European Journal of Pharmacology – Environmental Toxicology and Pharmacology Section, 228 (1992) 179–199

EJPTOX 40029

Assessment

Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept

Ulf G. Ahlborg [a], Abraham Brouwer [b], Marilyn A. Fingerhut [c], Joseph L. Jacobson [d], Sandra W. Jacobson [d], Sean W. Kennedy [e], Antonius A.F. Kettrup [f], Jan H. Koeman [b], Hermann Poiger [g], Christoffer Rappe [h], Stephen H. Safe [i], Richard F. Seegal [j], Jouko Tuomisto [k] and Martin van den Berg [l]

[a] *Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden,* [b] *Department of Toxicology, Agricultural University Wageningen, Netherlands,* [c] *National Institute for Occupational Safety and Health, Cincinnati, OH, USA,* [d] *Department of Psychology, Wayne State University, Detroit, MI, USA,* [e] *Canadian Wildlife Service, Environment Canada, Hull, Canada,* [f] *Institut für Ökologische Chemie, Gesellschaft für Strahlenforschung, Neuherberg, Germany,* [g] *Institute of Toxicology, University of Zürich, Schwerzenbach, Switzerland,* [h] *Institute of Environmental Chemistry, University of Umeå, Umeå, Sweden,* [i] *Department of Veterinary Physiology and Pharmacology, Texas A & M University, College Station, TX, USA,* [j] *New York State Department of Health, Wadsworth Center, Albany, NY, USA,* [k] *Division of Environmental Health, National Public Health Institute, Kuopio, Finland, and* [l] *Research Institute of Toxicology, State University of Utrecht, Utrecht, Netherlands*

Received 24 August 1992, accepted 26 August 1992

A scientific evaluation was made of the mechanisms of action of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls. Distinction is made between the aryl-hydrocarbon (Ah) receptor-mediated and non-Ah receptor-mediated toxic responses. Special attention is paid to the applicability of the toxic equivalency factor (TEF) concept.

Polychlorinated dibenzo-p-dioxins (PCDDs); Polychlorinated dibenzofurans (PCDFs); Polychlorinated biphenyls (PCBs); Ah receptors; Ah (aryl-hydrocarbon)-mediated effects; Non-Ah (aryl-hydrocarbon)-mediated effects; Toxic equivalency factor (TEF); Risk management; Neurobehavioural responses; Carcinogenicity; Endocrine responses; Kinetics; Metabolism; Species differences

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are complex mixtures of compounds with similar environmental chemical and toxicological properties. Although sources and routes of environmental contamination may differ for PCDD/Fs and PCBs, they are simultaneously present in various biological samples, including fish, wildlife, meat and dairy products. Differences do exist, however, in the abundance of individual congeners within, and in the level of contamination for each class of chemicals between various biological samples (Rappe and Buser, 1989; Norstrom et al., 1990; Duinker et al., 1989).

Correspondence to: Nicole G.C. Wevers, EERO – Assessment Unit, P.O. Box 182, 6700 AD Wageningen, Netherlands. Tel. +31-8370-84924; Fax +31-8370-84941.

Of the 75 positional isomers of PCDDs and 135 isomers of PCDFs, usually only the 2,3,7,8-substituted congeners are present in biota (Van den Berg et al., 1987a; Norstrom et al., 1990). In general, higher chlorinated congeners of 2,3,7,8-PCDD/Fs and PCBs are more abundant than lower chlorinated compounds in most biotic samples and this is further accentuated in biota at higher trophic levels of the food chain.

A considerable body of literature exists on the toxic and biochemical responses observed in laboratory animals experimentally exposed to PCDDs, PCDFs and PCBs (McConnell, 1989; Goldstein and Safe, 1989). These responses include dermal, immuno- and hepatotoxicity, carcinogenic, teratogenic and neurobehavioural effects, as well as numerous biochemical responses, such as the induction of several drug metabolizing enzymes.

Strong evidence exists that many of the above mentioned effects are mediated by an aryl-hydrocarbon (Ah) receptor mechanism of action (Poland and Knut-

son, 1982). The 2,3,7,8-substituted PCDD/F congeners are particularly potent in inducing Ah receptor-mediated toxic responses. In addition, good correlations have been found between their Ah receptor-mediated P4501A1 induction and toxic potency (Goldstein and Safe, 1989). On the basis of these and other observations the toxic equivalency factor (TEF) concept has been developed to express the toxic potency of complex mixtures of PCDD/Fs in biological samples by a single value, the toxic equivalency (TEQ) value (Safe, 1990).

A similar correlation between toxic response potencies and Ah receptor binding affinities has also been observed for PCBs. The most potent PCB congeners are the coplanar, non-ortho substituted PCBs e.g., 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta- and 3,3′,4,4′,5,5′-hexachlorobiphenyl, and to a lesser extent some relatively planar, mono-ortho PCBs (Safe, 1990). It is considered that these PCB congeners may therefore also be included in the TEF approach. However, it should be noted that, for PCBs in particular, a number of important toxic and biochemical responses induced in experimental animals, e.g., neurobehavioural, neurochemical, carcinogenic and endocrine effects may not, or only partly be mediated by the Ah receptor (Seegal et al., 1990a; Silberhorn et al., 1990; Brouwer, 1991).

