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**MEMORANDUM: RESPONSE TO ECOLOGICAL RISK ASSESSMENT FORUM
REQUEST FOR INFORMATION ON THE BENEFITS OF
PCB CONGENER-SPECIFIC ANALYSES**

by

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

SUBJECT: Response to the ERAF Request for Information on the Benefits of PCB Congener-Specific Analyses

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In August, 2001, the Ecological Risk Assessment Forum (ERAF) submitted a formal question to the Ecological Risk Assessment Support Center (ERASC) on the benefits of evaluating PCB congeners in environmental samples. This question was developed by ERAF members Bruce Duncan and Clarence Callahan. ERASC contacted NCEA's Exposure Analysis and Risk Characterization Group for assistance in responding to the request. The purpose of this memorandum is to formalize the response.

Central question being asked: *What information is provided by performing PCB congener analysis as compared with Aroclor or total PCB reporting of the results of environmental sampling?*

The production of PCBs ceased in the late 1970's. Monsanto produced commercial mixtures known as Aroclors that differed by the total amount of chlorine present as well as the PCB congener composition. In the environment, various biotic and abiotic processes shift the PCB congener composition from the original commercial products such that environmental samples no longer resemble PCB Aroclors. Hence using Aroclor technical standards to qualitatively determine PCB levels present in the environment may provide an inaccurate depiction of the current PCB congener mixtures present in environmental media and biota. This technical response is divided as follows:

¹ Revised March, and August 2004 in accordance with internal and external peer review comments

- I A review of the historical production and use of PCBs
- II A brief review of the PCB congener composition of Aroclors
- III Alterations of PCB congener profiles as a result of environmental weathering
- IV Alterations of PCB congener profiles as a result of bioaccumulation and biomagnification in ecological food chains
- V Utility of PCB congener-specific analyses - two examples
- VI PCB congeners common to environmental matrices
- VII Conclusions regarding PCB congener-specific analyses for environmental samples

I. Historical Production and Use of PCBs

As a prelude to the discussion of the benefits of congener-specific analysis, it is useful to briefly review the history of the domestic and global production and use of PCBs. PCBs were once perceived as highly valuable manmade chemicals. Their high boiling points and resistance to thermolytic breakdown made them useful in a broad array of industrial applications. Furthermore, since PCBs do not conduct electric current, they were useful for commercial purposes as insulating material and dielectric fluid. Global production of commercial PCB mixtures from 1929 to 1980 has been estimated to be greater than 1.1 million metric tons (Erickson, 1997). U.S. production has been estimated to be approximately 568,000 metric tons (U.S. EPA, 1976). Maximum U.S. production occurred in 1970 with a volume of 38,500 metric tons (IARC, 1978). In 1972, Monsanto Corporation, the major U.S. producer, voluntarily restricted the sale of PCBs to uses as dielectric fluids in "closed electrical systems." This restriction was prompted by growing evidence of PCBs' persistence in the environment, tendency to bioaccumulate in animal tissues and toxic effects. Annual production fell to 18,000 metric tons in 1974. Monsanto ceased PCB manufacture in mid-1977 and shipped the last inventory in October 1977 (Erickson, 1997). Regulations issued by EPA beginning in 1977, principally under the Toxic Substances Control Act (TSCA) (40 CFR 761), have strictly limited the production, import, use, and disposal of PCBs.

Monsanto Corporation marketed technical grade mixtures of PCBs primarily under the patented trade name Aroclor. The Aroclors are identified by a four-digit numbering code in which the last two digits indicate the approximate chlorine content of the formulation by weight percent. The exception to this coding scheme is Aroclor 1016, which contains only mono-through hexa-chlorinated congeners with an average chlorine content of 40 percent. The uses of Aroclors containing mixtures of PCB congeners can be classified into three categories (Erickson, 1997; U.S. EPA, 1976):

1. Completely closed electrical systems such as electrical capacitors and transformers. In these cases, PCB dielectric fluids are self-contained within the electrical apparatus in sealed steel vessels.
2. Semi-closed applications. In these cases, PCBs are used as lubricants in high temperature environments. Examples include hydraulic and heat transfer systems and vacuum pumps.

3. Open-ended applications. These uses are varied but refer to coatings, dyes, paints, inks, adhesives, pesticide extenders, plasticizers, synthetic rubber, carbonless copy paper, cutting oils, lubricating oils, and casting waxes.

