion	Not available

II.   Overview

There are a number of studies which indicate that developing fetuses and newborns, particularly breast-fed infants, represent a segment of the population particularly vulnerable to exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). Concern about dioxins and dioxin-like compounds is justified not only because of their toxicity but also because of their very long biological and environmental persistence. Emissions into the air, the majority of environmental emissions, of these toxic air pollutants results in subsequent deposition onto crops, grass and feed. Deposited dioxins are either eaten by humans directly or eaten by livestock and become a source of contamination for humans in beef, poultry and dairy products. In addition, these persistent compounds accumulate in breast milk, and are thus transferred to the feeding infant. Since human exposure *in utero* and in infancy to PCDDs, PCDFs and PCBs represents a serious concern for children's health, this class of chemicals is considered a priority for evaluation of potential differential effects on infants and children.

- Immunotoxicology is identified as one of the key toxicological endpoints of concern for infants and children (see Introduction Section III). Immune system toxicity appears to be among the most sensitive responses (Birnbaum, 1994). Effects on immune development from perinatal exposure to dioxin and dioxin-like chemicals may be more dramatic or persistent than that following exposure during adult life (Holladay, 1999). Functional developmental immunotoxicity was observed in children exposed both pre- and post-natally during the rice oil poisoning episode with PCDFs and PCBs in Yu-Cheng, Taiwan (Guo *et al.*, 1995; Schecter *et al.*, 1996). Similar effects were also observed in children as a result of background exposure to these chemicals (Gladen *et al.*, 2000; Nagayama *et al.*, 1998a; Nagayama *et al.*, 1998b; Papke, 1998; Patandin *et al.*, 1998; Patandin *et al.*, 1999b; Vartiainen *et al.*, 1998; Weisglas-Kuperus *et al.*, 2000).

- Developmental toxicity represents another key toxicological endpoint of concern for infants and children (see Introduction Section III). Dioxins and dioxin-like chemicals are potent teratogens in animals. Detectable concentrations of PCBs and dioxins have been found in amniotic fluid, placenta and fetal tissue samples, and infants who are breast-fed can have blood levels of PCBs and dioxins greater than the corresponding maternal levels (Feeley and Brouwer, 2000). Evidence of transplacental transfer has been obtained from analysis of PCDDs and PCDFs in fetal tissue (Kreuzer *et al.*, 1997; Schecter *et al.*, 1996).

- PCDDs, PCDFs, and PCBs are transferred to infants from the mother during breast feeding. This route appears to be the most important route of exposure for humans, resulting in about 50 times the daily dose of dioxin toxic equivalents (TEQ) in breast-fed infants compared to adults (Patandin *et al*., 1999a). Lanting *et al.* (1998a) clearly identified lactation as a major source of the PCB body burden of 42 month-old children. Forty-two month-old children who had been fully breast-fed for at least six weeks as babies, had plasma median PCB levels 4.5-times as high as that in formula-fed children (0.81 µg/L vs. 0.18 µg/L). Children receive greater exposures to environmental pollutants present in air, food, and water because they inhale or ingest more air, food, or water on a per kg

body weight basis than do adults (Mott, 1995; OEHHA, 2000). This holds true especially for lipophilic compounds like the PCDDs, PCDFs and PCBs because, in addition to the increased dose through inhalation and ingestion of contaminated food, these compounds are transferred through breast milk, which is often the sole source of nutrition in the infant.

- Exposure of infants and children to carcinogenic chemicals is a general concern since, as discussed in the introduction to this report, exposure to carcinogens early in life may result in higher tumor incidence and shorter latency than exposure as an adult. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a multisite, multispecies animal carcinogen, and is probably a human carcinogen. Populations occupationally or accidentally exposed to chemicals contaminated with dioxin have demonstrated increased incidences of soft-tissue sarcoma and non-Hodgkin's lymphoma (Mukerjee, 1998; Birnbaum, 1994). In addition, evidence from rodent studies indicates that *in utero* exposure to a single dose of TCDD was sufficient to promote mammary carcinogenesis when animals were dosed at day 50 with a mammary carcinogen (Brown *et al.*, 1998).

- Developmental neurotoxicity has been shown in animals, and there is evidence of this effect in humans in epidemiological studies of children exposed *in utero* to the non-coplanar PCBs. The PCBs can be grouped into two categories by mechanism of toxicity. The non-coplanar PCBs have predominantly neurotoxic effects. The coplanar PCBs have dioxin-like effects and act through the aryl hydrocarbon receptor. Like polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), the coplanar polychlorinated biphenyls (PCBs) appear to have a significant number of toxic effects mediated through their interaction with the aryl hydrocarbon (*Ah*) receptor. These PCB congeners are substituted in the para and at least 2 of the meta positions but not at any of the ortho positions, and are structurally similar to TCDD. PCBs with more than one chlorine in the ortho positions lack some effects exerted by non- and mono-ortho PCBs and show a partially different spectrum of toxic effects (Safe, 1994), indicating that a second mechanism of toxicity exists for non-coplanar PCBs, which probably acts outside of the *Ah* receptor pathway.

III.   Principal Sources of Exposure

PCDDs and PCDFs are generated as by-products from various combustion and chemical processes. PCDDs, PCDFs and PCBs are produced during incomplete combustion of chlorine containing wastes like municipal solid waste, sewage sludge, and hospital and hazardous wastes. Various metallurgical processes involving heat, and burning of coal, wood, petroleum products and used tires for energy generation also generate PCDDs. Industrial and municipal processes in which naturally occurring phenolic compounds are chlorinated can produce PCDDs; the best example is chlorine bleaching of wood pulp in the manufacture of paper products. These PCDDs and PCDFs end up as water contaminants and generally not air contaminants. It should be noted that most pulp mills in the U.S. have switched to a bleaching process that produces little or no PCDDs and PCDFs.

U.S. EPA (2000a) conducted an extensive review of contemporary formation (as opposed to reservoir) sources of dioxin release to the environment. The U.S. EPA report (U.S. EPA, 2000a) divides sources of PCDD/PCDFs into two subclasses: 1) contemporary formation sources (sources which have

essentially simultaneous formation and release) and 2) reservoir sources (materials or places that contain previously formed PCDD/PCDFs or dioxin-like PCBs that are re-released to the environment). The inventory of estimated dioxin releases in the United States prepared by U.S. EPA (2000a), classified by media and source category, for 1987 and 1995 is shown in Table 1. Values in the table are reported as dioxin equivalents, or TEQ. The equivalency factors (WHO98) used in the calculation are those in the 1998 World Health Organization update to the previously established equivalency factors (I-TEF) for dioxins, furans, and dioxin-like PCBs (Van den Berg *et al*., 1998). This inventory lists estimated emissions of chlorinated dioxins and furans only. Environmental releases of PCDD/PCDFs in the United States occur from a wide variety of sources, but these are dominated by releases to the air from combustion sources including waste incineration, industrial power generation, and vehicle fuel combustion. Vehicle fuel combustion is considered to make a significant contribution to general ambient dioxin levels in urban areas, and to contribute particularly to the higher dioxin levels experienced near freeways and similar high-traffic areas (Hunt *et al*., 1997). Among different classes of vehicles, diesel fueled vehicles contribute nearly five times as much dioxins (Table 1), in spite of their smaller numbers than gasoline fueled vehicles. The 1995 inventory indicates that quantifiable emissions of PCDD/PCDFs from combustion sources are more than an order of magnitude greater than quantifiable emissions from all other categories combined (U.S. EPA, 2000a). Total environmental releases of PCDD/PCDFs in the United States in 1995, expressed as TEQ-WHO, were estimated at 2,835 g TEQ. Air emissions contributed 2,705 g TEQ in that year, while emissions to water contributed 20 g TEQ and releases to land contributed 110 g TEQ. While there is substantial uncertainty in the emissions estimates for any specific source, it appears that air emissions represent more than 95 % of all emissions across all sources in the 1995 inventory of the PCDD/PCDFs in the United States.

U.S. EPA (2000a) compared emission of dioxins into the environment from quantifiable sources in 1987 and 1995, and found an approximately 80% reduction over this time interval. Their best estimates of releases of chlorinated dioxins and dibenzofurans to all environmental media (except products) were approximately 2,800 g TEQ (WHO) in 1995 and 13,500 g TEQ –(WHO) in 1987. This decrease was primarily due to decreased emissions of dioxins and related compounds into the atmosphere from municipal and medical waste incinerators. Emission reductions resulted partly from improved combustion and emission controls applied to these sources, in response to regulatory initiatives. For instance, the California Air Resources Board developed an airborne toxic control measure for dioxins from medical waste incinerators in 1990, which reduced emissions from these sources by 99%. In addition to improved controls on operating incinerators, a number of facilities were closed. More recently promulgated regulations and those currently under development by US EPA should result in some additional reduction in emissions from major combustion sources of dioxin-like compounds.

Ambient air concentrations for urban and rural areas in the United States were reported in 1995 to be 0.050 and 0.022 pg I-TEQ/m^3 for all measured PCDDs and PCDFs. TCDD levels for the same monitored area were 0.007 and 0.003 pg/m^3 (U.S. EPA, 2000b).

Table 1: Quantitative Inventory of Environmental Releases of Dioxins in the United States

Emission source category	Reference year 1995			Reference year 1987		
Confidence Rating[a]	A	B	C	A	B	C
RELEASES (g TEQ/yr) TO AIR						
Waste Incineration[f]						
Municipal waste incineration		1,250		8,877		
Hazardous waste incineration		5.8			5.0	
Boilers/industrial furnaces			0.39			0.78
Medical waste/pathological incineration			488			2,590
Crematoria			9.11[e]			5.5[e]
Sewage sludge incineration		14.8			6.1	
Tire combustion			0.11			0.11
Power/Energy Generation						
Vehicle fuel combustion - leaded[b]		2.0		37.5		
- unleaded			5.9			3.6
- diesel			35.5			27.8
Wood combustion - residential			62.8[e]			89.6[e]
- industrial		27.6			26.4	
Coal combustion – utility		60.1			50.8	
Oil combustion – industrial/utility			10.7			17.8
Other High Temperature Sources						
Cement kilns (hazardous waste burning)		156.1		117.8		
Lightweight aggregate kilns burning hazardous waste			3.3[e]			2.4[e]
Cement kilns (non hazardous waste burning)			17.8			13.7
Petroleum refining catalyst regeneration			2.21			2.24
Cigarette combustion			0.8			1.0
Carbon reactivation furnaces			0.08[e]			0.06[e]
Kraft recover boilers		2.3			2.0	
Minimally Controlled or Uncontrolled Combustion						
Forest, brush, and straw fires[d]		208[e]		170[e]		
Metallurgical Processes						
Metal Smelting/refining						
Ferrous: - Sintering plants		28.0				32.7
Nonferrous: - Primary copper		< 0.5[e]			< 0.5[e]	
- Secondary aluminum			29.1			16.3
- Secondary copper			271			983
- Secondary lead		1.72			1.29	
Drum and barrel reclamation			0.08			0.08
Chemical Manufacturing /Processing Sources						

Ethylene dichloride/vinyl chloride		11.2 [e]				
Total Quantified Releases To Air[c]	**2,705**			**13,081**		

Table 1: Quantitative Inventory of Environmental Releases of Dioxins in the United States (continued)

Emission source category	Reference year 1995			Reference year 1987		
Confidence Rating[a]	A	B	C	A	B	C
RELEASES (g TEQ/yr) TO WATER						
Chemical Manufacturing/Processing Sources						
Bleached chemical wood pulp and paper mills	19.5			356		
Ethylene dichloride/vinyl chloride		0.43 [e]				
Total Quantified Releases To Water[f]	**19.93**			**356**		
RELEASES (g TEQ/yr) TO LAND						
Chemical Manufacturing/Processing Sources						
Bleached chemical wood pulp and paper mill sludge	1.4			14.1		
Ethylene dichloride/vinyl chloride		0.73 [e]				
Municipal wastewater treatment sludge	76.6			76.6		
Commercially marketed sewage sludge	2.6			2.6		
2,4-Dichlorophenoxy acetic acid	28.9			33.4		
Total Quantified Releases To Land[c]	**110.23**			**126.7**		
OVERALL QUANTIFIED RELEASES TO THE OPEN and CIRCULATING ENVIRONMENT	**2,835**			**13,564**		

a. Characterization of the source category judged to be adequate for quantitative estimation with:
    A = High confidence in the emission factor and high confidence in activity level.
    B = Medium confidence in the emission factor and at least medium confidence in activity level.
    C = Low confidence in either the emission factor and/or the activity level.
b. Leaded fuel production and the manufacture of motor vehicle engines requiring leaded fuel for highway use have been prohibited in the United States.
c. TOTAL reflects only the total of the estimates made in U.S. EPA (2000a).
d. It is not known what fraction, if any, of the estimated emissions from forest fires represents a "reservoir" source. The estimated emissions may be solely the result of combustion.
e. Congener-specific emissions data were not available; the I-TEQ_{DF} emission estimate was used as a surrogate for the TEQ_{DF}-WHO98 emission estimate.
    f. Pulp and paper mill sludge incinerators were included within estimate for Wood Combustion - Industrial.

