Biomarkers for Great Lakes Priority Contaminants: Halogenated Aromatic Hydrocarbons

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One of the major goals of the Great Lakes Action Plan is to actively accumulate and assess toxicological information on persistent toxic substances found in the Great Lakes basin. As part of Health Canada’s commitment to this plan, a review of biomarkers for the environmental contaminants polychlorinated biphenyls (PCBs) and polychlorinated dibenzodioxins/dibenzo furans (PCDDs/PCDFs) was conducted. In general, while food consumption was identified as the major source of human exposure to both contaminant groups, certain commodities, such as fish, milk and dairy products, and meat, were found to predominate. Due to the ubiquitous nature of these environmental contaminants and their propensity to bioaccumulate, all humans will have detectable body burdens, which in certain cases can be positively associated with the consumption of particular foods (i.e., PCBs and freshwater fish from the Great Lakes). When dealing with environmental exposure only, relating specific effect biomarkers to contaminant exposure or tissue levels was difficult, due in part to the complex nature of the exposure and the nonspecific nature of the effect. For PCBs, the most likely biomarkers of effect included some form of alteration in lipid metabolism (serum triglyceride/cholesterol levels) and elevation of hepatic-related enzymes, aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT). Cross-species extrapolation also indicates the potential for neurotoxicologic effects to occur in humans. For PCDDs/PCDFs, dermatologic lesions (chloracne) and indications of hepatic enzyme induction have been documented, but primarily due to occupational or high acute accidental exposures. Recent evidence suggests that neonates may represent a potential at-risk population due to relatively high exposure to PCDDs/PCDFs, as with PCBs, during breast feeding as compared to standard adult dietary intake. Future areas of potential benefit for biomarker development include immunologic and endocrine effects, primarily based on biologic plausibility from experimental animal research. — Environ Health Perspect 103(Suppl 9):7–16 (1995)

Key words: biomarkers, environmental contaminants, humans, polychlorinated biphenyls, polychlorinated dibenzodioxins/dibenzo furans, exposure, effect, susceptibility

Introduction

The Great Lakes Water Quality Board of the International Joint Commission (IJC) has developed a list of 11 critical pollutants from the nearly 400 chemical contaminants that have been identified in the Great Lakes basin. In a 1991 report on toxic substances in the Great Lakes ecosystem, the IJC stated that due to the abundance of discernable adverse health effects documented in 14 Great Lakes wildlife species near the top of the food chain, “the Commission must conclude that there is a threat to the health of our children emanating from our exposure to persistent toxic substances” (1).

As part of Health Canada’s commitment to the Great Lakes Action Plan, a biomarker-related review was conducted for three major classes of environmental contaminants—organochlorine pesticides, toxic metals, and halogenated aromatic hydrocarbons—which incorporate the 11 critical pollutants. This report summarizes the findings on polychlorinated biphenyls (PCBs) and polychlorinated dibenzodioxins and dibenzo furans (PCDDs/PCDFs).

For the purpose of this review, biomarkers can be operationally defined as any biologic indication that exposure to a contaminant(s) has taken place. Three subclasses of biomarkers have been suggested (2): a) biomarkers of exposure—measurement of chemicals (including metabolites) in body fluids, tissues, or cells or the interaction products between the chemicals and an endogenous substance (Figure 1); b) biomarkers of effect—morphologic, physiologic, and biochemical changes that have occurred as the result of exposure to xenobiotics (depending on the dose—response relationship, a bioeffective or critical dose can be reached at a target organ/tissue, resulting in an alteration of homeostasis [compensatory responses]; when compensatory responses become overwhelmed, adverse health effects may occur); and c) biomarkers of susceptibility—any factor, usually intrinsic or genetic, that may result in an increased response to exposure. An example of a biomarker of susceptibility in humans is the genetic trait of glutathione-S-transferase (GST) deficiency. Approximately 50% of Caucasians have reduced levels of GST, an enzyme system that assists in the detoxification of reactive compounds, and

This paper was prepared for the Great Lakes Health Effects Program which is part of a Canadian Department of Health initiative established in 1989. Manuscript received 7 March 1995; manuscript accepted 1 September 1995.

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Abbreviations used: PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzodioxins; PCDFs, polychlorinated dibenzofurans; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; UC, International Joint Commission; GST, glutathione-S-transferase; HAHs, halogenated aromatic hydrocarbons; WHO, World Health Organization; GI, gastrointestinal; ppb, parts per billion; CDC, Centers for Disease Control and Prevention; BCF, bioconcentration factor; PCBs, polychlorinated biphenyls; t1/2, half-life; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; SCE, sister chromatid exchanges; LNR, laboratory normal range; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TEFs, toxic equivalency factors; TEQ, toxic equivalency; BMI, body mass index; NIOSH, National Institute for Occupational Safety and Health; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; ALT, alanine amino transferase; T4/T3, T-helper/T-suppressor; Thy-1, thymosin alpha-1; CD, clusters of differentiation; LH, luteinizing hormone; FSH, follicle-stimulating hormone; Ah, ah hydrocarbon; Ahh, ah hydrocarbon hydroxylase; EROD, ethoxyresorufin-O-deethylase; TBG, thyroid binding globulin; TSH, thyroid stimulating hormone; OCDD, octachlorodibenzo-p-dioxin.