Estimates of PCB usage in the United States by usage category during the period 1930-1975 are presented in Table 1. Prior to voluntary restrictions by Monsanto Corporation in 1972 on sales for uses other than “closed electrical systems,” approximately 13 percent of the PCBs were used in “semi-closed applications,” and 26 percent were used in “open-end applications.” Most of this usage of PCBs for “semi-closed” and “open-end” applications occurred between 1960 and 1972 (U.S. EPA, 1976). Table 2 shows the percentage of total Aroclor production contributed by specific Aroclors during the years 1957-1977. EPA has estimated that approximately 5 percent of the PCBs used in closed electrical systems were released into the open environment; 60 percent of the PCBs used in semi-closed applications were released; 25 percent of the PCBs used for plasticizers were released; and 90 percent of PCBs used for miscellaneous industrial uses had escaped into the environment (U.S. EPA, 1976). The reliability of these release estimates was assumed to be ± 30 percent (U.S. EPA, 1976). Prior to the enactment of legislation, approximately 132,000 metric tons of PCBs were buried in unsecured sanitary landfills (U.S. EPA, 1976). This total was comprised of 50,000 metric tons from capacitor and transformer production wastes, 36,000 metric tons from disposal of obsolete electrical equipment, and 46,000 metric tons from disposal of material from open-end applications. An additional 14,000 metric tons of PCBs, although still in service in various semi-closed and open-end applications in 1976 were estimated to ultimately be destined for disposal in landfills.

Table 1. Estimated U.S. Usage of PCBs by Use Category (1930-1975)

Category	Type of Product	Total Use
Completely closed electrical systems	Transformers, capacitors, electrical insulating and cooling applications	61% before 1971 100% after 1971
Semi-closed applications	Hydraulic fluids, heat transfer fluids, lubricants	13% before 1971 0% after 1971
Open-end applications	Plasticizers, surface coatings, ink and dye carriers, adhesives, pesticide extenders, carbonless copy paper, dyes	26% before 1971 0% after 1971

Source: NRC (2000)

Table 2. Percentage of Total Aroclor Production Contributed by Specific Aroclors During the Years 1957 - 1977

Aroclor	1957-1977 U.S. Production (%)
1016	12.88
1221	00.96
1232	00.24
1242	51.76
1248	06.76
1254	15.73
1260	10.61
1262	00.83
1268	00.33

Source: Brown (1994)

II. PCB Congener Composition of Aroclors

Frame et al. (1996) has reported on the detailed analyses of the PCB congener distributions present in Aroclors 1016, 1242, 1248, and 1254. Because of variation to the chlorination process during chemical synthesis, no two batches of the same Aroclor had identical PCB congener distributions. In fact, substantial differences in congener profiles between batches of the same Aroclor could lead to significant differences in biological effects (Kodavanti et al., 2001). Nevertheless the work by Frame et al. (1996) has yielded typical congener patterns present in the PCB formulations. The typical percent distribution of congeners by PCB homologue groupings is depicted in Table 3. Note that the most abundant homologue groups are the di- and tri-chlorinated biphenyls for the low chlorinated Aroclors (1016 and 1242) while penta-chlorinated biphenyls were more abundant in the higher chlorinated Aroclors (1248, 1254 and 1260). Tetra-chlorinated biphenyls were abundant in both low chlorinated and higher chlorinated Aroclors.

Table 3. Typical PCB Homologue Composition (% wt) of Five PCB Aroclors

PCB Homologue	Aroclor 1016 (%)	Aroclor 1242 (%)	Aroclor 1248 (%)	Aroclor 1254 (%)	Aroclor 1260 (%)
Mono-CB	0.7	0.8	0	0	0
Di-CB	17.5	15.0	0.4	0.2	0.1
Tri-CB	54.7	44.9	22.0	1.3	0.2
Tetra-CB	26.6	32.6	56.6	16.4	0.5
Penta-CB	0.5	6.4	18.6	53.0	8.6
Hexa-CB	0	0.3	2.0	26.8	43.4
Hepta-CB	0	0	0.6	2.7	38.5
Octa-CB	0	0	0	0	8.3
Nona-CB	0	0	0	0	0.7
Deca-CB	0	0	0	0	0

Source: Frame et al. (1996)