(Source : U.S. EPA, 2000a)

Emissions of dioxin-like compounds in California by county in 1999 are shown in Table 2. For the year 1999, Sacramento County had the highest emission for PCDDs and PCDFs with 5.4 lbs./year for chlorinated PCDFs alone. However, Contra Costa County had the highest emission of total PCBs, with 9.7 lbs./year. PCDD/PCDF emission data from California Air Resource Board (CARB) and U.S. Environmental Protection Agency (U.S. EPA) are not directly comparable, since CARB reports air emission in pounds per year of total and some isomers of PCDDs and PCDFs while U.S. EPA reports air emission of PCDDs and PCDFs as total g TEQ per year.

Table 2: CALIFORNIA EMISSION INVENTORY: DIOXINS, DIBENZOFURANS, PCBS
for data base year 1999

County	Pollutant	lbs/year	g/year	g TEQ/year (WHO-97)
LOS ANGELES	2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.000149	6.79E-02	6.79E-02
	Total TEQ of the 15 PCDDs/Fs*	-	-	7.56E-02
	PCDFs (chlorinated)	0.00056	2.55E-01	-
	PCDDs total $^{w/o}$	0.010803	4.92E+00	-
	PCDDs total ^{w}	0.001311	5.97E-01	-
	Polychlorinated biphenyls (PCBs)	4.17509	1.90E+03	-
SACRAMENTO	2,3,7,8-Tetrachlorodibenzo-p-dioxin	3.83E-06	1.75E-03	1.75E-03
	Total TEQ of the 15 PCDDs/Fs*	-	-	5.89E+01
	PCDFs (chlorinated)	5.42022	2.47E+03	-
	PCDDs total $^{w/o}$	2.4E-08	1.09E-05	-
	PCDDs total ^{w}	0.039012	1.78E+01	-
	Polychlorinated biphenyls (PCBs)	0.444033	2.02E+02	-
TUOLUMNE	PCDFs (chlorinated)	1.251765	5.70E+02	-
	PCDDs total ^{w}	0.020105	9.16E+00	-
KERN	2,3,7,8-Tetrachlorodibenzo-p-dioxin	3.46E-06	1.58E-03	1.58E-03
	Total TEQ of the 15 PCDDs/Fs*	-	-	7.18E-02
	PCDFs (chlorinated)	0.008751	3.99E+00	-
	PCDDs total ^{w}	0.000741	3.37E-01	-
	Polychlorinated biphenyls (PCBs)	1.73	7.88E+02	-
SAN BERNARDINO	2,3,7,8-Tetrachlorodibenzo-p-dioxin	1.76E-06	8.02E-04	8.02E-04
	Total TEQ of the 15 PCDDs/Fs*	-	-	1.89E-03
	PCDFs (chlorinated)	9.57E-05	4.36E-02	-
	PCDDs total $^{w/o}$	1.36E-05	6.20E-03	-
	PCDDs total ^{w}	0.001421	6.47E-01	-
MADERA	PCDFs (chlorinated)	0.01165	5.31E+00	-
	PCDDs total ^{w}	0.000035	1.59E-02	-
SHASTA	PCDFs (chlorinated)	0.010083	4.59E+00	-
	PCDDs total $^{w/o}$	6.01E-05	2.74E-02	-
	PCDDs total ^{w}	0.000847	3.86E-01	-
	Polychlorinated biphenyls (PCBs)	2.15501	9.82E+02	-

| CONTRA COSTA | Polychlorinated biphenyls (PCBs) | 9.681 | 4.41E+03 | - |

(See next page for footnotes.)

[w/o] Dioxins, total, excluding individual isomers reported (PCDDs)

[w] Dioxins, total, with individual isomers also reported (PCDDs)

* Total TEQ of the 15 PCDDs/Fs for which a Toxicity Equivalent Factor is applicable:

> 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin
> 1,2,3,4,6,7,8-Heptachlorodibenzofuran
> 1,2,3,4,7,8,9-Heptachlorodibenzofuran
> 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
> 1,2,3,4,7,8-Hexachlorodibenzofuran
> 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin
> 1,2,3,6,7,8-Hexachlorodibenzofuran
> 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin
> 1,2,3,7,8,9-Hexachlorodibenzofuran
> 1,2,3,7,8-Pentachlorodibenzo-p-dioxin
> 1,2,3,7,8-Pentachlorodibenzofuran
> 2,3,4,6,7,8-Hexachlorodibenzofuran
> 2,3,4,7,8-Pentachlorodibenzofuran
> 2,3,7,8-Tetrachlorodibenzo-p-dioxin
> 2,3,7,8-Tetrachlorodibenzofuran

(Source : CARB, 1999)

Concerns about dioxins and dioxin-like compounds are justified not only because of toxicity but also because of their very long biological and environmental persistence. These toxic air pollutants settle in grass and feed, which are then eaten and become a source of contamination for humans in livestock, poultry and dairy products. In addition, these persistent compounds accumulate in breast milk and then are transferred to the feeding infant.

Exposure to dioxins and dioxin-like compounds can occur through several pathways. The most important route for human exposure to PCDDs, PCDFs and PCBs is food consumption, contributing over 90% of total exposure, with products of animal origin and fish making the greatest contribution to this exposure (Liem *et al.*, 2000). Therefore, consumption habits may play a major role in the intake of dioxins and dioxin-like compounds. It must be stressed that the PCDDs and PCDFs as well as PCBs were largely originally airborne. The U.S. EPA report cited above (2000a) concluded that "*The environmental releases of CDD/CDFs ... are dominated by releases to the air from combustion sources. The current (i.e., 1995) inventory indicates that quantifiable emissions from combustion sources are more than an order of magnitude greater than quantifiable emissions from all other categories combined*".

U.S. EPA (2000b) examined the geographical distribution of emissions contributing to the total dioxin TEQ in the food supply. The major contributors to dioxins entering the human food supply in the U.S. (48 contiguous states) were identified by combining CDD/CDF/PCB concentration values from the EPA meat/milk surveys with food production data for beef, pork, chicken, eggs and dairy products by

county (from USDA and State agricultural records), expressed as production of animal fat. The 3,048 counties in the database were sorted in descending order and divided into four groups, with each group encompassing 25 percent of the total. The top 65 counties account for 25 percent of the total TEQ. The second, third, and fourth quartiles included 212, 498, and 2,303 counties, respectively.   These findings are shown in the map presented in U.S. EPA (2000b) and shown below (Figure 1). (For discussion of the TEQ definition used in this description and the accompanying figure, refer to the explanation on page 4 given in relation to the US emissions inventory)

Figure 1: Total TEQ production in meats, eggs and dairy products, categorized by quartile.
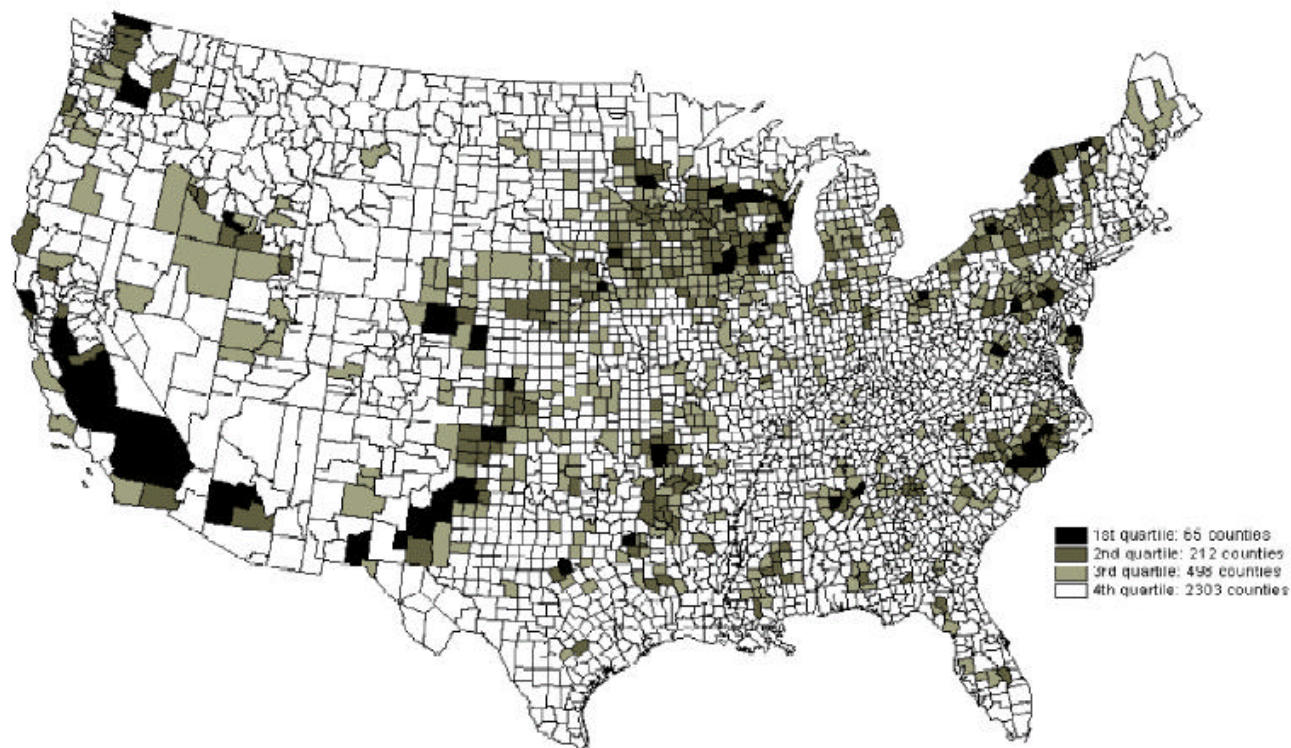
(Source : U.S. EPA, 2000b)

Most commercial food growing occurs in rural areas where there is no large dioxin reservoir source in the soil. (Some known contaminated sites contribute to dioxin levels in homegrown produce and livestock used by certain small communities, but such food materials do not enter the general commercial market. Also some reservoir sources in agricultural areas may result from earlier use of pesticides or herbicides, but inventory data [U.S. EPA, 2000a] suggest that this is not a major contributor to the overall input of dioxins into the food supply.) It therefore follows that the dominant pathway resulting in dioxin exposure for domestic meat and dairy animals is air deposition onto feed crops. Thus, the dominant sources of general population exposure to dioxin are the ambient air concentrations in the areas flagged by this analysis, and control of airborne contamination must occur to decrease PCDD, PCDF and PCB exposures via food intake. U.S. EPA, CARB and local air districts are currently engaged in measurement and analyses to further characterize these dioxin sources.