There is considerable uncertainty in the extrapolation of toxicological information on PCDD/Fs and PCBs from experimental animals to man, because of the observed large species differences in toxicokinetics, sensitivity and pattern of toxic responses (Kimbrough, 1987; Gallo et al., 1991). In addition, the information on possible adverse health effects in man and wildlife species associated with exposure to PCDD/Fs and PCBs is scarce and largely of epidemiological nature. Several national and international assessment reports have been made during the past 5 years on the adverse human health risks of exposure to PCDD/Fs, some of which are listed at the end of this report. This present assessment aims to specify the present state of knowledge on environmental impact and possible adverse human health risks of PCDD/Fs, including the PCBs, with special emphasis on applicability of the TEF concept.

Sources

PCDDs and PCDFs

Environmental contamination by PCDDs and PCDFs can be attributed to a series of primary sources, which are noted below, while animal and human exposure is primarily from secondary sources including food intake, drinking water, inhalation and skin contact. The primary sources can be divided into the following four categories.

- During many chemical reactions it has been found that PCDDs and PCDFs are formed as unwanted byproducts. As a result many pesticides and technical products, including chlorophenols, chlorophenoxy herbicides and PCBs, have been contaminated with these compounds. The production and use of these chemicals are currently banned or strictly regulated in most countries, but during the 1960s and 1970s they were widely used and still form a major source of contamination in the environment. Other chemical processes generating PCDDs and PCDFs include the bleaching of pulp with chlorine (Swanson, 1988) and the production of graphite electrodes (Rappe et al., 1991).
- Combustion processes are considered to be another important primary source of PCDDs and PCDFs. Most thermal reactions involving chlorinated organic or inorganic compounds result in the formation of PCDDs and PCDFs. Of special importance is the incineration of various types of wastes (municipal, hospital, hazardous) and the production of iron and steel and other metals (Cu, Mg, Ni). Some thermal sources may be non-anthropogenic, for example forest fires and burning of sea-weeds containing NaCl, but the background levels associated with these processes are relatively low (Clement et al., 1991; Kjeller et al., 1991).
- Photochemical reactions under atmospheric conditions can result in the formation as well as in the degradation of PCDDs and PCDFs. These reactions are of special interest, since most combustion and incineration sources produce emissions that undergo long-range transport (Tysklind et al., 1991).
- Besides abiotic formation processes, some biochemical processes can results in the formation of PCDDs and PCDFs. For example incubation of chlorophenols, hydrogen peroxide and a series of peroxidases resulted in the formation of PCDDs and PCDFs. In addition to this evidence from in vitro experiments, these reactions may also occur under environmental conditions in sewage sludge and compost (Öberg et al., 1992).

There is a question regarding the contributions of non-anthropogenic sources to the present background environmental levels of PCDDs and PCDFs. A few historical samples (sediment, soil, vegetation) have been analysed and the results indicate that only low background environmental levels of PCDDs and PCDFs are derived from non-anthropogenic sources (Czuczwa and Hites, 1984; Kjeller et al., 1991).

Although many sources of PCDDs and PCDFs have been identified, a substantial fraction of these chemicals in the environment may originate from as yet unidentified sources. Mass balance calculations indicate a gap between the observed deposition of PCDDs and PCDFs and known sources and this can be ex-

plained by long-range transport from unidentified sources (Rappe, 1991).

For most of the processes mentioned above, complex mixtures of PCDDs and PCDFs are emitted into the environment. Some processes, especially those involving combustion, have similar congener-specific patterns and profiles. Congener-specific patterns in the abiotic environment can to some extent be used for source identification, discriminating sources such as chlorinated phenoxy acids, pentachlorophenol, PCB combustion, chlorine production and pulp bleaching processes (Rappe and Buser 1989; Rappe et al., 1991). Source identification through biotic samples is much more speculative, as selective accumulation of 2,3,7,8-substituted congeners prevails (Van den Berg et al., 1987a; Norstrom et al., 1988, 1990)

PCBs

Extensive environmental contamination with PCBs has occurred during the period of their industrial use, i.e., from the early 30s until the 1980s. Although PCBs have been banned from industrial application since the early 1980s they are still entering the environment by:

- leakage from old so-called closed systems, such as capacitors and transformers;
- the disposal of materials contaminated with PCBs, such as old paints, painted construction materials, recycling of paper (copy papers), lubricant oils, sealing material and fire retardants in old fire extinguishers.

Recently it was shown, that de novo synthesis of PCBs possibly occurs during combustion processes. Among the congeners formed, were those with a non-ortho substitution pattern which exhibit 'dioxin-like' biological properties (Rappe and Buser, 1989; Van Bavel et al., 1992).