III. Alterations of PCB Congener Profiles as a Result of Environmental Weathering

In the environment PCBs occur as mixtures of congeners, but their composition will differ from the commercial Aroclors. The chemical and physical properties largely affects the environmental distribution of PCBs. The National Research Council (2000) recently reviewed the fate processes that tend to change the environmental mixtures of PCBs from what was initially released into the environment. The term 'weathering' refers to the sum effect of these fate processes. When released into the environment, PCBs tend to partition to the organic component of soil, water and sediment. Within an aquatic system, PCBs can exist in three abiotic phases: freely dissolved in water; associated with dissolved organic carbon in the water column; and sorbed to particles (NRC, 2000). Freely dissolved light molecular weight PCB congeners may volatilize from the water and into the atmosphere. PCB congeners sorbed to organic carbon in the water column can cross the sediment-water interface and can move below the surficial sediments through the process of diffusion (NRC, 2000). Particle-bound PCB congeners eventually settle to become incorporated into the sediments. The NRC (2000) has noted the following generalizations with respect to the environmental distribution of PCB congeners:

1. The less chlorinated PCB congeners are more water soluble, more volatile and more susceptible to biodegradation. This tends to cause lower concentrations of these PCB congeners in sediments as compared with their distribution in the Aroclor that was discharged into the environment.
2. Higher chlorinated PCB congeners are less soluble, sorb more readily to organic substrates, are less volatile and less susceptible to biodegradation. These PCB congeners are persistent in sediments and tend to bioaccumulate in ecological food webs.
3. At the interface of surface water and the atmosphere, the lower chlorinated PCB congeners have higher vapor pressures and tend to volatilize from the water body into the atmosphere. By this process the atmosphere tends to become enriched in the lower chlorinated congeners relative to the water column, leaving the water body depleted of low molecular weight PCB congeners.
4. As a result of weathering, the PCB congener mixtures that occur in the environment differ substantially from the PCB congener composition of the original industrial release (Bazzanti et al., 1997).

IV. Alterations of PCB Congener Profiles as a Result of Bioaccumulation and Biomagnification in Ecological Food Chains

Measures of Aroclor mixtures or total PCB concentrations may not provide adequate data on PCB exposure and health risks to wildlife. Although there are 209 possible congeners, only about half are prevalent in the environment and, of those, only a limited number both accumulate in animal tissues and exhibit significant toxic effects (McFarland and Clarke, 1989; Chiu et al., 2000; Letcher et al., 2000). Furthermore, differences in uptake, metabolism and bioaccumulation

of PCB congeners can lead to significant differences between congener profiles in predators feeding high on aquatic and terrestrial food webs, and congener profiles in contaminated sediments and soils in the same ecosystems or in the original Aroclor mixtures (NRC, 2000; Chiu et al., 2000; Wagman et al., 2001).

Toxicity of different congener groups

Accurate information on specific congener body burdens is important as toxicological effects vary greatly. PCB congener-specific toxic effects in mammals are believed to include developmental and reproductive toxicity, dermal toxicity, endocrine effects, hepatotoxicity and carcinogenesis (Safe, 1993; ATSDR, 2000). Coplanar congeners, those having two para-, two or more meta- and no ortho- chlorine substituents, and their mono-ortho analogs may be responsible for much of the observed toxicity of PCB mixtures present in the environment (Safe, 1994; van den Berg et al., 1998). Coplanar PCBs have similar chemical structures to dioxins, have a common mechanism of toxicity, and invoke a similar battery of toxic responses as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Because of a shared mechanism of toxicity with certain dioxins and dibenzofurans, the concept of toxicity equivalence has been applied for translating complex mixtures of chlorodibenzo-*p*-dioxins, chlorodibenzofurans and coplanar PCBs present in tissues or diet into 2,3,7,8-TCDD toxicity equivalent concentrations (TEC) using a toxicity equivalence factor (TEF) (Safe, 1994). A TEF is an estimate of the potency, relative to 2,3,7,8-TCDD, of an individual polychlorinated dibenzo-*p*-dioxin, dibenzofuran or biphenyl congener, using careful scientific judgment after considering all available relative potency data. Coplanar PCBs behave like TCDD by binding to the aryl hydrocarbon (Ah) receptor, a common pathway for dioxin-like toxicity. The dioxin/PCB-receptor complex then enters the cell nucleus and induces a variety of toxicologically-relevant changes, including enzyme induction (e.g., aryl hydrocarbon hydroxylase, AHH) and possibly competition for other nuclear proteins that regulate cell differentiation. Thus the PCB congeners that also induce AHH activity are referred to as dioxin-like. Several different methods for calculating TEFs have been developed using particular animal species and toxic endpoints (Leonards et al., 1995). In conducting human health and wildlife risk assessments, EPA generally recommends the use of the TEF procedure developed by the World Health Organization (WHO) as reviewed by van den Berg et al. (1998). Table 4 displays the WHO-TEFs developed for mammals, birds and fish. The toxicity equivalence concept is applied by multiplying the TEF of each congener present in a mixture by the respective mass concentration and the products are summed to represent the 2,3,7,8-TCDD TEC of the mixture, as in the following equation:

$$TEC \cong \sum_{i=1}^n (\text{Congener}_i \times TEF_i) + (\text{Congener}_j \times TEF_j) + \dots (\text{Congener}_n \times TEF_n)$$