In general on a body weight basis, intake of dioxins and dioxin-like compounds is highest during childhood, drops during adolescence, and stabilizes in adults of about 20 years of age (Liem *et al.*, 2000; Patandin *et al.*, 1999a; Schecter *et al.*, 2001). When normalized by body weight, exposure is found to decrease with childhood age due primarily to increasing body weight (Liem *et al.*, 2000). In the U.S., estimated daily intake in WHO-TEQ (World Health Organization – toxic equivalents) declines with age. Schecter *et al.* (2001) estimated a mean daily intake of 42 pg TEQ/kg body weight for breast-fed infants during the first year of life. For children aged 1-11 years, males and females aged 12-19 years, and adult men and women aged 20-79 years, the estimated daily TEQ intakes were 6.2, 3.5 and 2.7, and 2.4 and 2.2 pg/kg body weight, respectively (Schecter *et al.*, 2001). Note that the oral Reference Exposure Level for dioxins is 10 pg/kg-day. Infant exposures exceed this oral REL by four-fold.

For newborns and fetuses however, maternal exposure and body burden need to be considered since *in utero* and lactational exposure represent important exposure pathways (Feeley and Brouwer, 2000). To better understand exposure of infants via lactation, at least two parameters need to be considered: the level of chemicals in breast-milk in the United States, and elimination kinetics from the mother during breast-feeding. Over 80% of the human milk samples examined by Angulo *et al.* (1999) contained PCB congeners #28, 138, 170 and 180, and over 70% of the human milk samples contained PCB congeners #52, 153, 187 and 188. PCB #28 demonstrated the highest milk concentration with 1.626 ppb, and PCB #183 the lowest with 0.109 ppb. In this study, PCB congener levels were significantly associated with birthplace, the location of industrial facilities, smoking, the consumption of a varied diet, meat, fish or industrially processed foods, number of children and lactation periods. Dioxins in breast milk were monitored in Tennessee in 1990, and averaged 18.8 ppt WHO-TEQ (per gram fat). In the Los Angeles region, data collected in 1987 showed dioxin levels in breast milk of 20.2 ppt WHO-TEQ (LaKind *et al.*, 2001). Schecter *et al.* (2001) reported breast milk levels for PCDDs, PCDFs and coplanar PCBs of 0.257, 0.089 and 0.075 pg TEQ/g whole weight with a mean lipid content of 3.70 % in 1996 in Binghamton, NY.

Newborns of active smoking mothers had higher plasma PCB levels than newborns of passive smoking mothers during pregnancy. Prenatal uptake of PCB was significantly less in newborns of non-smoking families compared to infants borne to active smoking mothers and passive smoking mothers (Lackmann *et al.*, 2000).

Although intake from inhalation of dioxins and dioxin-like compounds is low, there are some cases where inhalation can be a potential contributor to the uptake of these chemicals. It has been reported that indoor air is a more important source of dioxin-like compounds (Currado and Harrad, 1998). In a limited survey in Birmingham and Midlands, United Kingdom, on average, 9.0 ng total PCB/m^3 were measured in indoor air versus 0.31 ng total PCB/m^3 in outdoor air. This trend was not modified even when samples were collected near and away from a harbor during dredging of contaminated sediments (Vorhees *et al*., 1997). Similarly, there was more PCB contamination in house dust than in the yard soil in a neighborhood close to contaminated sediment fill from a harbor (Vorhees *et al*., 1999). Although the yard soil and the house dust showed similar profile for PCB contamination, house dust contained almost 10 times as much PCB as the yard soil (260-23,000 ng/g in house dust versus 15-1800 ng/g in

yard soil). Thus it appears that when considering inhalation as an uptake route for PCBs, indoor air can be an important contributor to human contamination.

IV.   Potential for Differential Effects

A.   *Summary of the Key Human Studies*

a)   *PCDD, PCDF and coplanar PCB-induced effects*

(1)   *Immunotoxicological effects*

Dioxins and dioxin-like PCBs have been associated with immunotoxicity. It was reported that, in Dutch preschool children, the immunotoxic effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases (Weisglas-Kuperus *et al.*, 2000). In this prospective study, on 207 healthy mother-infant pairs, prenatal PCB exposure was associated with an increased number of lymphocytes, T-cells, and $CD_3CD_8(+)$ (cytotoxic), $CD_4(+)CD_{45}RO(+)$ (memory), T-cell receptor (TcR) $\alpha B(+)$, and $CD_3(+)HLA\text{-}DR(+)$ (activated) T cells (Table 3) and lower antibody levels to mumps and measles at preschool age. Alteration in the developing stage of immune cells (change in cell population ratio) may indicate detrimental effects on the immune system. Any intrinsic (hormonal) or extrinsic (chemical) insult on thymocyte maturation during critical periods of thymocyte selection for self-recognition may have significant and detrimental consequences on immune function in postnatal life (Blaylock *et al.*, 1992). For instance, exposure to PCBs and dioxins may change the kinetics of thymocyte maturation and skew the thymocyte differentiation toward CD8+ phenotypically more mature TcR $\alpha B(+)$ T cells.

Prenatal PCB exposure was also associated with fewer cases of shortness of breath with wheeze. In addition, current PCB body burden was related to a higher prevalence of recurrent middle-ear infections and of chicken pox and to a lower prevalence of allergic reactions. A higher breast milk dioxin TEQ was associated with a higher prevalence of coughing, chest congestion, and phlegm. These results are consistent with suppression of the immune system. In this study, the median concentration in breast milk for dioxins was approximately 35 pg TEQ/g milk fat. Planar PCB and mono-ortho PCB median concentrations were both approximately 14 pg TEQ/g milk fat. The median plasma concentrations of PCBs in maternal, umbilical cord and 42 month old children were 2, 0.4, and 0.39 µg/L, respectively.

Chao *et al.* (1997) reported a higher incidence of middle-ear diseases, in comparison to control, in a follow-up study of the episode of poisoning from ingestion of rice oil contaminated with PCB and PCDF in Yu-Cheng, central Taiwan during 1978 and 1979. These children were born between 1978 and 1985 of mothers who had consumed contaminated oils before their children were born. The 8–9 year old children had a risk ratio for middle-ear diseases of 5.8 ($p = 0.051$) and the group of 10 – 11 year olds had a risk ratio of 4.1 ($p = 0.032$) (Chao *et al.*, 1997). These children had serum blood levels of 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) ranging from 1200-1400 ng/kg lipid and of 1,2,3,4,7,8-hexachloro-dibenzofuran (HxCDF) ranging from 2800 - 3200 ng/kg lipid. The reference group, Yu-Cheng children with normal middle ear, had PnCDF and HxCDF serum blood levels ranging from 200-400 and 400–800 ng/kg lipid respectively. Although blood PCDF was associated with an increase

middle-ear infection rate, blood PCB levels were not found to be associated with middle-ear disease in this study.

Table 3: Results of the white blood cell counts and the immunologic marker analysis (n = 85) in relation to prenatal PCB exposure.

| | Absolute counts Percentiles (10^9/L) | | | Prenatal PCB exposure | | | |
| | | | | S PCB maternal | | S PCB cord | |
	5th	50th	95th	Pearson correlation[a]	p Value	Pearson correlation[a]	p Value
White blood cells							
Monocytes	0.3	0.5	0.9	0.04	0.73	0.09	0.48
Granulocytes	2.2	4.1	7.5	0.14	0.22	0.15	0.20
Lymphocytes	2.2	4.1	6.6	0.25	0.02*	0.22	0.05*
T-cells markers							
CD3+	1.4	2.7	4.6	0.25	0.02*	0.21	0.07
CD3+CD4+	0.8	1.7	2.7	0.19	0.08	0.16	0.17
CD3+CD8+	0.4	0.9	1.7	0.27	0.01*	0.24	0.04*
CD4+CD45RA+	0.3	1.0	1.9	0.12	0.26	0.04	0.77
CD4+45RO+	0.2	0.4	0.6	0.25	0.02*	0.26	0.02*
TcR aB+	1.1	2.5	4.2	0.25	0.02*	0.20	0.08
TcR yS+	0.1	0.2	0.4	0.17	0.12	0.15	0.20
CD3 +HLA-DR+	0.1	0.3	0.5	0.26	0.02*	0.32	0.005*
B-cell markers							
CD 19/20+	0.4	0.9	1.7	0.12	0.28	0.15	0.20
NK-cell markers							
CD16+ n/or CD56+/CD3-	0.1	0.3	1.1	0.13	0.23	0.11	0.31

a : After logarithmic transformation of both variables involved. * Significant at the $p \leq 0.05$ level.
(Sources: Weisglas-Kuperus *et al.*, 2000)

In an investigation of 36 Japanese mother-children pairs, Nagayama *et al.* (1998b) reported a positive correlation between the PCDD, PCDF and coplanar PCB concentrations (TEQ) in breast milk and the CD4+/CD8+ lymphocyte ratio for these breast-fed babies. One year after birth, peripheral blood samples were obtained from 36 healthy babies to measure lymphocyte subsets by immunofluorescence using monoclonal antibody against CD3 (mature T cells), CD4 (helper T cells), CD8 (suppressor/cytotoxic T cells), CD20 (B cells), and HLA-DR (activated T cells). Breast milk samples taken about 3 months after birth were analyzed for PCDDs, PCDFs, and PCBs. Breast milk TEQ concentrations averaged 27 ppt on a fat weight basis. Postnatal exposure was then estimated as a product of breast milk intake and breast milk concentration. Analysis of variance was applied to evaluate the relationship between postnatal breastmilk exposure to PCDD, PCDF and coplanar PCB and lymphocyte subsets in peripheral blood. Mean TEQ intake was 34 ng/kg-day (range 6-84). The authors report that TEQ intake correlated positively with the percentage of CD4+ T cells and negatively

with CD8+ T cells. The ratio of CD4+ to CD8+ T cells showed a significant increase with increase TEQ intake (p = 0.025). This study indicates an impact of dioxin TEQ on the functioning of the immune system in infants. In contrast to the result reported by Nagayama *et al.* (1998b) in infants exposed to dioxins, a reduction in CD4+ T helper cells was observed (U.S. EPA, 2000c) in several human studies of cohorts exposed to polyhalogenated aromatic hydrocarbons (PHAHs). Although the fluctuations in the immune cell population were generally within the "normal" range in cohorts exposed to PHAHs, and may not translate into clinical effects, it is important to note that such cells have an important role in regulating immune responses. For instance, reduction/increase in immune cell population in clinical diseases is associated with immunosuppression. The CD4+/CD8+ T cell ratio is an indication of the stage of development of T-helper cells and cytotoxic T lymphocytes. Nagayama *et al.* (1998b) also note that CD4+/CD8+ T cell ratio is one of the most sensitive biomarkers for the exposure to highly toxic PCDDs, PCDFs and coplanar PCBs.