Figure 1. Biomarkers of exposure. External exposure to contaminants results in a measurable internal dose (parent xenobiotic, metabolite, etc.).
chlorine content (21–68%) with each batch containing a unique combination of congeners (isomers) distinguished by the chlorine substitution pattern on two biphenyl rings. Whereas theoretically 209 separate congeners are possible in the commercial mixtures, only approximately 130 are likely to occur (7). It should also be noted that, due to selected biodegradation processes (e.g., reductive dechlorination, photochemical hydrolysis), environmental PCB mixtures differ in their congener profile from the commercial PCB mixtures. The chemical stability of PCBs combined with their extreme lipophilicity has led to environmental persistence and bioaccumulation in almost all ecosystem trophic levels.

The majority of humans are exposed to PCBs through consumption of contaminated foods, particularly meat, fish, and dairy products. In a survey of fatty foods from a Canadian dietary study (market basket), fish, particularly freshwater fish, contained the highest specific PCB congener levels (8). Market basket surveys in Ontario have estimated that of a total human yearly PCB intake of approximately 300 μg, fresh food accounted for 98.6%, and fish accounted for 70%. This observation was confirmed in a recent Finnish dietary review in which up to 72% of the theoretical maximum intake of an average consumer was attributable to fish (9). The total yearly intake of 300 μg/person is comparable to U.S. total diet studies that estimate the average daily PCB intake as 8 ng/kg body weight (bw) (9). A World Health Organization review, however, indicated that based on a 70-year lifespan, the average person would ingest a maximum of 0.2 μg/kg bw/day (10).

PCBs are effectively absorbed from the gastrointestinal (GI) tract and respiratory system of mammals and are then rapidly distributed in a biphasic manner from the blood and lymph. It has been estimated that the GI tract absorption of the majority of PCB congeners is greater than 90% (10). In an experiment involving consumption of PCB-contaminated fish by human volunteers, serum PCB levels were shown to rise within 4 hr after fish ingestion, peak by 10 hr, and return to baseline values by 7 days (11). The magnitude of the serum PCB increase was directly related to the extent of the fish contamination. Similar investigations have shown an increase in blood PCB levels following consumption of PCB-contaminated food can be detected as early as 2 hr postingestion (12). Following clearance from the blood, PCBs are primarily distributed to the liver and lipid fractions of various organs, with liver being the main site of metabolism. The main excretory routes for PCBs in humans are bile and breast milk, with additional routes including feces, hair, and urine (minor).

### Biomarkers of Exposure

Due to the ubiquitous nature of PCBs in the environment, the majority of humans will have detectable levels of PCBs in blood, adipose tissue, and breast milk. Based on PCB concentrations found in domestically produced foods, it is estimated that the general population in North America will have PCB whole blood values in the range of 0 to 10 μg/kg (ppb) (13). In a series of samples collected in 1986 to 1987 from Ontario urban centers, 79% of 750 samples fell within this range (mean whole blood PCB of 9.2 ppb), indicating that the remaining 21% of samples (10–100 ppb) potentially had exposure from other sources (freshwater fish consumption, occupational) (14). The U.S. Centers for Disease Control and Prevention (CDC) has estimated the mean blood PCB level in the United States as 5 to 7 ppb. In a preemployment screen of a selected nonoccupationally exposed cohort of U.S. adults (n=738), the mean PCB level in blood plasma was 5 ppb (15). PCB serum levels in humans have also been shown to increase with age, possibly in relation to age-related increases in serum cholesterol and triglyceride levels, and are in general lower in females (16). Fish consumption in North America has been positively correlated with blood PCB levels (13,17). In certain species of fish, the bioconcentration factor (BCF) for PCBs has been estimated at up to 100,000 (PCB in fish vs PCB in water). Historically for the Great Lakes, Lake Michigan had been associated with some of the highest PCB–fish levels. Analysis by the Michigan State Department of Public Health has shown that serum PCB levels in a Michigan anglers cohort were positively correlated to history, quantity, and type of Lake Michigan fish consumed. People who consumed up to and greater than 10.91 kg of fish per year had upper serum PCB ranges almost 24-fold higher than reported fish nonconsumers (25–366 ppb vs less than 5–41 ppb, respectively) (11). In contrast, a sample of 1631 Michigan residents associated with farming had mean (geometric) serum PCB levels of 6.3 ppb (18). There have been two major epidemiologic episodes in which humans have been exposed to PCBs through
consumption of food other than fish. Over 1200 people in Japan (Yusho, 1968) and 2000 in Taiwan (Yu-Cheng, 1979) consumed cooking oil contaminated with degraded PCBs, with an average estimated PCB intake between 0.633 and 1.84 g (157–200 μg/kg bw/day) (19). Clinical symptoms of intoxication, largely attributable to PCDFs, polychlorinated quarternaryphenols (PCQs), and chlorinated diphenyl ethers, occurred by 2 to 4 months. Blood PCB values for the Yusho subjects were only twice that of background (6.7 ppb) while Yu-Cheng individuals had appreciably higher levels (89.1 ppb). This is in comparison to occupational cohorts involved with capacitor plants and transformer repairs for which mean serum PCB values of greater than 300 ppb have been recorded (20).