The assumption of additivity is inherent to the TEF approach. There are limitations to the predictive value of the TEF approach for PCB mixtures as the biological activity of some congeners may change in the presence of other congeners (Safe, 1994, 1998; van den Berg et al., 1998). Nonetheless, considerable experimental data for ecologically relevant exposures and toxicity endpoints, such as early life stage mortality, support the additivity assumption with no evidence of antagonism or synergism (Walker and Peterson, 1991; Walker et al., 1996; Zabel et al., 1995; Tillet et al., 1996). In general, the four congeners considered most toxic, based on

dioxin-like activity, are the non-ortho (coplanar) PCBs with the IUPAC numbers 77 (chlorinated on carbons 3,3',4,4'), 81 (3,4,4',5), 126 (3,3',4,4',5) and 169 (3,3',4,4',5,5') (van den Berg et al., 1998). Some congeners may cause toxic effects indirectly through a variety of mechanisms involving the cytochrome P450 (CYP) mixed-function oxidase system (Letcher et al., 2000). While induction of CYP may help to remove contaminants, it may also harm the organism by turning otherwise nontoxic contaminants into cytotoxic or genotoxic metabolites (McFarland and Clarke, 1989). In addition, ortho-substituted congeners may have neurotoxic effects (Kodavanti et al., 2001).

Table 4. World Health Organization Toxicity Equivalence Factors (TEF) for Mammals, Birds and Fish

Congener	TEF Mammals	TEF Birds	TEF Fish
2378-TCDD	1	1	1
12378-PeCDD	1	1	1
123478-HxCDD	0.1	0.05	0.5
123678-HxCDD	0.1	0.01	0.01
123789-HxCDD	0.1	0.1	0.01
1234678-HpCDD	0.01	0.001	0.001
OCDD	0.0001	0.0001	0.0001
2378-TCDF	0.1	1	0.0001
12378-PeCDF	0.05	0.1	0.05
23478-PeCDF	0.5	1	0.5
123478-HxCDF	0.1	0.1	0.1
123678-HxCDF	0.1	0.1	0.1
123789-HxCDF	0.1	0.1	0.1
234678-HxCDF	0.1	0.1	0.1
1234678-HpCDF	0.01	0.01	0.01
1234789-HpCDF	0.01	0.01	0.01
OCDF	0.0001	0.0001	0.0001
IUPAC PCB-81	0.0001	0.1	0.0005
IUPAC PCB-77	0.0001	0.05	0.0001
IUPAC PCB-126	0.1	0.1	0.005
IUPAC PCB-169	0.01	0.001	0.00005
IUPAC PCB-105	0.0001	0.0001	0.000005
IUPAC PCB-114	0.0005	0.0001	0.000005
IUPAC PCB-118	0.0001	0.00001	0.000005
IUPAC PCB-123	0.0001	0.00001	0.000005
IUPAC PCB-156	0.0005	0.0001	0.000005
IUPAC PCB-157	0.0005	0.0001	0.000005
IUPAC PCB-167	0.00001	0.00001	0.000005
IUPAC PCB-189	0.0001	0.00001	0.000005

Source: van den Berg et al. (1998)

Persistence and bioaccumulation of different congeners

PCB mixtures change as these contaminants move through abiotic media into food chains (Chiu et al., 2000; NRC, 2000). Some congeners exhibit preferential uptake, metabolism or bioaccumulation by different organisms (Goerke and Weber, 2001; Jackson et al., 1998, 2001; Traas et al., 2001; Willman et al., 1997). Organisms acquire PCBs either through contact with or ingestion of contaminated sediments, water or soil, or through their food. Once the PCBs enter an organism, individual congeners are either cleared through metabolism and/or excretion or are sequestered, usually in lipid tissues. The rate of clearance of a PCB congener through non-metabolic processes is usually inversely proportional to its preferential solubility in lipids, measured as the octanol-water partition coefficient K_{ow} (Thomann, 1989). Metabolic clearance also depends on chemical structure of the congener and the induction of appropriate enzyme pathways in the organism.