(2)   Developmental effects

A number of developmental effects including teratogenicity have been associated with exposure to dioxins in animals. Several investigations of humans have tried to evaluate developmental effects in people exposed environmentally. Patandin *et al.* (1998) evaluated growth of 207 children by measuring birth weight, and weight, height and head circumference at 10 days, and 3, 7, 10, and 42 months of age and evaluating any association of these parameters with background exposure to PCBs and dioxins. About half the children were breast-fed and half were formula-fed. Prenatal exposure (based on umbilical cord and maternal plasma levels) to "background" PCB level was significantly associated with reduced growth for the first 3 months as measured by weight, length, and head circumference. However, the same association was not noted for the breast-fed children (estimated from the analysis of PCB and dioxin concentrations in milk), which the authors note could be interpreted as a protective effect of breast-feeding nutrition on a number of health outcomes in infants (Patandin *et al.*, 1998). In this study, cord and maternal plasma PCB levels (based on PCB congeners #118, 138, 153 and 180) were both significantly associated with lower growth rate. Furthermore, infants with high cord plasma PCB levels (0.80 µg/L, the 90th percentile) weighed significantly less (165 g less; p ≤ 0.05) compared with infants with low cord plasma PCB levels (0.20 µg/L, the 10th percentile).

Similarly, in a study of 167 pregnant women, breast milk contamination by PCDDs/PCDFs (a measure of the mother's body burden and indirectly of prenatal exposure) was tentatively associated with low birth weight (Vartiainen *et al.*, 1998). Breast milk was analyzed for the seventeen 2,3,7,8-substituted PCDDs and PCDFs, three coplanar PCBs, and 23 mono-ortho, di-ortho and non-coplanar PCBs. Concentrations of PCDDs and PCDFs in breast milk averaged 26 pg/g fat and the sum of PCB concentrations averaged approximately 500 ng/g fat. Using Pearsson's correlation 2-tailed test, the birth weight for all children grouped together (p<0.02), and in boys separately (p<0.04) but not girls, was slightly decreased with increasing concentrations of 2,3,4,7,8-pentachlorodibenzofuran, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. However, when the analysis was restricted to primiparae, there was no statistically significant correlation between birth weight and the concentrations of PCDDs/PCDFs in the mother's milk (Vartiainen *et al.*, 1998).   Also, in the same study, no correlation was found between the weight of the child and PCBs, PCB-TEQs, or individual

PCB congeners in the whole group or among primiparae, or among boys or girls. The authors note that the correlation of birth weight in boys and dioxin contamination of the mother (as assessed by breast milk concentrations) may or may not be real due to the lack of correlation in girls or in primiparae.

Altered sex ratio of offspring has also been reported as a health effect of environmental exposure to PCDDs and PCDFs. Mocarelli *et al.* (2000), in a follow-up study of the Seveso, Italy, accident, found an association between lower sex ratio (male/female) in children and increasing TCDD concentrations in serum samples from their fathers ($p = 0.008$). This effect started at concentrations less than 20 ng/kg body weight, and fathers exposed when they were younger than 19 years of age sired significantly more girls than boys (sex ratio 0.38 [95% CI 0.30-0.47]). The median concentration of dioxin in fathers in this study was similar to doses that induce epididymal impairments in rats, and is about 20 times the estimated average concentration of TCDD currently found in human beings in industrialized countries.

(3)   *Thyroid hormone effects*

In the same cohort described above by Nagayama *et al.* (1998b), Nagayama *et al.* (1998a) reported a negative correlation between polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls concentrations in breast milk (in TEQ) and the levels of triiodothyronine (T_3) and thyroxin (T_4) in the blood of 36 breast-fed Japanese babies. Blood samples, taken one year after birth, were analyzed for serum T_3, T_4, and TSH by radioimmunoassay. Analysis of variance indicated a significant negative correlation between total TEQ intake and serum T_3 ($p< 0.037$) and serum T_4 ($p< 0.018$). In this study, breast milk TEQ concentration averaged 27 ppt on a fat weight basis.

In an epidemiological study on exposure of 320 children 7-10 years of age to a toxic waste incineration plant, Osius *et al.* (1999) found a statistically significant positive association between the mono-ortho congener PCB 118 in blood and thyroid stimulating hormone (TSH) as well as statistically significant negative relationships between PCBs 138, 153, 180, 183, and 187 and free T_3. Free T_4 level was not associated with the PCB congeners considered. The geometric mean of blood concentrations for PCBs 138, 153 and 180 was 0.39 µg/L.

b)   *Non-coplanar PCB-induced effects*

(1)   *Neurobehavioral effects*

The non-dioxin-like PCBs have been associated with developmental neurotoxicity in animals, an effect of particular concern for infants and children. There are a number of epidemiological studies suggesting that PCB exposure is associated in humans with developmental neurotoxicity.

Winneke *et al.*, (1998) administered the Bayley Scale Index Development, version II (BSID II) mental development index test to 7-month old infants in a cohort covering 171 healthy mother-infant pairs. The BSID II is an established psychodevelopmental tool which has been applied in several PCB studies. The BSID consists of three scales: the Mental Development Index (MDI), the Psychomotor Development Index (PDI) and the Behavior Rating Scale. Winneke *et al.* (1998) administered only the

first two tests. The MDI assesses the child's level of cognitive development (memory, learning and problem solving), language development (expressive/receptive language, vocalization), and personal/social development. The motor scale assesses fine and gross motor development.

Winneke *et al*. (1998) reported that cord plasma and breast milk mean sum PCB-concentrations for the three non-coplanar di-ortho substituted PCBs monitored (PCB congeners #138, 153 and 180) were 0.55 ng/ml and 427 ng/g fat respectively. These concentrations correspond to those reported in recent studies of central European cohorts (Lanting *et al.*, 1998c; Schade and Heinzow, 1998). Winneke *et al*. (1998) found a significant negative association between the PCB contamination of maternal milk and the mental development index of 7-month old infants (p < 0.05).

These results agree with a similar study in North Carolina (Gladen *et al.*, 1988). In the Winneke *et al*. (1998) and the Gladen *et al*. (1988) studies, only maternal milk PCB was related to cognitive/motor outcome; cord plasma PCB was not. On the other hand Jacobson *et al.*, (1985) in Michigan found an association of impaired mental development with PCB levels in cord plasma but not in milk.

A cohort of 418 Dutch children, half breast-fed and half formula-fed, were evaluated for potential effects on neurological development of background exposure to PCBs (Huisman *et al*., 1995). Maternal and cord blood were analyzed for the non-coplanar PCB congeners # 118, 138, 153, and 180, as a measure of prenatal exposure. Of these PCB congeners, only PCB #118 is a mono-ortho substituted PCB; the remaining PCB congeners are di-ortho substituted with no or very little affinity for the Ah receptor. Breast milk samples were also obtained and the fat analyzed for PCBs and dioxins (seventeen 2,3,7,8-chlorinated dioxins and furans, three coplanar PCBs and 23 non-coplanar PCBs). Formula milk was also obtained for the formula-fed babies and analyzed for the same set of congeners. At 18 months of age, neurological examinations of the children were conducted focusing on motor function. Each toddler was classified as normal, mildly abnormal (e.g., presence of signs such as slight asymmetry, hyper- or hypotonia), or abnormal (e.g., presence of overt neurological problems). A list of 57 neurological items was scored for "optimality" and a total score calculated for each child. Special emphasis was placed on fluency of movement, which the authors note is a good indicator of the integrity of brain function. Chi-square, Student's T-test, and the Mann-Whitney U tests were used to compare groups. The effect of PCB and dioxin exposure was evaluated by multiple linear regression analyses. The independent variables were PCB and dioxin levels, social, perinatal, and obstetric variables. The dependent variables were the neurological optimality score and fluency score. A small but statistically significant effect of PCB exposure *in utero* on the neurological optimality score was noted. There was also an effect of paternal smoking on neurological optimality score. There was no association between PCB or dioxin exposure via breast milk with the neurological optimality score, despite the higher doses of dioxins and PCBs in these children. The authors speculate this could be a protective effect of breast-feeding on brain development.

Lanting *et al*. (1998b) evaluated the neurological condition of the same cohort used by Huisman *et al* (1995) and reported that although some small neurological effects were observed in prenatally exposed children at 18 months of age, no effect of pre- or postnatal exposure to PCBs or dioxins was associated with neurological adverse effect in children 42 months of age. In this cohort, the median summed concentrations of PCBs were 0.4 and 2.0 µg/L for umbilical cord and maternal plasma, and 0.4 µg/L in

plasma of 42-month old children. Concentrations of dioxins in breast milk were 28.8 ng TEQ dioxin/kg fat, and of coplanar PCBs were 14.5 ng TEQ /kg fat.

However, others have reported persistent neurological effects of non-coplanar PCB exposures. Patandin *et al.* (1999b), in a follow-up of the Dutch PCB/dioxin study, reported that exposure *in utero* to "background" PCB concentrations is associated with poorer cognitive functioning (cognitive abilities and verbal comprehension) in preschool 42-month-old children (n = 395). Prenatal PCB exposure was estimated from the sum of PCBs 118, 138, 153, and 180 (ΣPCB) in maternal plasma during pregnancy. The investigators used the Kaufman Assessment Battery for Children (KABC), and Reynell Language Developmental Scales (RLDS). The KABC tests both sequential problem solving and simultaneous processing ability; the RLDS is primarily an assessment of language ability. After controlling for confounders, prenatal PCB exposure, measured as the maternal plasma PCB levels, was significantly associated for all children with lower scores on the overall cognitive scale (p < 0.005) and the sequential and simultaneous processing scales (both p < 0.02) of the KABC. This was highly significant for the formula-fed babies, but the breast-fed babies showed much less of an effect and most associations were not significant. In the formula-fed group, there was also a significant association between prenatal exposure to PCB and low scores on the verbal comprehension scale of the RLDS (p < 0.03). Cord plasma PCB concentrations were also significantly associated with the simultaneous processing score of the KABC in the whole group, and significantly associated with the RDLS verbal comprehension scale in the formula-fed group. The highest exposed group (ΣPCB $\geq$ 3 μg/L) scored 4 points lower than the lowest exposed group (ΣPCB < 1.5 μg/L) on all 3 scales of the KABC (p < 0.05). In this study, lactation and current (42-month-old infant estimated body burden) exposure to PCBs and dioxins were not related to 42-month cognitive performance. Thus, the prenatal exposure appears to be more important for effects on cognitive development.

In a follow-up study of a group of 418 infants from birth up to 6 years of age, Boersma and Lanting (2000) concluded that prenatal exposure to PCBs has subtle negative effects on neurological and cognitive development of the child up to school-age. They also showed evidence that breast-feeding, despite a greater intake of PCBs and dioxins compared to formula-fed babies, counteracts these adverse developmental effects of *in utero* exposure to PCBs and dioxins. Median maternal and umbilical cord plasma sum PCB-concentrations were 2.2 and 0.43 μg/L respectively. For breast milk, sum PCB and sum dioxins median concentrations were 405 μg/kg fat and 29 ng TEQ/kg fat respectively.

Moreover, Jacobson and Jacobson (1996), in a follow-up of the Michigan study (Jacobson *et al.*, 1990) administered a battery of IQ and achievement tests to 212 eleven-year-old children. These children were born to mothers who were known to have consumed Lake Michigan fish contaminated with PCBs. Each species of fish was weighted according to degree of contamination with PCBs as reported in the database from the U.S. Environmental Protection Agency. Each child was tested individually at 11 years of age with the Wechsler Intelligence Scales for Children IQ Test, the spelling and arithmetic subtests of the Wide Range Achievement Test and the word- and passage-comprehension subtests of the Woodcock Reading Mastery Tests. The authors reported a significant association between prenatal exposure to PCBs and lower full-scale and verbal IQ scores. The

strongest effects were related to memory and attention. In this study, children most highly exposed ($\geq$ 1.25 µg PCB/g of fat expressed in terms of maternal milk contamination) were three times as likely to have low average IQ scores and twice as likely to be at least two years behind in reading comprehension.