Due to their lipophilicity, PCBs will accumulate in organs and tissues on the basis of lipid content. Half-lives for PCBs in humans have been estimated at up to 4.8 years and are dependent on both the degree of congener chlorination and the initial serum concentration (21). Concentrations of PCBs in breast milk and adipose tissue in humans are generally 100- to 200-fold higher than PCBs in serum (10). Based on results from the National Human Adipose Tissue Survey in the United States, it has been estimated that as of 1983 almost 100% of the U.S. population has detectable adipose PCB levels (up to 1 ppm) and less than 10% have adipose PCB levels greater than 1 ppm (22). Analysis of 108 human autopsy adipose samples from across Canada supported the generalized observations regarding PCB exposure biomarkers: 100% of the samples had detectable PCB residues with a mean level (geometric) of 0.410 ppm (wt weight), mean residue levels in females were approximately 20% lower than in males, and PCB levels increased with age (the over-51-year-old group had adipose PCB levels approximately 65% higher than the 0 to 21-year-old group [0.306 ppm vs 0.504 ppm, respectively] (23). The average concentration of PCBs in human breast milk samples from industrialized countries has been shown to be in the range of 0.5 to 1.5 mg/kg milk fat or approximately 15 to 45 μg/kg of whole milk (24). Surveys of Canadian breast milk samples for PCBs have shown values ranging up to 1.31 mg/kg of milk fat (approximately 40 μg/kg whole milk) with a median value of 0.168 mg/kg (25). In breast milk samples collected from Ontario residents (n=348) between 1975 and 1985, almost 90% of the PCB values occurred in the range of 5.1 to 50 μg/kg of whole milk (26).

Increased consumption of food with high levels of PCBs has been shown to lead to increased body burdens. In a survey of Inuit women whose diet includes primarily native foods (marine mammals, fresh and salt water fish), the mean whole milk PCB level was almost 4-fold higher than in Caucasian controls (111.3 μg/kg vs 28.4 μg/kg, respectively) (27).

**Biomarkers of Effect**

Based on results in experimental animals, the liver is thought to be the primary organ associated with PCB effects. Observations have included dose-dependent hepatic enzyme induction, hypertrophy due to smooth endoplasmic reticulum proliferation, steatosis, and necrosis. Indications of nonspecific hepatic enzyme induction have been seen in workers involved with capacitor manufacturing. In workers with at least 4 years exposure to Aroclors (no biologic monitoring), antipyrine half-lives (t1/2) were reduced by approximately 30% compared to nonexposed control workers (t1/2 10.8 vs. 15.6 hr, respectively) (28). This confirmed previous observations of increased antipyrine metabolism in workers involved with pesticide manufacturing (e.g., DDT, lindane). Antipyrine is an analgesic agent that in humans is completely metabolized by hepatic microsomal enzymes. In a human population exposed to PCBs through fish consumption (serum PCB 22.2 μg/kg), plasma triglyceride and cholesterol levels were positively correlated to serum PCB values (13). In a cross-sectional study of 120 male workers occupationally exposed to PCBs through transformer fluids, plasma PCB values, serum aspartate aminotransferase (AST) activity, and triglyceride levels were significantly correlated (29). The mean plasma PCB value for the most highly exposed group was 33.4 μg/kg. It was speculated that, although there was no evidence of overt hepatotoxicity, an interference in lipid metabolism at the hepatic microsomal level may have been occurring. The incidence of abnormal serum triglyceride values (greater than 140 mg/dl) was also increased in Japanese capacitor factory workers with blood PCB levels 50 μg/kg and greater (30). Similar indications of physiologic effects of PCBs on the liver were seen in a study of workers employed in power capacitor plants. Serum PCB measurements were positively correlated with serum AST, γ-glutamyltransferase (GGT), and plasma triglyceride and high density lipoprotein cholesterol levels (31). PCB concentrations in worker’s serum ranged from approximately 100 to 500 ng/ml (ppb) (up to 50-fold higher than the community background level). In a group of 38 transformer repairmen with slightly elevated serum PCBs (12.2 ppb, current exposure), serum GGT activity and PCB levels were significantly correlated in comparison to the control group (4.6 ppb) (32). Based on the various epidemiologic studies, it has been suggested that alterations (outside laboratory normal range [LNR] values) of serum AST or GGT “appear to be the most sensitive indicators of PCB exposure in humans” (10,33).

In a modification of the antipyrine assay, caffeine metabolism rates (indirective of hepatic CYP1A2 activity) were assessed by the caffeine breath test in a small group of Michigan fisheaters (mean serum PCB of 24 vs 5.9 μg/kg in controls) (34). The fisheaters metabolized approximately 70% more of the administered caffeine dose compared to the control group (4.6%/hr vs 2.7%/hr, respectively).

Indications of the inductive properties of PCBs on hepatic enzymes were also seen in factory workers (n=67) involved with the manufacturing of transformers and capacitors. With an average exposure interval of 12 years, PCB blood levels ranged from 162 to 1319 ng/ml (mean 386 ng/ml) compared to up to 20 ng/ml in the controls (n=67). Quantitative increases were seen in the urinary excretion of porphyrin congeners, indicating enzymatic stimulation and/or inhibition of the hepatic heme biosynthetic pathway (35). The average total urine porphyrin concentration for the workers was 94.5 μg/l versus 48.3 μg/l in the controls. In children transplacentally exposed to PCBs during Yu-Cheng, total urine porphyrin content was increased versus controls (95.2 μg/l vs 80.7 μg/l, respectively) (36). No specific heme precursors (porphyrin congeners) were measured.

A variety of adverse reproductive effects have been attributed to PCB exposure. Alterations in birth weight, gestational age, and fetal development (physical and neurologic) have been assessed in infants born to women who were either occupationally or environmentally exposed to PCBs. In a cohort composed of women who had consumed Lake Michigan fish before and during pregnancy, mean infant birth weights and gestational ages were reduced (153–190 g and 4.9–8.8 days premature,
respectively) and correlated to cord blood PCB levels (up to 3.0 ng/ml [ppb]) (10,37).