Several factors tend to influence the bioaccumulation tendency of different congeners, including the number of chlorine (Cl) substituents and their positions on the two phenyl rings. In general, the more chlorinated congeners exhibit a higher level of persistence in vertebrates and will concentrate to higher levels in tissues of predators than in the surrounding environmental media or prey organisms. This phenomenon has been observed in field populations of fish (salmon - Jackson et al., 2001), birds (cormorants - Guruge and Tanabe, 1997; seabirds - Braune et al., 2001) and mammals (seals - Bernt et al., 1999) and corroborated in controlled laboratory studies (rats - Kodavanti et al., 1998). Wagman et al. (2001) observed similar congener specificity for persistence and elimination in invertebrates (earthworms) exposed to PCB mixtures. In earthworms, elimination was observed to be enhanced for lower chlorinated PCB compounds (e.g., PCB-45) and slowest for nonpolar PCB congeners having 6 or 7 chlorine substituents (e.g., PCB-169). The position of Cl substituents on the ortho, meta or para positions also affects bioaccumulation (Boon et al., 1994). Congeners can be classified as either persistent or readily cleared based on the absence or presence, respectively, of neighboring meta-para hydrogen substituents (m,p-H) on at least one phenyl ring (kestrels - Drouillard et al., 2001; weasel/otters - Leonards et al., 1998; cormorants - Guruge and Tanabe, 1997; crustaceans and fish - Porte and Albaiges, 1994). At the bottom of the food chain, phytoplankton were observed to preferentially take up coplanar congeners rather than those with ortho- chlorine substituents (Swackhamer and Skoglund, 1991). The effect of degree of chlorination at ortho positions on bioaccumulation is more complex and variable in vertebrates (Bruhn et al., 1995; Boon et al., 1994). In general, bioaccumulation in both vertebrates and invertebrates changes the congener profile relative to the surrounding environment.

It should be stressed that patterns of persistence of certain congeners do not hold across species. For example, the highly toxic non-ortho PCB congeners IUPAC 77, 126 and 169 are metabolized and not abundant in body tissues of some predators experiencing high levels of PCB exposure (seal - Nakata et al., 1997; salmon - Willman et al., 1997; porpoise - Bruhn et al., 1995) but congeners 126 and 169 do appear to preferentially bioaccumulate in other predator species (weasel/otters - Leonards et al., 1998; cormorants - Guruge and Tanabe, 1997). Some general patterns may, however, exist. In their review of the occurrence and abundance of PCB congeners across species, including humans, McFarland and Clarke (1989) observed that tetra-, penta- and

hexa-chlorinated congeners seemed to predominate in biological tissues. In addition, accumulated PCBs are further bioconcentrated at each succeeding trophic level in a food web, causing high-order predators to experience the highest overall environmental exposure to PCBs. The frequency and severity of adverse effects, however, depend not just on the level of exposure but also on the particular species' sensitivity to PCBs.

Biomagnification of different congeners in aquatic food chains

More highly chlorinated congeners tend to bioaccumulate most readily, and PCBs tend to biomagnify in the food chain, reaching relatively high, toxic concentrations at higher trophic levels, such as in piscivorous birds (e.g., gulls, terns, and cormorants) and mammals (e.g., minks, otters, seals, and sea lions) (ATSDR, 2000). As PCBs are passed through a food chain, certain congeners are more likely to biomagnify than others (Jackson et al., 1998; Trowbridge and Swackhamer, 2002). How congener patterns change depends upon the number of trophic levels in a food web and the varying capacities of the different species to metabolize the contaminants. In an aquatic food web that included plankton, macro invertebrates, alewife and salmon in Lake Michigan, Jackson et al. (1998, 2001) found that the degree of biomagnification generally increased with the degree of congener chlorination. More specifically, the largest components of the total PCB mixture in plankton, the base of the pelagic food web, were tetra- and pentachlorobiphenyls, but in the macro invertebrates *Mysis* and *Diporeia* and in salmon, the PCB mixture was predominantly hexachlorobiphenyls. A similar pattern was observed in earlier research in Lake Michigan by Willman et al. (1997), in which penta-, hexa- and heptachloro congeners were more concentrated while trichloro congeners were depleted as the PCB mixtures moved from sediments to plankton to fish. Within groups of congeners with the same degree of chlorination (homologues), congeners with no or few ortho- chlorine substituents have shown a tendency to bioaccumulate in lower trophic levels in a study by Swackhamer and Skoglund (1991) but not in a study by Willman et al. (1997). In a follow-up study, Trowbridge and Swackhamer (2002) confirmed that dioxin-like PCB congeners preferentially bioaccumulate in the trophic transfer from the dissolved phase to phytoplankton and from phytoplankton to zooplankton of freshwater lakes. Trowbridge and Swackhamer (2002) found that the biomagnification of dioxin-like PCB congeners are twice the rate of other PCBs for each of the trophic transfer steps. This observation is consistent with the hypothesis that the environmental distribution and trophodynamics of mono- and non-ortho substituted PCB congeners differs from other PCB congeners, with the result that dioxin-like PCBs preferentially bioaccumulate in lower trophic levels. In a Lake Ontario study focusing on mono- and non-ortho congeners, Metcalfe and Metcalfe (1997) found that these more toxic congeners were a larger percentage of the PCB profiles in plankton than in higher organisms in the food web. These congeners did preferentially bioaccumulate at certain trophic transfer points, specifically between invertebrates and fish, and between fish and herring gulls.