Stewart *et al.* (2000) demonstrated that neonates born of mothers (n=141) who consumed at least 40 lbs of Lake Ontario fish over their lifetime demonstrated a significant linear relationship between the most heavily chlorinated PCBs measured in umbilical cord plasma and performance impairments on the Habituation and Autonomic clusters of the Neonatal Behavioral Assessment Scale (NBAS) at 25-48 hours after birth (Table 4). The controls consisted of 152 women known not to have eaten fish from Lake Ontario. The most highly prenatally exposed neonates, as evaluated by the umbilical cord PCB level, exhibited poorer performance in a significantly greater proportion of the NBAS scales (Stewart *et al.*, 2000). Less chlorinated PCBs, DDE, Mirex, HCB, lead, and mercury were not related to NBAS performance. These results corroborated earlier findings; the most heavily chlorinated PCB congeners (hepta-, octa-, and nonachlorinated biphenyls) are most strongly correlated with breast milk levels. It appears that the chlorination and persistence of PCBs may be important factors both for exposure assessment and for neurobehavioral toxicity.

Table 4: Dose-response relationships between the concentration of highly chlorinated PCBs (ng/g fat) and performance on the habituation, autonomic, and reflex clusters of the Neonatal Behavioral Assessment Scale (NBAS) at 25 - 48 h after birth

NBAS performance	Highly chlorinated PCBs (ng/g fat)				Linear trend analysis
	0 (ND)	**> 0**	**> 24**	**> 133**	
Habituation (48 h postnatal)	7.34	7.60	7.06	6.80	$F_{(1, 221)} = 3.95$ $p < 0.05$
Autonomic (48 h postnatal)	6.02	6.35	5.48	5.72	$F_{(1, 261)} = 4.40$ $p < 0.05$
Abnormal reflexes	2.3	2.75	3.0	2.85	$F_{(1, 262)} = 2.81$ $p = 0.095$

(Source : Stewart *et al.*, 2000)

B.   *Summary of the Key Animal Studies*

a)   *PCDD, PCDF and coplanar PCB-induced effects*

(1)   *Immunotoxicological effects*

Delayed immunotoxicological effects were demonstrated in experiments on TCDD-exposed dams (Nohara *et al.*, 2000). Pregnant dams were administered a single oral dose of 12.5-800 ng /kg body weight TCDD on gestation day (GD) 15. The thymus and spleen of pups, from dams exposed to 800

ng/kg TCDD, contained 102.0 and 62.7 pg TCDD/g tissue on post-natal day (PND) 21, respectively, and the amounts decreased thereafter. In the thymus, dose-dependent CYP1A1 mRNA induction was clearly observed on PND 5 in pups of dams exposed to 50-800 ng/kg TCDD. The induction was gradually decreased on PND 21 and 49. CYP1A1 mRNA induction in the spleen was very weak. Splenocyte number, on PND 49 (puberty), decreased in a dose-dependent manner in pups of dams exposed to 12.5-800 ng/kg TCDD. The alteration in spleen cellularity by TCDD was not detected on PND 21 (weaning) or 120 (adulthood). The results showed an effect of perinatal exposure to low doses of TCDD on the immune system, which is apparent in the spleen around puberty and likely to be unrelated to Ah receptor-dependent gene expression (Nohara *et al.*, 2000).

Exposure *in utero* to TCDD can cause persistent immunotoxicological effects. In Gehrs *et al.* (1997), timed-bred pregnant F344 rats were dosed with 0 or 1.0 µg/kg TCDD by gavage on GD 14. One day after birth, litters were cross-fostered to produce control, placental-only, lactational-only, and placental/lactational exposure groups. The organ weights and thymic and splenic phenotypes of these pups were assayed 1, 2, or 3 weeks post-partum, while the delayed-type hypersensitivity (DTH) response was assessed in 5-month-old males. Increased liver/body weight ratios, decreased percentages of thymic $CD3^+/CD4^-CD8^-$ cells, and increased percentages of thymic $CD3^+/CD4^-CD8^+$ cells were seen through 3 weeks old in both genders after TCDD exposure. These data are presented in Table 5 through Table 9. The severity of the effects was related to the route of exposure (i.e. placental/lactational > lactational > placental). The delayed-type hypersensitivity (DTH) response to bovine serum albumin (BSA) was suppressed in the males receiving both placental and lactational exposure. In a second set of experiments, TCDD exposure (3.0 µg/kg) increased spleen/body weight ratio, decreased the thymus/body weight ratio (in males), and decreased the percentage of splenic $CD3^+/CD4^-CD8^-$ cells in both the TCDD-exposed male and female pups when tested at 14 - 17 weeks (Table 9). TCDD suppressed DTH response to BSA in both genders (Gehrs *et al.*, 1997).

Table 5: Effects of TCDD on 1-week-old male rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al*., 1997)

	Route of TCDD exposure[b, c]			
	Control	Placental	Lactational	Placental/lactational
Body weight (g)	7.73 ± 0.65	7.77 ± 0.41	7.92 ± 0.42	7.28 ± 0.38
Relative organ weights (mg/g body wt.)				
Spleen	3.63 ± 0.23	3.76 ± 0.21	3.63 ± 0.14	3.13 ± 0.13
Thymus	1.60 ± 0.13	1.69 ± 0.08	1.55 ± 0.11	1.37 ± 0.09
Liver	27.2 ± 1.8	29.4 ± 1.4	$33.8 \pm 1.8^*$	31.1 ± 1.3
Splenic cellularity ($\times 10^6$)	9.8 ± 1.6	10.0 ± 0.9	8.6 ± 1.2	7.9 ± 1.3
Thymic cellularity ($\times 10^6$)	21.0 ± 3.9	19.7 ± 3.2	16.0 ± 2.9	14.0 ± 2.1
Thymocyte phenotype				
Percentage $CD3^+$	24.8 ± 0.7	24.5 ± 1.1	27.1 ± 0.5	28.0 ± 0.9
% $CD4^+CD8^-$	11.4 ± 0.6	11.5 ± 0.4	10.8 ± 0.4	11.5 ± 0.4
% $CD4^+CD8^+$	75.6 ± 0.4	73.0 ± 1.2	72.7 ± 2.2	$70.3 \pm 1.2^*$
% $CD4^-CD8^-$	2.4 ± 0.1	$1.8 \pm 0.3^*$	$1.1 \pm 0.1^{**}$	$1.0 \pm 0.1^{**}$
% $CD4^-CD8^+$	10.6 ± 0.4	13.8 ± 1.3	15.6 ± 2.1	$17.3 \pm 1.3^*$

[a] Results expressed as means $\pm$ S.E.

[b] Dams were given 1.0 µg TCDD/kg or vehicle control.

[c] There were five animals (1/litter) in each exposure group. For the placental and the lactational exposure groups, the litters were cross-fostered on postnatal day 1.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 6: Effects of TCDD on 1-week-old female rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al.*, 1997)

	Route of TCDD exposure[b, c]			
	Control	**Placental**	**Lactational**	**Placental/lactational**
Body weight (g)	9.38 ± 0.20	8.92 ± 0.54	8.89 ± 0.24	8.07 ± 0.53
Relative organ weights (mg/g body wt.)				
Spleen	3.52 ± 0.26	3.58 ± 0.34	3.64 ± 0.06	3.17 ± 0.21
Thymus	2.09 ± 0.09	2.02 ± 0.14	1.70 ± 0.10	1.77 ± 0.14
Liver	25.9 ± 1.0	28.8 ± 1.4	$31.8 \pm 1.8^{*}$	$32.8 \pm 1.1^{*}$
Splenic cellularity ($\times 10^{6}$)	17.7 ± 1.9	12.5 ± 1.8	16.2 ± 2.0	11.8 ± 2.4
Thymic cellularity ($\times 10^{6}$)	33.7 ± 1.9	24.1 ± 3.4	$21.9 \pm 2.7^{*}$	$17.4 \pm 2.6^{**}$
Thymocyte phenotype				
Percentage $CD3^{+}$	31.6 ± 0.6	32.7 ± 0.7	30.0 ± 0.6	32.1 ± 1.0
% $CD4^{+}CD8^{-}$	5.8 ± 0.3	5.7 ± 0.4	5.5 ± 0.5	5.3 ± 0.4
% $CD4^{+}CD8^{+}$	84.6 ± 0.6	83.0 ± 0.8	82.9 ± 1.1	$78.9 \pm 2.0^{*}$
% $CD4^{-}CD8^{-}$	1.1 ± 0.1	1.1 ± 0.1	$0.6 \pm 0.0^{**}$	$0.7 \pm 0.1^{**}$
% $CD4^{-}CD8^{+}$	8.6 ± 0.4	10.3 ± 0.8	11.1 ± 1.1	$15.1 \pm 1.5^{**}$

[a] Results expressed as means $\pm$ S.E.

[b] Dams were given 1.0 µg TCDD/kg or vehicle control.

[c] There were five animals (1/litter) in each exposure group. For the placental and the lactational exposure groups, the litters were cross-fostered on postnatal day 1.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 7: Effects of TCDD on 2-week-old rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al.*, 1997)

	Male pups[b]		Female pups[b]	
	Control	**Perinatal TCDD[c]**	**Control**	**Perinatal TCDD[c]**
Body weight (g)	19.7 ± 0.6	17.4 ± 0.6	20.0 ± 0.9	16.2 ± 1.1
Relative organ weights (mg/g body wt.)				
Spleen	3.31 ± 0.1	3.55 ± 0.11	3.27 ± 0.1	3.22 ± 0.15
Thymus	2.56 ± 0.1	1.98 ± 0.09	2.60 ± 0.1	2.27 ± 0.26
Liver	27.1 ± 0.5	$30.0 \pm 0.5^*$	29.3 ± 0.1	$33.2 \pm 0.9^*$
Splenic cellularity ($\times 10^6$)	32.6 ± 4.3	28.5 ± 1.7	25.7 ± 2.4	15.4 ± 2.8
Thymic cellularity ($\times 10^6$)	81.3 ± 3.5	$51.9 \pm 3.4^{**}$	82.2 ± 7.7	55.5 ± 9.0
Thymocyte phenotype				
Percentage CD3$^+$	25.0 ± 0.4	23.4 ± 0.3	23.8 ± 0.2	25.5 ± 1.2
% CD4$^+$CD8$^-$	17.0 ± 1.5	$11.9 \pm 0.4^{**}$	10.1 ± 0.6	$7.4 \pm 0.5^*$
% CD4$^+$CD8$^+$	70.1 ± 1.6	73.1 ± 0.8	75.0 ± 0.5	77.7 ± 0.9
% CD4$^-$CD8$^-$	1.4 ± 0.1	$0.8 \pm 0.0^{**}$	1.2 ± 0.1	$0.7 \pm 0.1^{**}$
% CD4$^-$CD8$^+$	11.6 ± 1.0	14.3 ± 0.5	13.7 ± 0.2	14.3 ± 0.3

[a] Results expressed as means $\pm$ S. E.

[b] There were five or six animals (1/litter) in each exposure group.