The median maternal serum PCB level has been estimated at up to 10.3 ppb. Missed abortions and premature delivery have also been associated with elevated serum concentrations of PCBs and organochlorine pesticides. Mean serum PCB levels were approximately 4- to 5-fold higher in the women (n=17) with reproductive pathology versus the control group (103–128 vs 19.25–20.69 ng/ml, respectively) (38,39). Appreciable levels of total DDT in serum were also found in the affected women (119.6 vs 26.5 ng/ml). In contrast to the Michigan findings, in a cohort of 912 North Carolina infants exposed transplacentally to DDT and PCBs (maternal serum PCB levels of 7.0–9.1 ppb), no relationship was observed between either contaminants and infant size or age (40). In addition, it has been suggested that these women who had consumed large quantities of Great Lakes sport fish may be a "culturally distinct subset of the population" and this may be associated with the observed physical and neurological developmental deficits (41).

In the Yusho and Yu-Cheng poisoning episodes, the earliest toxicologic symptoms noted were primarily dermatologic effects (hyperpigmentation, hyperkeratosis, conjunctivitis, chloracne). Additional generalized adverse health effects included hepatomegaly, bronchitis, and peripheral neuropathy (20). In the Yu-Cheng cohort, increased upper respiratory tract infection rates were associated with decreased serum IgA and IgM plus increased IgG levels (42). Recent reassessment of 118 children born during and up to 6 years after Yu-Cheng has suggested continued evidence of delayed cognitive development (43). However, analysis of both contaminated rice oil mixtures from Yusho and Yu-Cheng has determined that the primary etiologic agents responsible were not PCBs but probably PCDDs and other contaminants (44,45). Duration of occupational exposure to PCBs has been correlated with increasing incidences of chromosomal aberrations and sister chromatid exchanges (SCE) detected in peripheral blood lymphocytes. Workers who had been employed for 16 to 25 years (blood PCB concentrations = 420 ± 220 μg/l) had a 5.85% frequency of aberrant cells and 12.6 SCE per cell as compared to 1.60% and 6.9 SCE per cell in a control population (blood PCB concentrations = 3 ± 1.6 μg/l) (46).

Biomarkers of Susceptibility

From epidemiologic investigations, it appears that the developing fetus is at greatest risk from PCB exposure. In the Michigan fish eaters cohort, physiologic and neurobehavioral deficits were noted in the infants born to mothers who had consumed large quantities of fish (maternal PCB serum levels of 25–366 ppb). In addition, serum PCB levels obtained from the same infants when they were 4 years of age were associated with decreases in the composite activity ratings (infant serum PCB levels of 9–23.3 ng/ml were associated with the largest decrease in activity) (37). In a cohort of 930 Wisconsin infants, PCB breast milk levels of 3.5 mg/kg of milk fat or greater (approximately 100 μg/kg of whole milk) were associated with increased incidences of hypotonicity and hyporeflexia (40); however, it was theorized by the authors that the developmental or other toxic effects noted were not attributable to breast feeding but to in utero exposure. Unlike the oral rice oil episodes, the developmental delays observed in these infants at up to 2 years of age were no longer evident at ages 5.5 to 10.5 years (47).

Analysis of workers potentially exposed to PCBs through transformer cleanup revealed that 8 of 16 had expression of serum oncogene proteins (48). All liver function tests were within the LNR while 3 workers with high serum triglycerides (>140 mg/dl) were included in the oncogene protein positive group. Only one worker had a serum PCB value greater than 10 μg/l.

Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-dibenzofurans are two subsets of the halogenated aromatic hydrocarbon (HAH) family of ubiquitous anthropogenic environmental contaminants. Both groups are formed as impurities during the synthesis of various chlorinated compounds such as chlorophenoxy herbicides (e.g., Agent Orange, 2,4-dichlorophenoxyacetic acid), PCBs, pentachlorophenol, and hexachlorobenzene. Significant amounts also continue to be released into the environment through pulp and paper mill activities and solid waste incineration (e.g., municipal, hospital, hazardous) (49). It is estimated that combustion of any organic or chlorinated material can result in the formation of PCDDs and PCDFs (50). Analysis of archived soil, vegetation, and human tissue samples has revealed that the majority of PCDDs and PCDFs have entered the environment during the 20th century (51,52), although detectable quantities can still be found in biotic material from the mid-1800s. As with PCBs, PCDDs and PCDFs are classified according to their chlorine substitution pattern, with a total of 75 possible PCDD congeners and 135 PCDF congeners. The 2,3,7,8 chlorine substituted PCDD/PCDF congeners (n=17) are considered to be the most toxicologically significant, with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) being of greatest concern. For risk assessment purposes, all of the 2,3,7,8-substituted PCDD/PCDF congeners are related to TCDD through the use of toxic equivalence factors (TEFs), numerical potency ratings developed by international consensus (53). The concentration of each PCDD/PCDF congener is multiplied by its respective TEF and then summed, giving a TCDD toxic equivalency (TEQ) value. This methodology has proven particularly useful for the determination of human body burdens because only the 2,3,7,8-substituted congeners are routinely detected with a high frequency in human samples (50,54).