V. Utility of PCB Congener-specific Analyses - Two Examples

Example 1. Reproductive toxicity of PCBs in mink

The limitations of wildlife risk assessments based only on total PCBs is illustrated in the case of mink (*Mustela vison*). Mink are fish-eating mammals and may be exposed through their diet to high levels of PCBs in contaminated ecosystems (Giesy et al., 1994). Mink are highly

sensitive to PCBs and experience adverse reproductive effects including reduced litter size and high (up to 100%) kit mortality even when exposed to fairly low levels of PCB contamination in their diet (Heaton et al., 1995; Restum et al., 1998; Brunstrom et al., 2001). PCB concentrations in the food sources of wild populations of mink have been assessed in the Great Lakes region, while the practice of mink farming has allowed controlled laboratory experiments to determine the effects of PCBs on mink directly. Estimates of reproductive risks from PCB congeners can be based on analyses of diet or on mink body burdens. The reproductive effects appear to be caused by the dioxin-like congeners (Brunstrom et al., 2001). Ortho-congeners such as IUPAC 153 (2,2',4,4',5,5') and IUPAC 136 (2,2',3,3',6,6') may exhibit neurological effects (Aulerich et al., 1985).

In their review of recent literature, Giesy and Kannan (1998) observed that laboratory mink feeding studies using commercial Aroclor mixtures calculated higher low/no observable effect levels (LOEL/NOEL) and EC_{50} than did studies using weathered PCB mixtures as found in wild food sources (fish). In other words, weathered PCB mixtures were more toxic than the commercial mixtures, indicating that the congener profile changed as the contaminants moved through abiotic media and into the aquatic food web (e.g., Giesy et al., 1994). In an earlier review of experimental data, Leonards et al. (1995) found it difficult to determine a dose-response curve or reference dose for reproductive effects such as litter size or kit survival based on total PCB in the mink diet. However, a dose-response curve that matched laboratory observations could be calculated based on mink whole-body concentration of dioxin-like PCB congeners estimated using TEF calculations developed by Safe (1993). Further laboratory work by Brunstrom et al. (2001) confirmed that reproductive effects in mink are correlated with high levels of non- and mono-ortho-substituted-biphenyls (dioxin-like congeners) in their food, while no reproductive effects are observed among mink exposed to high concentrations of 2-4-ortho-substituted-biphenyls only. These studies clearly indicate the importance of using more refined measurements than total PCB concentrations to assess risks to sensitive wildlife.

Example 2. A study of the effects of PCB on the hormonal system of polar bears

Braathen et al. (2004) studied the relationship between the uptake of PCB congeners and thyroid hormones (THs) and retinal within male and female polar bears. This provides a second example of the use of PCB congener-specific analysis to understand the toxicity of PCBs on mammals. The authors noted that alterations of THs can have observable effects on behavior and on the neurological system. In addition, hypothyroidism is associated with menstrual dysfunction, anaovulation and miscarriages.

The polar bear (*Ursus maritimus*) is at the top of the Arctic food web and preys on marine mammals, e.g., ringed and harp seals. PCB exposures to the polar bear are through their diet in that marine mammals have a tendency for accumulating PCBs present in the environment through the process of biomagnification up trophic systems. This study examined the relationship of the body burden of twenty-eight PCB congeners in polar bears (PCB-99, 105, 118, 138, 153, 156, 157, 170, 180, 194, 206 and 209 were listed by the authors) to alterations in their hormonal system. Six congeners were analyzed to represent the twenty-eight since many of the PCB congeners were found to be correlated to one another, that is, when one is present the other is also present in polar bear tissues (i.e., PCB-99 correlated with 138; 118 with 105; 156 with