[c] Dams were given 1.0 µg TCDD/kg or vehicle control. The perinatal TCDD groups refer to placental/lactational exposure. In general, results in the placental and the lactational groups were intermediate between those in the control and the placental/lactational groups.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 8: Effects of TCDD on 3-week-old rat pups whose dams were dosed orally on gestational day 14[a] (From Gehrs et al., 1997)

	Male pups [b]		Female pups [b]	
	Control	**Perinatal TCDD[c]**	**Control**	**Perinatal TCDD[c]**
Body weight (g)	**34.9 ± 1.8**	**33.1 ± 0.6**	**36.3 ± 0.4**	**31.1 ± 0.9[**]**
Relative organ weights (mg/g body wt.)				
Spleen	3.18 ± 0.03	3.31 ± 0.07	3.85 ± 0.12	3.77 ± 0.15
Thymus	3.19 ± 0.06	2.91 ± 0.08	3.54 ± 0.24	3.l2 ± 0.15
Liver	40.2 ± 1.0	48.1 ± 1.5[**]	43.1 ± 0.8	50.2 ± 1.3[**]
Splenic cellularity ($\times 10^6$)	58.4 ± 5.7	41.6 ± 2.0	80.4 ± 6.5	63.0 ± 4.3
Thymic cellularity ($\times 10^6$)	185.3 ± 12.4	142.2 ± 20.5	240.8 ± 23.0	172.7 ± 14.2[*]
Thymocyte phenotype				
Percentage $CD3^+$	30.0 ± 0.9	34.9 ± 1.1[**]	33.7 ± 0.5	35.1 ± 1.3
% $CD4^+CD8^-$	14.5 ± 0.7	14.0 ± 0.3	24.8 ± 1.1	21.6 ± 0.8[*]
% $CD4^+CD8^+$	68.5 ± 0.7	66.0 ± 0.7	52.6 ± 1.0	55.1 ± 1.2
% $CD4^-CD8^-$	1.0 ± 0.1	0.6 ± 0.0[**]	1.3 ± 0.1	0.9 ± 0.1[*]
% $CD4^-CD8+$	16.1 ± 0.3	19.6 ± 0.6[**]	21.4 ± 0.2	22.4 ± 0.3[*]

[a] Results expressed as means ± S. E.

[b] There were five or six animals (1/litter) in each exposure group.

[c] Dams were given 1.0 µg TCDD/kg or vehicle control. The perinatal TCDD groups refer to placental/lactational exposure. In general, results in the placental and the lactational groups were intermediate between those in the control and the placental/lactational groups.

* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Table 9: Effects of TCDD on 14-week-old rats whose dams were dosed orally on gestational day 14[a] (From Gehrs *et al*., 1997)

	Male rats[b]		Female rats[b]	
	Control	Perinatal TCDD	Control	Perinatal TCDD
Body weight (g)	278.6 ± 5.0	264.5 ± 5.9	166.1 ± 2.6	167.7 ± 2.4
Relative organ weights (mg/g body wt.)				
Spleen	1.99 ± 0.03	$2.44 \pm 0.06^{**}$	2.40 ± 0.05	$3.01 \pm 0.09^{**}$
Thymus	0.96 ± 0.04	$0.70 \pm 0.02^{**}$	1.09 ± 0.05	1.04 ± 0.07
Liver	43.0 ± 1.2	$37.3 \pm 1.5^{*}$	36.2 ± 0.5	36.8 ± 0.3
Splenic cellularity ($\times 10^6$)	289.8 ± 7.5	269.3 ± 10.8	233.5 ± 15.2	276.8 ± 16.4
Splenocyte phenotype				
Percentage IgM$^+$	44.2 ± 0.6	41.8 ± 1.3	40.5 ± 0.9	38.4 ± 1.7
Percentage CD3$^+$	42.1 ± 1.1	40.9 ± 1.0	47.3 ± 1.1	48.2 ± 1.0
% CD4$^+$CD8$^-$	75.4 ± 0.8	77.1 ± 0.4	74.1 ± 1.0	76.1 ± 0.3
% CD4$^+$CD8$^+$	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.3	3.0 ± 0.2
% CD4$^-$CD8$^-$	2.1 ± 0.0	$1.8 \pm 0.1^{**}$	2.0 ± 0.1	$1.5 \pm 0.1^{**}$
% CD4$^-$CD8$^+$	19.6 ± 0.8	18.2 ± 0.3	21.3 ± 0.8	19.4 ± 0.5

[a] Results expressed as means $\pm$ S.E.

[b] There were seven animals in each exposure group. The perinatal TCDD groups refer to placental/lactational exposure. The dams of these rats received 3.0 µg/kg TCDD on GD14. The dams of the control rats were untreated.
* $p < 0.05$ versus vehicle control; ** $p < 0.01$ versus vehicle control.

Gehrs and Smialowicz (1999) in a similar experiment demonstrated that the suppression of the DTH response in rats associated with perinatal TCDD exposure is persistent through late adulthood, occurs at a low dose (i.e. 0.1 µg TCDD/kg to the dam), and is more pronounced in males than females. In the first experiment, DTH response to BSA was tested in animals previously shown to have a suppressed DTH response at 4 months of age following 3 µg TCDD/kg dose to the dams at GD 14. The animals were retested at 8, 12, and 19 months of age. Male offspring had significantly suppressed DTH response at 4, 8, and 19 months of age (p<0.05); the trend in females was towards a suppressed DTH response but was only significant at 4 mo of age. In a second experiment, dams were given 0, 0.1, 0.3, or 1.0 µg TCDD/kg on GD 14, and lactational exposure was allowed through 4 weeks in the pups. Suppression of the DTH response to BSA was evident in males at 0.1 and 0.3 µg TCDD/kg (to the dam) group. Thus, this study shows that the perinatally exposed animals continued to have a suppressed immune response into late adulthood. DTH reactions are important in protection against invading microorganisms and certain tumors, as well as having a role in allergy. Thus, supression of DTH response results in an increased risk for infectious disease, and also neoplasms, while decreasing the risk of autoimmune disease.

In a chicken embryo study where eggs were injected with PCB #126 at various incubation stages, Fox and Grasman (1999) reported that lymphoid cell numbers were more sensitive to PCB #126 than immune organ masses. They also observed that the bursa of Fabricius (a dorsal outpocketing of the cloaca that controls antibody-mediated immunity in young birds) tended to be more sensitive than the thymus. Doses necessary to reduce the number of viable lymphoid cells in the thymus and bursa were

at least one order of magnitude lower with full-term incubation as compared to exposure only during later stages of incubation. The LD_{20} and LD_{50} for lymphocyte viability was estimated to be 0.21 and 1.01 ng PCB #126/g, respectively. Thymus mass dropped sharply between 0.13 and 0.32 ng/g, and lymphoid cell numbers in the thymus fell sharply between 0.051 and 0.13 ng/g. Bursa mass began to decrease at the lowest dose of 0.051 ng/g and reached a minimum at 0.32 ng/g. The number of viable cells decreased slightly at 0.051 ng/g and reached a minimum at the 0.13- and 0.32-ng/g doses.

(2)   Developmental effects

In animal experiments, offspring of pregnant Long Evans rats treated on GD 15 with 1.0 µg/kg TCDD by gavage showed signs of reproductive developmental toxicity (Hamm *et al.*, 2000). These changes are indicative of disruption of the proper hormonal environment in the offspring. The effects seen may parallel those in adults, but whereas the responses may be reversible in adults, exposure of the fetus results in irreversible effects, including both anatomical and functional abnormalities. Starting at PND 32 male pups showed impaired growth of their seminal vesicles, which was associated with a dramatic decrease in the development of the epithelium. Gray *et al.* (1997) administered TCDD to Long Evans pregnant rats at gestational day 15 at dosage levels of 0.05, 0.2 or 0.8 µg/kg. Female rat offspring (80 days of age) had morphological reproductive tract alterations ($p < 0.05$) such as cleft phallus (significant at 0.8 µg TCDD/kg), temporary or permanent vaginal thread formation (significant at 0.2 and 0.8 µg TCDD/kg) and delayed vaginal opening (significant at 0.8 µg TCDD/kg).

In a cross-fostering study (Crofton *et al.* 2000) examined the progeny of rats treated with 6 mg/kg/day Aroclor 1254 (A1254) from GD 6 to PND 21. On the day of birth, half of the treated litters and half of the control litters were cross-fostered, resulting in the following groups: Ctrl/Ctrl (controls); A1254/A1254 (perinatal exposure); A1254/Ctrl (prenatal exposure only); and Ctrl/A1254 (postnatal exposure only). Rats exposed during their development, exhibited ototoxicity however, that effect was mostly observed in the group exposed during lactation (Table 10). They concluded that the critical period for developmental ototoxicity from Aroclor 1254 exposure is within the first few postnatal weeks in the rat.

Table 10: Perinatal Arochlor 1254 (6 mg/kg/day) treatment caused low frequency hearing loss that was due solely to postnatal exposure.

Frequency	Threshold, dB SPL (mean ± SE)			
	Ctrl/Ctrl	**A1254/A1254**	**A1254/Ctrl**	**Ctrl/A1254**
1 kHz	24	42[*]	28	46[*]
40 kHz	16	17	17	21

* indicates significant difference from the 1 kHz control group, $p < 0.05$; $n = 11 –14$ litter/group
(Source : Crofton *et al.*, 2000)

Offspring of pregnant Wistar rats administered a single oral dose of 10 µg/kg body weight PCB #126 or 100 µg/kg of PCB #77 on GD 15 showed signs of developmental toxicity (Faqi *et al.*, 1998). Male

offspring were killed on postnatal days 65 or 140. In the PCB #126 group, the age of vaginal opening was delayed in the female pups. Testis and brain weights, and daily sperm production were permanently increased and seminal vesicle weight was decreased in male offspring of the PCB #77-treated group. In male rats of the PCB #126 group, brain weights were permanently increased and ventral prostate weights permanently reduced. In both PCB groups, however, serum testosterone concentration was reduced only at adulthood. All these responses were significant at $p<0.05$. Faqi *et al.* (1998) concluded that PCB #126 elicited some TCDD-like developmental toxicity on the reproductive tract after exposure *in utero*. For the PCB #77, these authors hypothesized that the reproductive effects of *in utero* exposure to PCB #77 on male offspring may be attributed to neonatal hypothyroidism induced by the substance during early fetal development.

(3) Thyroid hormone effects

A number of studies indicate that dioxins and dioxin-like compounds decrease circulating thyroid hormone levels. A reduction of maternal serum thyroxin (T_4) levels can impair the brain development of the offspring (Glorieux *et al.*, 1988; Rovet *et al.*, 1987; Haddow *et al.*, 1999). Brain developmental damage appears to be inversely related to maternal serum T_4 levels in the first and second trimesters. Maternal serum free T_4 is able to pass through the placenta and is converted to tri-iodothyronine (T_3) in the fetal brain. The T_3 generated in situ is believed to be necessary for the development of brain, specifically the cerebral cortex, the extrapyramidal system, and the cochlea (Porterfield, 1994). The availability of a minimum level of maternal free T_4 is crucial for proper fetal brain development in the first and second trimesters, as the fetal thyroid is not fully mature and functional during that time period. A number of human studies have shown that pregnancy itself puts stress on the thyroid (Crooks *et al.*, 1967; Glinoer *et al.*, 1990; Brent, 1999). Consequently, insults on the maternal thyroid condition are particularly relevant to the issue of increased sensitivity of infants and children to dioxins and dioxin like compounds.

In a cross fostering study, Crofton *et al.* (2000) demonstrated that progeny of rats gavaged with 6 mg/kg/day Aroclor 1254 (A1254) from GD 6 to PND 21, exhibited hypothyroxinemia. On the day of birth, half of the treated litters and half of the control litters were cross-fostered, resulting in the following groups: Ctrl/Ctrl (controls); A1254/A1254 (perinatal exposure); A1254/Ctrl (prenatal exposure only); and Ctrl/A1254 (postnatal exposure only). Compared to the control, serum T_4 concentrations of offspring were sharply reduced at GD 21 in all A1254-exposed groups ($p < 0.05$). On PND 3, 7, 14, and 21, T_4 decrease was also significant in the A1254/A1254 and the Ctrl/A1254 groups ($p < 0.05$). Smaller but significant decreases in T_4 were observed in the A1254/Ctrl group on PND 3, 7, and 14. Thus, decrease in serum T_4 was mostly observed in the lactationally exposed group.