The chemical stability and lipophilicity of PCDDs/PCDFs equal or exceed that of PCBs and, as with the majority of HAHs, it can be expected that these compounds will undergo extensive biomagnification. Data from Lake Ontario indicate that the BCF for TCDD in some species of fish may exceed 140,000 (55). The major route of human exposure to PCDDs and PCDFs is through consumption of contaminated foods, particularly fatty foods such as meat, dairy products, and fish. Analysis of food groups in the Netherlands has shown that all dairy products account for approximately 33% of the daily intake, fish 25%, and other meats 17% (56). Exposure assessment for Canadian populations also has identified dairy products (39.7%), meats (26.2%), and fish (12.2%) as the principal food groups involved in human exposure to PCDDs/PCDFs (57). PCDDs and PCDFs have been detected in bleached paper products (e.g., newsprint, filters, milk cartons), suggesting migration from packaging materials could also be considered a significant source of exposure (58). The possibility of occupational exposure still exists through the accidental incineration of PCBs in transformers and capacitors. Total PCDD/PCDF concentrations in soot samples resulting from a 1981 transformer fire in Binghamton, New York, were up to 4700 ppm, with possible adverse human health effects attributed to
Although TCDD human exposure to PCDDs and PCDFs, it has recently been estimated that up to 99.9% of the total environmental burden exists in soils and sediments (64).

Based on human experimental data, the majority of PCDDs and PCDFs ingested in food are expected to be efficiently absorbed from the gastrointestinal tract. When a human volunteer self-ingested 1.14 ng of TCDD per of body weight, fecal excretion analysis revealed that approximately 87% of the dose was absorbed (65). Following absorption, PCDDs/PCDFs are sequestered to various body tissues and organs based on lipid content. On a whole weight basis, abdominal and subcutaneous fat contain the greatest concentrations followed by liver, muscle, and kidney. On a lipid basis, all organs contained approximately the same level of PCDDs/PCDFs (49). Available information from most mammalian species suggests that PCDDs/PCDFs are metabolized very slowly; the major excretory routes are feces, breast milk, and urine. PCDDs/PCDFs have been detected in liver samples from stillborn human infants, which indicates that placental transfer from mother to fetus does occur (66). The current TCDD half-life estimation for humans occupationally exposed (Vietnam veterans) is up to 10 years, with shorter half-life estimations for certain PCDF congeners (67).

**Biomarkers of Exposure**

Extensive analysis of adipose tissue samples from a number of countries has concluded that almost all humans contain TCDD at concentrations up to and greater than 3 ng/kg of lipid (ppt) (63). TCDD levels in U.S. adipose samples ranged from 1.4 to 20.2 ppt (mean = 6.4 ppt) with 95% of the levels 16.6 ppt or less. On a lipid basis, total PCDD/PCDF concentrations are comparable for adipose tissue, blood, and breast milk with generally only the 2,3,7,8-substituted congeners being routinely detected. Analysis of plasma and adipose samples from the same patients has shown that, on a lipid basis, the concentrations of the lower chlorinated PCDD/PCDF congeners are similar, but the higher chlorinated congeners are found at elevated levels in the plasma samples. Based on whole blood, PCDD/PCDF values on a lipid basis are found at relatively equal concentrations to adipose tissue (52). PCDDs are generally found at higher concentrations in human samples than PCDFs, with individual congeners increasing by approximately two orders of magnitude on increasing chlorination (tetra to octa) (65). The most highly chlorinated PCDD congener, octachlorodibenzo-p-dioxin (OCDD), has been detected in Canadian adipose samples at over 900 ppt (lipid based) while TCDD is routinely detected in nonoccupationally exposed individuals at 6.4 to 7.1 ppt. Increased consumption of contaminated food has been positively correlated with increased concentration of PCDDs/PCDFs in humans. There was a consistent and statistically significant association found between reported human fish consumption and plasma levels of various PCDD/PCDF congeners (68). Median levels of TCDD in plasma samples from high (1214 g/week), moderate (349 g/week), and nonfish consumers were 8.0, 2.6, and 1.8 pg/g (ppt) of plasma lipid, respectively. In general, the trends for human breast milk content of PCDDs/PCDFs from industrialized countries is similar to adipose samples; i.e., only 2,3,7,8-chlorinated congeners are routinely detected. PCDDs occur at greater levels than PCDFs, the average concentration of the most toxic congener, TCDD, is 1 to 10 ng/kg of milk lipid; and the most common congener is OCDD which occurs at levels of 100 to 800 ng/kg of milk lipid (42). Recent analysis of 100 Canadian human milk samples supported these observations; the concentration of TCDD was 2.2 ng/kg of milk lipid and OCDD was the most abundant congener detected (173 ng/kg of milk lipid) (69). The primary factors affecting PCDD/PCDF concentrations measured in nonoccupationally exposed humans are age, diet, and body mass index (BMI). In U.S. adipose tissue samples collected between 1971 and 1987, the concentration of TCDD increased with respect to age; the 15- to 44-year-old age category, TCDD adipose levels ranged from approximately 3 to 6 pg/g of lipid while the over-45 age category ranged from 6 to 15 pg/g of lipid (70). Humans tend to bioaccumulate increasing levels of PCDDs/PCDFs with respect to age, which presumably is related to intake of animal protein, fats, and fish. A direct relationship has been established between the amount of animal fat consumed on a daily basis and the PCDD/PCDF content of human breast milk. Upper limits of TCDD TEQ content of breast milk samples (40 ng TEQ/kg of milk lipid) were positively associated with daily intake of animal fats and protein intake of up to 100 g (71). In addition, as the total PCDD/PCDF intake is distributed on the basis of body fat content, measured congener concentrations (based on total TEQ) have been shown to be negatively associated with BMI (72).