157; 180 with 170; and 194 with 206+209). Blood samples were collected (17 female and 29 male mature bears) for PCB, TH and retinal analysis. When evaluating a possible cause and effect relationship between PCBs and the alteration of TH, these six PCB congeners were summed to represent all twenty-eight congeners. The investigators observed a statistically significant negative correlation between plasma PCB-levels and the plasma levels of five and two TH variables in female and male polar bears, respectively, i.e., decreases in plasma levels of specific thyroid hormones. Females were found to be more susceptible to TH suppression by PCBs than males, and the TH hormone, T₃, was decreased to the greatest extent. The authors noted a need for research to relate the apparent TH imbalance in females caused by PCBs to adverse functional effects on the endocrine system. No statistically significant effect was observed between plasma PCB-levels and retinal in either male or female bears. In this study, the utility of PCB congener specific analysis was to identify PCB congeners that should be further studied for their propensity for endocrine disruption.

VI. PCB Congeners Common to Environmental Matrices

Hansen (2001) identified a series of PCB congeners that are most commonly detected in environmental matrices, including sediments, soils and biota. Hansen based his list of congeners, summarized in Table 5, on a review of numerous data sets published in the scientific literature. Together, the congeners in Table 5 account for >60% of all PCBs measured. This table may serve as a basis for agreement on PCB congeners in need of analytical delineation.

Table 5. Hansen's List of Major PCB Congeners Detected and Characteristic of Steady State PCB Residues

IUPAC No.	Ring Chlorination	IUPAC No.	Ring Chlorination
153	245-245	201	2345-2356
138	234-245	56 + 60	23-34 + 234-4
180	2345-245	66 + 95	24-34 + 236-25
118	245-34	163	2356-34
74	245-4	128 + 167	234-234 + 245-345
146	235-245	194	2345-2345
170	2345-234	196 + 203	2345-2346 + 23456-245
99	245-24	70 + 76	25-34 + 345-2
187	2356-245	77	34-34
28 + 31	24-4 + 25-4	81	345-4
156	2345-34	126	345-34
105 + 132	234-34	169	345-345
183	2346-245		

Notes: Two congeners separated by a + sign indicates that they are seldom resolved and coelute.

Source: Hansen (2001)

VII. Utility of PCB Congener Patterns Analysis in Environmental Samples.

PCB congener-specific analysis can provide information necessary to resolve likely sources of environmental contamination. Termed "fingerprinting," congener patterns present in

aquatic sediments or other environmental media can be matched against a database of PCB congener profiles of source releases to link sources with receptors.

For example, Ikonomou et al. (2004) compared the PCB congener distribution in Dungeness crab (*Cancer magister*) to the PCB congener distributions measured in sediments at various locations in Victoria Harbor, British Columbia. The crab was used as a biomonitor with the intent of identifying sources of PCB contamination. Statistical analysis (factor analysis and principal component analysis) was applied to the PCB congener patterns discerned in crab and sediment. A remarkable similarity in the PCB congener patterns was found between crab and sediment close to the outfall of a former bleached chlorine pulp and paper mill. In both matrices the congener pattern resembled a 45:55 mixture of Aroclors 1254 and 1260. Ikonomou et al. (2004) applied a direct mixing model to the PCBs associated with the mill operations, and, considering the weathering of PCBs in sediments with the passage of time, they predicted the PCB congener distributions in the sediments. From this procedure, the investigators found a good fit between the PCB congener profile measured in the sediments at the outfall to the wastewater discharge at the mill with the predicted congener profile resulting from the mixing model. With these two lines of evidence, i.e., a PCB fingerprint match between crab and sediment, and a PCB fingerprint match between measured and predicted sediment PCB distribution, Environment Canada concluded that the likely source of PCB contamination in Victoria Harbor was the past operation of the paper mill.

A study of the Lower Fox River in Wisconsin (Stratus Consulting, 1999) provides a second example of the utility of PCB congener pattern analysis. In an effort to identify the source of PCB contamination in the river sediments, the investigators relied on the comparison of PCB congener patterns in relation to four locations within the watershed area: Lower Fox River (LFR), Inner Green Bay, Outer Green Bay, and Lake Michigan. A suspected source of contamination was the congregation of several paper mills along the LFR reach. These mills were the center of the production of carbonless copy paper in which PCBs were used as a coating. Based on reviews of historical facility records, industrial processes and waste disposal practices, and PCB concentrations in paper products and waste, it was estimated that paper mills along the LFR had released a total of 300,000 kg of PCBs into the Lower Fox River. Sediment concentrations were lowest upstream of the location of the mills and reached maxima near the cluster of the mills on the LFR. The spatial and temporal pattern of PCB contamination in LFR sediments was found to be consistent with the transport and weathering of PCBs discharged from the paper mills. PCB congeners 028+031 (coeluted), 056+060, 066+095, and 070+076 were common to all sampling areas. However, the LFR was found to be more enriched in the lower chlorinated congeners (those with low congener numbers) than the other three sampling areas. Lake Michigan samples appeared to be relatively enriched in the higher chlorinated congeners (those with high congener numbers). Analysis of PCB congener patterns indicated that PCBs in Green Bay were derived from those in the LFR (and ultimately the paper mills), and Lake Michigan PCBs probably originated from outer Green Bay.