Viluksela *et al.* (1997) demonstrated that rats exposed orally to 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) showed a dose-dependent statistically significant decrease (78% at the highest dose, 6,000 and 10,000 µg/kg HpCDD for females and males respectively) in the serum T_4 concentrations. The animals were divided into 7 treatment groups (n = 20 per sex per group). HpCDD dosages (Group 1 = 0; Group 2 = 18.5 females (F), 30.9 for males (M); Group 3 = 222 (F), 370 (M); group 4 = 1,333 (F), 2,222 (M); Group 5 = 4,000 (F), 6,667 (M); Group 6 = 6,000 (F), 10,000 (M)) in µg/kg were divided into four daily loading doses and six biweekly maintenance doses for 13 weeks. In group

7, the rats were administered TCDD in one total dose of 41.9 and 70 µg/kg for female and male rats respectively. Half the animals were sacrificed after the 13 week dosing schedule and the other half allowed a 13-week off-dose schedule. Dose-dependent enzyme induction was noted in the liver by measuring EROD activity ($p < 0.05$ for all treatment groups relative to controls). Serum T_4 levels were decreased in a dose-dependent manner at the three highest HpCDD doses and by TCDD ($p < 0.01$ to 0.001). There was a maximal decrease of 78 % in the males and 44 % in the females at the end of the 13-week dosing period. This decrease in serum T_4 continued through the off-dose period and was maximally 65 and 60 % in males and females in the HpCDD treatment groups. Serum T_4 came back towards normal in the TCDD group, which is consistent with its shorter half-life in this species. Serum T_3 concentrations were only slightly affected, and not significantly in either males or females. In a similar study, Viluksela *et al.* (1998) described a subchronic experiment in rats given a mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentaCDD (PCDD), 1,2,3,4,7,8-hexaCDD (HxCDD), and 1,2,3,4,6,7,8-heptaCDD (HpCDD), and in rats given PCDD or HxCDD (cumulative dosage 10 – 100 µg/kg). The dosing period was 13 weeks, and half of the animals were then put on a 13-week off-dose period. They reported a dose-dependent statistically significant decrease of serum T_4 concentrations (maximally by 69 %), with some reversibility in males during the off-dose period. Serum T_3 levels were not significantly affected.

Treatment with 25 µmol (single oral dose) tetrachlorobiphenyl (TCB, PCB #77) significantly reduced plasma T_4 levels up to 7 days after administration in non-pregnant rats and up to 4 days after administration in pregnant rats (Morse *et al.*, 1995). By 7 days after administration, plasma T_4 levels had returned to control levels in the TCB-treated pregnant rats. However, fetal plasma T_4 levels were significantly decreased from TCB-treated dams 7 days after TCB administration. This decrease in fetal T_4 was attributed to the 4-hydroxylated metabolite of TCB. Hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity was significantly induced in TCB-treated dams relative to controls at 4 and 7 days after administration, while no EROD activity was detected in hepatic microsomes from control or TCB treated fetal rats at day 20 of gestation.

An age-related effect was reported for the toxicity of Aroclor 1254 on thyroid hormone levels (Provost *et al.*, 1999). Dams were exposed to 1.25 or 12.5 ppm Aroclor 1254 from conception through postnatal day 15 or 30, and their offspring were tested at 15 or 30 days of age. T_4 concentrations were slightly elevated in 15-day-old pups of 1.25 ppm Aroclor 1254 exposed dams and significantly depressed ($p < 0.05$) in 15- and 30-day-old pups of 12.5 ppm Aroclor exposed dams. T_3 concentrations were not altered in 15-day-old rats but were significantly depressed ($p < 0.05$) in 30-day-old pups of dams treated with 1.25 and 12.5 ppm Aroclor 1254.

        b)   *Non-coplanar PCB-induced effects*

        (1) Neurobehavioral effects

Exposure to the non-coplanar PCBs has been associated with neurodevelopmental toxicity in animals and humans. Roegge *et al.* (2000) exposed pregnant Long-Evans females to 0 or 6-mg/kg/day Arochlor 1254 (A1254) (p.o. in corn oil) from gestation day (GD) 6 to weaning at postnatal day (PND) 21. Results indicate that perinatal exposure to Aroclor 1254 (6 mg/kg) in Long Evans pregnant

rats may cause sex-specific deficits in spatial learning and memory in adult offspring (120 – 150 days of age). Compared to control males, the A1254-exposed males made significantly ($p < 0.01$) more working memory errors (2.15 +/- 0.13 and 3.20 +/- 0.18 errors (+/- SEM) for A1254 and control males, respectively) and reference memory errors (3.17 +/- 0.10 and 4.13+/-0.14 errors (+/- SEM) for control and A1254 males, respectively) on a 12-arm radial maze (RAM). A1254-exposed females were not impaired relative to control females on the RAM.

Pregnant rats were gavaged with 8 or 32 mg/kg/day 2,2',3,5',6-pentachlorobiphenyl (PCB #95) on gestation days 10-16 (Schantz *et al.*, 1997). Spatial learning and memory was assessed using an eight arm radial maze working memory task at 60 days of age and a T-maze delayed spatial alternation task at 140 days of age. Locomotor activity was evaluated at 35 and 100 days of age using an automated open-field. PCB #95-treated rats did not differ from controls on the T-maze delayed spatial alternation task. Offspring of rats dosed with PCB #95 showed normal levels of activity in the open-field test as juveniles, but were hypoactive as adults ($p < 0.05$). Interestingly, these rats also showed a faster acquisition of the working memory task on the radial arm maze, as measured by the number of errors made in subsequent sessions in the maze ($p < 0.05$). The authors attribute this effect to the affected rats using a different strategy to learn the maze, a "response patterning" which is seen in certain types of brain damage. It should be noted that in earlier experiments by these investigators (Schantz *et al.*, 1995), treatment of pregnant dams with other ortho-substituted PCBs (118, 153) produced offspring that were significantly hyperactive as adults and impaired in learning in the radial arm maze ($p < 0.05$)

When pregnant rats were gavaged with 6 mg/kg/day Aroclor 1254 from GD 6 to postnatal day 21 in a cross-fostering study, no difference between the Aroclor 1254-treated group and controls could be established when spatial learning was tested in the offspring at 3 months of age (Gilbert *et al.*, 2000). Provost *et al.* (1999) demonstrated dose- and age-dependent alterations in choline acetyltransferase (ChAT, an enzyme involved in the biosynthesis of acetylcholine) activity and in learning and memory in 15- and 30-day old offspring of dams exposed to 1.25 or 12.5 ppm Aroclor 1254 beginning at conception (Table 11)

Dietary exposure of dams to 1.25 ppm Aroclor 1254 during gestation and lactation significantly ($p < 0.05$) elevated ChAT activity in the hippocampus and basal forebrain of 15-day old offspring. Rats exposed to 12.5 ppm of Aroclor 1254 until 15 days of age demonstrated significant elevations of ChAT activity in the basal forebrain. At 30 days both 1.25 and 12.5 ppm Aroclor 1254 treatment groups displayed significantly depressed ChAT activity in both areas of the brain, indicating persistency of the PCB effect. Only the 12.5 ppm Aroclor 1254-treated group showed decrements in spatial learning, when rats were tested between 25 and 29 days of age.

Table 11. Effect of Arochlor 1254 on ChAT activity (nmol/mg protein/hr) in hippocampus and basal forebrain.

Arochlor 1254, ppm	Hippocampus		Basal Forebrain	
	15 Day	30 Day	15 Day	30 Day
0 (control)	57 ± 8 [a]	79 ± 5	146 ± 6	257 ± 10
1.25	130 ± 5 [*]	62 ± 2 [*]	307 ± 10 [*]	195 ± 12 [*]
12.5	63 ± 7	57 ± 3 [*]	162 ± 13	196 ± 11 [*]

- Significantly different from control ($p < 0.05$)

[a] Mean $\pm$ SEM of 64 rat pup brains

(Data extracted from figure 1; Provost *et al.*, 1999)

V.   Additional Information

A.   *Carcinogenic effects*

There are only a few cases where dioxin exposure of the general population has been documented; the incident in Seveso, Italy is one of them. In 1976, a chemical plant producing 2,4,5-trichlorophenol, experienced an explosion and fire releasing several chemicals including TCDD into the atmosphere in the vicinity of Seveso. The Seveso incident represents a unique event in the sense that exposure to dioxins was not limited to occupational exposure by workers but the whole population was affected by the TCDD release in the area surrounding the chemical plant. The population was exposed to different degrees depending on the distance and direction from the origin of the plume. Fifteen years after the industrial accident, Bertazzi *et al.* (1997) examined the cancer mortality among residents (20 to 74 years old) of Seveso by comparing populations living in dioxin contaminated areas (divided into three zones: highest, lower and lowest zone of exposure to dioxin, zone A, B, and R, respectively) with a population from neighboring noncontaminated areas (zone nonABR). No increase for all-cancer mortality, or major specific sites like respiratory cancer among males and breast cancer among females, was found. However, elevation in other specific cancer mortality was observed and could be associated with dioxin exposure. Table 12 summarizes cancer mortality for men and women living in zone B.

Table 12: Female and male deaths in zone B for selected causes, 1976-1991, ten years or more since first exposure (latency) and duration of exposure (length of stay in contaminated area)*

		Latency > 10 years		Length of stay > 10 years	
		Female	Male	Female	Male
All cancers	OBS	23	31	20	29
	RR	1.4	1.0	1.4	1.1
	(95% CI)	(0.9 –2.1)	(0.7 –1.4)	(0.8 –2.1)	(0.7 – 1.6)
Digestive cancer	OBS	10	12	9	12
	RR	1.5	1.0	1.6	1.2
	(95% CI)	(0.7 – 2.7)	(0.5 – 1.8)	(0.7 – 2.9)	(0.6 – 2.1)
Stomach cancer	OBS	5	X	4	
	RR	2.4	X	2.3	
	(95% CI)	(0.8 – 5.7)		(0.6 – 6.0)	
Lymphatic and hemopoietic	OBS	4	4	3	4
	RR	2.8	2.5	2.4	3.0
	(95% CI)	(0.7 – 7.1)	(0.7 –6.4)	(0.5 – 7.1)	(0.8 – 7.7)
Multiple myeloma	OBS	3		2	
	RR	15.9		11.0	
	(95% CI)	(3.2 – 46.5)		(1.2 – 39.6)	
Rectal cancer	OBS		4		4
	RR		6.2		7.2
	(95% CI)		(1.7 – 15.9)		(1.9 - 18.4)
Leukemia	OBS		2		2
	RR		3.4		3.9
	(95% CI)		(0.4 – 12.3)		(0.4 – 14.1)

* OBS = observed deaths.
(Source : Bertazzi *et al.*, 1997)

Increased mortality from stomach cancer (RR = 2.4; 95% CI = 0.8-5.7) was reported 10 years after the accident in women living in zone B although the RR did not reach statistical significance. In men, a statistically significant increase in mortality from rectal cancer was observed and was highest for latency greater than 10 years (RR = 6.2; 95% CI = 1.7-15.9), and length of stay greater than ten years (RR= 7.2; 95% CI = 1.9-18.4). Leukemia in men also appears elevated in Zone B (RR=3.1, 95%CI = 1.3-6.4) when evaluated as observed versus expected total cases over the years 1976-1991. The relative

risk for leukemia in men did not reach statistical significance when broken out by latency or length of stay in the contaminated area (RR = 3.4; 95% CI = 0.4 – 12.3), leading the authors to conclude that there was no clear time-related trend. Statistically significant elevated rates of multiple myeloma were observed in women in Zone B with the highest risks in those with > 10 years latency (RR = 15.9; 95% CI = 3.2 - 46.5). Hodgkin's disease in both genders (RR = 3.3; 95% CI = 0.4 - 11.9 in men; and RR = 6.5; 95% CI = 0.7 - 23.5 in women) appeared elevated although the elevation was not statistically significant.