Various cohorts have been exposed to high levels of PCDDs/PCDFs as the result of the manufacture and application of chlorophenoxy herbicides and industrial accidents. In the large (n = 5172) U.S. National Institute for Occupational Safety and Health (NIOSH) study (73), which identified U.S. workers potentially exposed to TCDD-contaminated products, the mean serum TCDD level for all workers measured (n = 253) was 233 pg/g of lipid; the mean level for all workers with 1 year or more of potential exposure was 418 pg/g of lipid. In comparison, a reference population of unexposed workers had a mean serum TCDD level of 7 pg/g of lipid. When the latency period since the last known exposure was taken into account (15–37 years), the worker serum TCDD levels, at the time of last exposure, were estimated to be almost 3400 pg/g of lipid in the highest exposed group or 500-fold higher than background populations (73). In the U.S. Air Force Ranch Hand Study, military personnel exposed to TCDD through pesticide application had blood lipid TCDD levels approximately 3-fold higher than the comparison population (12.8 ppt vs 4.2 ppt; range to 618 ppt vs 54 ppt) (74). Current blood TCDD levels greater than 33.3 ppt were classified as belonging to the high exposure category. A cohort from Seveso, Italy, exposed to TCDD as the result of an industrial accident, has the highest known human serum TCDD values (75). Individuals from the geographical zone of highest contamination (soil TCDD levels up to 54 ppb) had an average serum TCDD value of 12,558 pg/g of lipid (range 828–56,000 pg/g).

**Biomarkers of Effect**

In a recent summary of possible adverse human health effects associated with PCDD/PCDF exposure, it was concluded, based on a weight-of-evidence classification scheme, that there was limited to sufficient evidence to attribute variations in serum lipid levels, chloracne and related dermatologic effects, microsomal enzyme induction, and gastrointestinal alterations as biologic effects (76).
Chloracne and related dermatologic effects have been described as one of the more consistent and reproducible consequences of PCDD/PCDF exposure in humans (49,77–80). Over 465 cases of chloracne have been documented worldwide in workers involved in industrial accidents during trichlorophenol production. In the Oriental rice oil poisonings, one of the earliest toxicologic symptoms was mild chloracne associated with an average TCDD TEQ intake of 1.5 to 3.0 ng/kg/day for 2 to 4 months (49). Total estimated TCDD TEQ body burdens were 150 μg or approximately 200-fold greater than the average current level of TCDD TEQs in North American adults (81). In comparison, the estimated chloracne-inducing dose in a Spanish family who ingested PCDD/PCDF-contaminated olive oil for approximately 6 to 7 months was 32 to 76 ng/kg/day (82). The discrepancy between the two estimated chloracne-inducing doses may be the result of congener patterns; in the rice oil poisonings, PCDFs were more prevalent, but PCDDs were more predominant but in the olive oil example. In addition, 8 of 10 human volunteers who had 107 μg TCDD/kg/day applied dermally to their backs (15 alternative day treatments) developed chloracne, which persisted for 4 to 7 months (83). Ten children from the highest contaminated zone of Seveso who developed chloracne had serum TCDD levels of 828 to 56,000 ng/kg of lipid with the average chloracne-inducing dose estimated at 3.1 μg/kg. In workers involved with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) production, light chloracne was evident with median blood lipid TCDD concentrations of 305 pg/g (extrapolated to 1640 pg/g at the end of exposure) (79). The severity of chloracne and current serum TCDD levels were closely associated in employees of BASF chemical plants in Germany. Individuals with severe chloracne were more likely to have serum TCDD values greater than 100 pg/g of serum lipid (84). These values are comparable to the estimated intake of 15 pg of TCDD TEQ per kilogram per day in a high fish consumer and blood lipid TCDD levels of less than 20 pg/g.

In a cross-sectional survey of workers employed in U.S. Monsanto plants involved with 2,4,5-T production, chloracne was seen in 52% of the estimated exposed cohort versus 0% in the unexposed cohort (85). The mean serum GGT levels and the incidence rate of abnormal serum GGT values (greater than 29 International Units [IU]) were also increased in the chloracne group (26.3 IU, 23%) as compared to the nonchloracne cohort (17.4 IU, 9%) (86). Additionally, after accounting for various confounding variables, the incidence rate of upper GI tract ulcers continued to be significantly elevated in the exposed chloracne group versus the referent population (16.6% vs 5.5%). Various neurologic abnormalities (e.g., peripheral-sensory neuropathy, decreased nerve conduction velocity, decreased libido, depression, insomnia) have been observed in humans occupationally or accidentally exposed to PCDDs/PCDFs. In three chemists involved with the synthesis of TCDD, decreased libido, sleeping difficulties, depression, and moderate to severe chloracne were the first symptoms noted (49). All symptoms, with the exception of the severe chloracne, were considered transient and disappeared within 1 to 2 years after elimination of the exposure. Individuals exposed to PCDDs/PCDFs from contaminated soil (originating from a metal reclamation plant) for an average of 21 years had a higher incidence rate of self-reported noncognitive complaints (e.g., emotional instability, irritability) than the control group (87). In addition, the group with the highest exposure estimation (residency time) also displayed reduced cognitive performance and psychomotor slowing. Although the range of TCDD TEQs found in the blood samples were within the national average (16–80 ng/kg of blood lipid; mean value 31 ng/kg), elevated levels of specific congeners (penta-, hexa-, and heptachlorinated PCDDs) that were also common to the contamination in question were found. Screening for hepatic and gastrointestinal effects in the NIOSH cohort revealed that the risk for an out-of-range serum GGT value (GGT referent value of 96 IU/L) in those TCDD-exposed workers who consumed alcohol increased with increasing serum TCDD levels (odds ratio 2.27) (88). The mean GGT level was also significantly higher in the workers versus the control group (58.5 IU/L vs 47.4 IU/L). Transient alterations in serum biochemistry were also observed in children exposed to TCDD at Seveso. In children from the geographic zone of highest TCDD fallout, slight increases in serum alanine transferase (ALT) and GGT were noted, primarily in males (89). The differences were restricted to inside the established reference limits and were less prevalent 1 year after the accident. Additional evidence of TCDD’s ability to induce transient hepatic alterations were seen in this study population. Urinary D-glucaric acid excretion, an indirect indication of hepatic enzyme activity, was increased in people living within Seveso as compared to Cannero, a nonindustrialized reference town (27.1 vs 19.8 μmol/g of creatinine) (90). In children with chloracne from the highest exposure area of Seveso, the median concentration of urinary D-glucaric acid was also elevated compared to children without skin lesions (39 vs 20.5 μmol/g of creatinine, respectively). In four children who were evacuated from the zone of contamination, the average amount of D-glucaric acid excreted decreased by approximately 56% in 4 to 6 months.