VIII. Conclusions

- PCB congener-specific data provide the best and most scientifically defensible basis for evaluating the ecological hazards that may be associated with PCB contamination in the environment. Information and examples presented in this memorandum give scientific support to this premise.
- The weathering of PCBs, coupled with PCB bioaccumulation in ecosystems, considerably alters the mixture of PCB congeners present in the environment. This means that, with the passage of time, the PCB congener patterns that initially comprised commercial PCB formulations are less likely to resemble the PCB congener patterns currently observed in the environment.
- Specifying the PCB congener profiles in environmental samples adds important information to the risk assessment process that otherwise would be obscured by only reporting Aroclor equivalents or total PCBs in the samples. PCBs exhibit a wide spectrum of toxicologic effects in various species, including humans (ATSDR, 2000). This has created two major groups of PCB congeners: 1) the dioxin-like (co-planar) PCBs, and (2) the non-dioxin-like PCBs. In specifying the PCB congener profiles for purposes of human health and ecological risk assessments, such groupings are useful.
- PCB congener fingerprinting techniques can lead to the identification of likely sources of PCB contamination. This involves the matching of the PCB congener patterns present in the environment to the PCB congener profiles associated with the use of specific PCB Aroclors by local point sources, and accounting for the weathering of PCBs. The application of statistical pattern recognition techniques to the data (e.g., principal component analysis) may greatly assist this effort.
- A central question for this analysis is whether PCB congener distributions can be estimated for historical environmental measurements in which the data is reported in Aroclor equivalents or the sum of PCBs present in the sample. The work of Ikonomou et al. (2004) has demonstrated that it is theoretically possible to predict PCB congener patterns based on an assumption of the weathering of specific Aroclors that may have been used by specific sources. This can be achieved through the application of sophisticated environmental mixing models. It may be possible to run these models backwards from the current measurement of the PCB congener patterns present in the environment to a prediction of the distribution of PCB congeners that may be present in environmental matrices in cases where environmental measurements are represented only as Aroclor equivalents. However, such analysis would be highly uncertain and results would be difficult to verify. Until research has reached a point where one can readily identify clear and unambiguous PCB congener patterns in all environmental matrices, and the physical/chemical processes of weathering are more accurately defined, it is not possible to reach a firm conclusion as to the scientific validity of retrospectively reconstructing the likely PCB congener distributions in these historical environmental studies.

- It is recommended that the results of analyses for PCB contamination in environmental matrices be expressed in terms of PCB congener-specific, total PCB, and Aroclor equivalent concentrations. This will provide useful information on the proportion of specific PCB congeners relative to the sum of all congeners that is needed in order to scientifically address the use of historical data. A second benefit of this approach is that ecological risk assessment metrics have, in many cases, been developed around exposures to total PCBs and Aroclor equivalents in the environment, and not on a single congener. The reporting of total PCBs and Aroclor equivalents in environmental studies will provide continuity in terms of the utilization of available bioaccumulation factors and ecotoxicity benchmarks in site-specific ecological risk assessments.
- Current state-of-the-art laboratory methods makes it possible to analytically identify and quantify the presence or absence of all 209 PCB congeners in environmental samples (e.g., EPA Method 1668A; U.S. EPA, 1999). The PCBs that can be determined by this Method are the 12 polychlorinated biphenyls (PCBs) designated as having dioxin-like activity and the remaining 197 PCBs. Approximately 125 of the PCB congeners are resolved adequately on an SPB-octyl gas chromatographic column to be determined as individual congeners. The remaining 70 congeners are determined as mixtures, e.g., they coelute with other congeners. The overarching question then shifts away from analytical chemistry capabilities to one of defining a subset of 209 PCB congeners that should be routinely identified as PCB congener mixtures in specific environmental media and biota. Hansen (2001; Table 5 of this document) has provided an interim list of major (commonly reported) congeners characteristic of steady state PCB residues.

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