In the young population (20,000 subjects aged 0 to 19 years old), some cases of cancer were also found (Pesatori *et al.*, 1993), including two ovarian cancers and Hodgkin's lymphoma; myeloid leukemia was elevated although not statistically significant (RR = 2.7; 95% CI = 0.7 - 11.4). Two cases of thyroid cancer were also reported (RR = 4.6; 95% CI = 0.6 - 32.7) in younger people.

None of the elevated cancer incidences in zone A, the area with the highest exposure, were statistically significant; however, this area also had the smallest population. Additionally, it should be noted that the Seveso population was exposed to 2–3 orders of magnitude the level of dioxin normally experienced by the general population of industrialized countries. In 1997, individuals living in the contaminated area at the time of the accident still experienced high level of plasma TCDD 20 years after the industrial accident in Seveso. Geometric means for plasma TCDD concentration for individuals who lived in zone A, B and nonABR (control zone) in 1976 were 53.2, 11.0 and 4.9 ppt, respectively. Women in these three groups represented the gender with the highest plasma TCDD contamination (Landi *et al.*, 1997). The authors concluded that the results indicate a positive association between dioxin exposure and certain cancers, but further study is needed to clarify this association. It should be noted that the length of follow-up of 15 years is still short. In addition, potential exposure miscalssification, and small sample size complicate the analysis.

Because dioxin is a potent potentiator but a weak initiator of cancer processes, exposure early in life theoretically should have less impact than when exposed later. However, Brown *et al.* (1998) suggested that prenatal exposure to dioxin and related compounds may increase sensitivity in adulthood to other chemical carcinogens. In an investigation of predisposition to mammary cancer, Brown *et al.* (1998) treated pregnant Sprague-Dawley rats on gestational day 15 with 1 µg/kg TCDD. Results indicate that prenatal TCDD exposure significantly increased terminal end buds and decreased lobules II in 50-day-old offspring. No alterations in mammary gland differentiation were observed in 21-day old offspring. Additionally, prenatal TCDD treatment was associated with an increased number of chemically induced (by DMBA) mammary adenocarcinomas in rats. These authors concluded that prenatal exposure to TCDD increased susceptibility to mammary cancer, which correlated with alteration of mammary gland differentiation based on the increased number of terminal end buds.

B. *Mechanism of toxicity*

Among the dioxin-like compounds that exert their toxic effects through the Aryl hydrocarbon (*Ah*) receptor are the coplanar polychlorinated biphenyls (PCB). These PCB congeners substituted in the para and at least 2 of the meta positions but not at any of the ortho positions are the most toxic PCBs. These congeners are structurally similar to TCDD. Introduction of one chlorine in the ortho position

results in a decrease in toxic potency, and PCBs with more than one chlorine in the ortho positions lack some effects exerted by non- and mono-ortho PCBs and show a partially different spectrum of toxic effects (Safe, 1994). PCB congeners that have little or no activity at the *Ah* receptor (non-dioxin-like PCBs) have been shown to accumulate in the brain following in vivo exposure and decrease dopamine content. These non-dioxin-like PCBs interfere with calcium homeostatic mechanisms and intracellular second messenger systems *in vitro* in neuronal cultures and brain subcellular fractions. Structure-activity relationship (SAR) studies based on measures of PCB-induced alterations in protein kinase C (PKC) translocation and Ca^{2+}-buffering, indicate that congeners with chlorine substitutions at the ortho-position are active *in vitro*, while non-ortho congeners are relatively inactive. Subsequent research has found that chlorine substitution patterns that favor non-co-planarity are associated with *in vitro* neurotoxicity (Tilson and Kodavanti, 1998). These results therefore seem to indicate a mechanism of toxicity for non-coplanar PCBs that is different than the interaction with the *Ah* receptor pathway.

C.   *Mechanistic evidence of age-related susceptibility in animals*

Dioxin and dioxin-like compounds act primarily through the aryl hydrocarbon (*Ah*) receptor. The presence of the *Ah* receptor in the rat varies with the stage of development. *Ah* receptor protein levels in developing rat ventral and dorsolateral prostate decrease with age, declining approximately 70 % between postnatal days (PND) 1 and 21. ARNT (*Ah* receptor nuclear translocator) protein levels also decrease with age in dorsolateral, but not ventral prostate (Sommer *et al.*, 1999). This decrease is associated with a decrease in *Ah* receptor and ARNT mRNA. TCDD treatment in adult male rats (0.2, 1, 5, or 25 µg/kg by gavage, 24 h) decreased *Ah* receptor but not ARNT protein in ventral and dorsolateral prostate, vas deferens, and epididymis (Sommer *et al.*, 1999). This study also reported that *in utero* and lactational TCDD exposure of offspring (1.0 µg/kg to dam by gavage, on gestation day (GD) 15) did not alter ARNT levels but reduced prostatic *Ah* receptor protein levels on PND 7 and delayed the developmental decrease in *Ah* receptor protein in ventral and dorsolateral prostate. Also, pretreatment of rat pups for 24 hours with TCDD (5 µg/kg ip) down-regulated prostatic *Ah* receptor protein on PND 7, but not on PND 1. Thus, the authors concluded that prostatic *Ah* receptor and ARNT protein and mRNA levels are regulated with age, whereas only *Ah* receptor protein concentration is altered by TCDD exposure.

Age-related induction of cytochrome P4501A1, as indicated by the activity of hepatic microsomal ethoxyresorufin-O-deethylase (EROD), was reported by Morse *et al.* (1995). Hepatic EROD activity in PCB #77-treated dams was significantly induced relative to controls at 4 and 7 days after administration. No EROD activity was detected at GD 20 in hepatic microsomes in fetal rats from control or PCB #77-treated dams. Provost *et al.* (1999) demonstrated the influence of age on thyroid hormone levels in the offspring of dams treated with Aroclor 1254. T_3 concentrations were not altered in 15-day-old offspring, but were significantly depressed in 30-day-old offspring of 1.25 ppm and 12.5 ppm-treated dams.

Another indication of age-related difference comes from the *in vitro* testing of Ca^{2+}-uptake by subcellular brain preparations from Long-Evans rats. Aroclor 1254 inhibited Ca^{2+}-uptake by brain microsomes, and the inhibition increased with age (PND 7 < PND 21 < or = adults; IC50s = 21-34, 8-

20 and 10-14 µM, respectively) (Sharma *et al.*, 2000). In general, microsomal and mitochondrial Ca^{2+}-uptake in selected brain regions increased with age (PND 7 < PND 21 < or = adults).

D.   Regulatory Background

a)   Chronic RELs

For TCDD (OEHHA, 2000)

- The inhalation reference exposure level is 0.00004 $\mu g/m^3$ (40 pg/m^3).

- The oral reference exposure level is 10 pg/kg/day.

The critical effects for these RELs, which are based on studies by Kociba *et al.* (1978) are: mortality, decreased weight gain, depression of erythroid parameters, increased excretion of porphyrins and d-aminolevulinic acid, increase serum activities of alkaline phosphatase, gamma glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues in rats (OEHHA, 2000).

b)   Cancer Risk

(1)   Cancer Risk for Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans

See Table 13

(2)   Cancer risk for PCBs

Unit risk factor: 5.7 E-4 $(\mu g/m^3)^{-1}$ (for use in cases where congeners with more than four chlorines do not comprise less than one-half percent of total PCBs.)

Unit risk factor: 2.0 E-5 $(\mu g/m^3)^{-1}$ (for use in cases where congeners with more than four chlorines comprise more than one-half percent of total PCBs.)

The Scientific Advisory Board (SAB) for the U.S. Environmental Protection Agency (US EPA) peer reviewed and approved the report *Dioxins reassessment: Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* in May 2001. This report identifies dioxin as a cause of cancer in laboratory animals and possibly in humans.

Table 13: Health Assessment Values for Dioxins and Dibenzofurans (OEHHA, 1999)

Congener	Unit Risk $(\mu g/m^3)^{-1}$	Oral Slope Factor $(mg/kg/day)^{-1}$
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	3.8×10^1	1.3×10^5
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	1.9×10^1	6.5×10^4
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	3.8	1.3×10^4
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	3.8	1.3×10^4
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	3.8	1.3×10^4
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	3.8×10^{-1}	1.3×10^3
1,2,3,4,5,6,7,8-Octachlorodibenzo-*p*-dioxin	3.8×10^{-2}	1.3×10^2
2,3,7,8-Tetrachlorodibenzofuran	3.8	1.3×10^4
1,2,3,7,8-Pentachlorodibenzofuran	1.9	6.5×10^3
2,3,4,7,8-Pentachlorodibenzofuran	1.9×10^1	6.5×10^4
1,2,3,4,7,8-Hexachlorodibenzofuran	3.8	1.3×10^4
1,2,3,6,7,8-Hexachlorodibenzofuran	3.8	1.3×10^4
1,2,3,7,8,9-Hexachlorodibenzofuran	3.8	1.3×10^4
2,3,4,6,7,8-Hexachlorodibenzofuran	3.8	1.3×10^4
1,2,3,4,6,7,8-Heptachlorodibenzofuran	3.8×10^{-1}	1.3×10^3
1,2,3,4,7,8,9-Heptachlorodibenzofuran	3.8×10^{-1}	1.3×10^3
1,2,3,4,5,6,7,8-Octachlorodibenzofuran	3.8×10^{-2}	1.3×10^2

[Linearized multistage procedure (GLOBAL79), fitted to male mouse hepatic adenoma and carcinoma data (NTP, 1982), body weight scaling, cross-route extrapolation (CDHS, 1986).]

VI.   Conclusions

There are numerous reports indicating that *in utero* and postnatal exposure to PCDDs, PCDFs and PCBs can result in significant toxicity in young animals and infants and children. OEHHA has therefore placed chlorinated dioxins and dibenzofurans in Tier 1. Because airborne exposures to PCBs are extremely low, OEHHA has placed the PCBs in Tier 2. The deleterious outcomes of exposure to dioxins and PCBs even at low exposure levels can persist long after birth. Immunological and neurobehavioral adverse effects in children perinatally exposed to dioxins and dioxin-like compounds and non-coplanar PCBs, respectively, have been reported to persist up to school age. Hormonal changes demonstrated in animals exposed to PCDD and PCDF, particularly on thyroid hormones, may be related to birth weight decrease, alterations in brain development, and delayed sexual maturation. In addition, there is some evidence from animal studies that the presence of the *Ah* receptor (through which dioxin toxicity is mediated) and cytochrome P450 CYP1A1 (which is greatly induced by dioxins) may vary during development. Thus, differential susceptibility to the dioxins and dioxin-like chemicals throughout development seems plausible. Interaction with *Ah* and steroid receptors are possible mechanisms for the observed effects.

Current background levels of human exposure to dioxins in particular are within the range at which various toxic responses have been observed in animals. Exposure *in utero* is the direct consequence of the accumulated maternal body burdens, and food chain contamination, including contamination of breast milk, are sources of continuing exposure. Regulatory efforts should focus on the identification and control of environmental airborne sources, which are currently the major origin of food chain contamination.

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