Although PCDDs and PCDFs are potent animal immunotoxicants, limited evidence is available to support this observation in humans. In an initial study of individuals exposed to TCDD from contaminated soil (average exposure time of 2.8 years), the exposed group displayed an increased frequency of anergy (11.8% vs 1.1%) and relative anergy (35.3% vs 11.8%) on skin testing compared to the unexposed controls (91). B-lymphocyte cell numbers were not measured, but the number of non-T peripheral lymphocytes was increased in the exposed group by approximately 16%. However, follow-up of these subjects 12 to 16 months later revealed no differences in anergy or relative anergy rates between the two groups, an observation attributed by the authors as probably due to weak potency of the initial skin tests (92). Abnormal T-helper/T-suppressor (T₄/T₈) ratios (less than 1.0) were found with greater frequency in the exposed group versus the controls (4/23 vs 0/15). In the same cohort, diminished levels of serum Thymosinalpha-1 (Thyα-1) were found in the group presumed to be exposed to TCDD-contaminated soil versus the unexposed controls (93). Ninety-four exposed people had mean serum Thyα-1 levels of 977.3 pg/ml versus 1148.7 pg/ml in the controls. Thyα-1 is a peptide produced by the thymus gland whose function is to modulate lymphokine expression and the differentiation of thymocytes. Although the biologic significance of the finding was unclear, the authors suggested that some form of mild insult may have occurred to the thymus and lymphoid tissue, which is consistent with the ability of TCDD to induce thymic atrophy in the majority of experimental animals tested (94). In a group of 89 workers involved in the decontamination of a German chemical plant, immunologic evaluations were performed,
with intragroup comparisons based on blood TCDD values. Low, medium, and high level controls were defined as having blood TCDD values between 1 and 23 ppt (lipid based) and all persons with blood TCDD values of greater than 24 ppt were considered exposed (95). Based on experimental studies with nonhuman primates, specific cell surface receptors on lymphocyte subpopulations were investigated. No differences were found between the various subgroups in the total number of leukocytes, granulocytes, or lymphocytes. However, 33% of both the exposed group (4/12) and the high level controls (10–23 ppt TCDD; 5/15) did show a trend of increases in the percentage of lymphocyte cells carrying the CD4 and CD45RO surface receptors. In addition, 40% of the same groups had CD19+CD20+CD21+ (mature) B-cell numbers lower than the reference population. The subtle changes observed in the different lymphocyte subpopulations was described as being “of no medical relevance” (95).

Indications of possible endocrine alterations due to TCDD exposure have been documented in the National Institute for Occupational Safety and Health (NIOSH) and U.S. Air Force Ranch Hand cohorts (75,96). Factory workers classified as diabetic had a mean serum TCDD level of 640 ppt (ng/kg of serum lipid) versus 170 ppt in workers without diabetes. In the Ranch Hand study (75), the incidence rate of diabetes was elevated 2-fold in the high current-serum dioxin group (greater than or equal to 33 ppt) versus the comparison group (16.6% vs 8.3%, respectively) (75). In relation, study participants classified as obese had a mean current-serum-dioxin level greater than the normal/lean classification group (22.4 ppt vs 12.9 ppt, and 4.4 ppt vs 3.3 ppt in the controls, respectively). The finding of low serum testosterone (less than 10.4 nmol/l) in the NIOSH cohort (96) was also positively associated with serum TCDD levels. In workers with present serum TCDD values of greater than 241 ppt (n=61), the incidence was approximately 16.4% versus 11.7% in the group with serum TCDD of 76 to 240 ppt (n=62). The referent population (n=231) (mean serum TCDD = 6.08 ppt) prevalence rate for low serum testosterone was 4.8%. In addition, there was a greater prevalence of higher serum luteinizing hormone (LH) values (greater than 28 IU/l) observed in the workers with serum TCDD greater than or equal to 20 ppt and high serum follicle-stimulating hormone (FSH) values (greater than 31 IU/l) in workers with serum TCDD values greater than 241 ppt. Of the workers with present serum TCDD values of 20 to 75 ppt (n=60), 10% had high LH values versus 6.5% of the referent population (96).

Overall, Egegard et al. (96) concluded that present serum TCDD values in the workers were positively related to LH and FSH values and inversely related to total serum testosterone levels.

**Biomarkers of Susceptibility**

It is now generally accepted that most, if not all, of the toxic effects attributed to PCDDs/PCDFs are mediated through a cytosolic aryl hydrocarbon (Ah) receptor (97–99). One of the initial responses detectable following TCDD interaction with the Ah receptor is increased enzyme activity (cytochrome P450 1A1 and 1A2 isozymes (CYP1A1, CYP1A2)). In a method analogous to steroid hormones, PCDDs/PCDFs bind to or activate the Ah receptor, which results in the increased transcription/translation of Ah-specific gene products (aryl hydrocarbon hydroxylase [AHH] or the related ethoxyresorufin-O-deethylase [EROD] enzyme activity). Induction of EROD activity in human lymphocytes in vitro by TCDD showed an approximately 25-fold variation in 39 subjects (99). In human placenta samples obtained from women of Yu-Cheng, there was an approximate 100-fold induction of AHH activity, with an overall variation of up to 60-fold (100). The magnitude of enzyme induction did not correlate with the measured concentrations of PCBs and PCDFs in the placental samples. Induction of EROD/AHH activity in human lymphocytes has previously been associated with increased susceptibility to lung cancer in humans (101). Recently, polymorphism in the CYP1A1 gene locus has been shown to be correlated with EROD induction in human lymphocytes (102). Heterozygosity for a particular polymorphism (MspI) in the CYP1A1 gene was associated with significantly greater induced EROD activity. Previously, this same polymorphism had been linked with increased risk for lung cancer. These examples of human phenotypic/genotypic polymorphisms in principle Ah receptor events would serve to further complicate biomarker development and interpretation.

Increased attention has been given to the possibility of adverse health effects attributed to in utero and lactational exposure of infants to PCDDs/PCDFs. PCDD/PCDF concentrations were measured in the breast milk of 38 Dutch mothers and related to thyroid hormone regulation in newborns. Infants consuming breast milk with an average of 3.75 ng of TCDD TEQ per kilogram of lipid (high group) had statistically significant increases in serum thyroxin and thyroxin/TBG (thyroid binding globulin) at 1 and 11 weeks post partum when compared to infants who consumed breast milk with an average of 18.6 ng of TCDD TEQ per kilogram of lipid (low) (103). Thyroid stimulating hormone (TSH) was also increased by 27% at 11 weeks of age in the high group. The current Canadian average breast milk TCDD TEQ is 15.6 ng of TCDD TEQ per kilogram of lipid (n=100 from 1986–1987) (69). The authors concluded that in utero and lactational exposure to PCDDs/PCDFs is capable of affecting the hypothalamo-pituitary-thyroid regulatory system in human infants (69). In a similar study (104), breast milk TCDD concentrations were related to early/late hemorrhagic disease in newborns. In a limited sample, four mothers whose infants developed some form of bleeding after birth had a mean breast milk TCDD content of 12.13 ng/kg of milk lipid as compared to 9.01 ng/kg in 10 mothers with asymptomatic infants (104). Although slightly lower, the breast milk TCDD TEQ value of the former group was not significantly different from the controls. Again, in comparison, the average TCDD concentration in Canadian breast milk from 1986 to 1987 samples is 2.2 ng/kg of milk lipid.

**Summary**

Biomarkers of exposure (external and internal), effects, and susceptibility were reviewed for PCBs and PCDDs/PCDFs; when possible, comparisons were made to the Great Lakes basin. After evaluation of the available data, it can be concluded that a variety of confounding factors might prevent the establishment of definitive biologic dose–response relationships between external exposure/internal dose and biologic effects. These factors included the complex nature of environmental contaminant exposure, probable exposure misclassification due to lack of internal biomarkers of exposure (in certain cases), nonspecificity of biomarkers of effect, and unknown or inadequately defined biomarkers of susceptibility. In two recent reviews dealing with human exposures to PCBs, it was concluded that there was uncertainty as to what, if any, PCB level in humans might be associated with an adverse health effect (105,106). While considerable evidence
exists from the Oriental rice oil poisonings
that humans who have been highly exposed
to a complex mixture of HAHs will develop
numerous adverse health effects, attempts to
identify similar effects (e.g., immunologic,
reproductive, developmental) in low or
moderately exposed cohorts have been
largely equivocal.

In the absence of definitive epidemiologic
data, biomarkers may still assist the
health assessment process by initially
providing evidence of increased exposure
to contaminants and therefore of possible
increased risk. After a determination that
some facet of exposure has resulted in a
quantifiable increased body burden, research
can then focus on whether an internal biol-
logically effective dose level has also been
reached. The majority of biomarkers of
effect for PCDDs/PCDFs and PCBs that
are presently available can be described as
adaptive or as representative of some form
of alteration in homeostatic function. While
current biomarker data for these two groups
of contaminants may not be at the interpr-
etation stage for developing risk assessment
guidelines, certain data do provide direction
for future research efforts (i.e., endocrine
effects in neonates). However, through con-
tinued characterization and validation,
earlier biomarkers of effect along the expos-
ure–disease continuum could eventually be
used to improve regulatory decisions regard-
ing environmental contaminant exposure
and protection of human health.

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