In the environment a number of secondary sources of PCBs can also be identified. These include transport of river sediments, leakage from dump sites, dumping of sewage sludge and long-range atmospheric transport.

Analytical methods

In general, any environmental analysis has to follow the concept of good analytical practice, including quality assurance of results as well as standardization by inter-laboratory comparison. For this it is important to establish standard procedures regarding sampling, sample conservation, extraction, separation and identification, quantification, data processing and evaluation.

Samples collected from the environment must be representative of the sampling area. If biota are collected the species sampled must represent the required trophic level or position in the food chain. Sampling of more than one specimen may be needed for estimating individual differences in, e.g., metabolism and kinetics. If the number of analyses is limited, then mixing of different samples may be required to obtain representative averages.

The analytical results should be assessed in terms of detection limits, signal-to-noise ratio, recovery rate, standard deviations and concentration ranges. The significance of these results should be evaluated relative to background concentrations, exposures, bioaccumulation, bioavailability and environmental effects.

PCDDs and PCDFs

High resolution chemical analysis of PCDDs and PCDFs has now been developed. Combinations of high resolution gas chromatography (HRGC) with high or low resolution mass spectroscopy (HRMS or LRMS), using either EI^+ or NCI^-, are the generally accepted techniques. For the analysis of (a)biotic environmental samples the use of carbon enrichment procedures for cleanup yields the best analytical data. The use of silica or alumina oxide based adsorbents is sufficient for biotic tissue analysis. ^{13}C-labelled standards must be added to crude analytes so that the recovery yields of the individual congeners can be determined and used for calculating the concentrations. Liquid-liquid extraction, solid phase extraction and supercritical fluid extraction are commonly used sample extraction methods which should be specified (Smith et al., 1984; Rappe and Buser, 1989)

PCBs

Techniques for the analysis of individual PCBs have not been as well developed as those for PCDD and PCDF congeners (Ballschmiter et al., 1989). Traditionally total PCB levels in a sample have been quantified by comparing the peak pattern of a sample with that of a commercial mixture. This method is adequate only when the sample under investigation has been directly contaminated with a commercial mixture. Because PCB patterns differ significantly between biological samples and technical products, the method leads to errors in the quantification of PCBs from biological samples and to differences in results between laboratories. Thus comparisons can be made with confidence only between results from one laboratory or between different laboratories when strict interlaboratory controls have been made.

Similar to PCDDs and PCDFs, congener-specific PCB analysis is required for risk management purposes. The standard PCB congeners, nos. 28, 52, 101, 138, 153 and 180 are usually quantified by using electron capture detector gas chromatography (GC-ECD). From an environmental and toxicological point of view the coplanar and mono-ortho PCB congeners nos. 77, 105, 118, 126, 156, 157, 167 and 169 are considered

most relevant for the risk assessment of dioxin-like activity.

Currently, a major analytical problem concerns the quantitation of congeners 77, 126 and 169 in environmental biota. These three non-ortho congeners are present in much smaller concentrations than those observed for most of the mono-ortho PCB congeners. These low concentrations in combination with limited congener-specific separation on capillary columns make the use of a standard PCB cleanup and GC-ECD analysis unreliable. Present analysis of these congeners involves a carbon enrichment and separation procedure, not unlike that used for the analysis of PCDDs and PCDFs (Kubiak et al., 1989; Liem et al., 1992). After purification and separation from other PCB congeners, the three non-ortho congeners can be analyzed either on GC-ECD, provided that sufficient separation of the other PCB congeners is obtained. However HRGC mass spectroscopy (MS) is preferable above the former method. The five mono-ortho congeners mentioned in the preceding paragraph are also preferably analyzed by the latter method. When using HRGC-MS, the EI^+ mode is more sensitive than the NCI^- method for congeners 77 and 169. The planar PCBs are widespread in the environment and in view of their relatively high toxicity compared to 2,3,7,8-TCDD (Safe, 1990) more 'dioxin' laboratories should be urged to analyze them.

Quality assurance

The World Health Organization in Europe (WHO/EURO) has coordinated two interlaboratory quality control studies on levels of PCDDs/PCDFs in human milk and blood (World Health Organization, 1989; Stephens et al., 1992) to improve the comparability of interlaboratory data. A third round of these studies includes the analysis of PCDDs and PCDFs and 'dioxin-like' PCBs in cow's milk, fish, human milk and blood. The results will be available in late 1992. In general, results from these studies had a coefficient of variation smaller than 50%. In view of the minute amounts analyzed in these studies, $< 10^{-12}$ g, these results can be considered as a good analytical achievement. However, continuing action is required to maintain this analytical quality.

Bioassays

Cell culture bioassays have been developed for qualitative and quantitative estimation of the dioxin-like activities of PCDDs, PCDFs, PCBs and related compounds. These bioassays have the advantage in that they can provide quantitative information on the toxic potency of complex mixtures of congeners (Safe, 1990). Bioassays have the potential to obviate the need for expensive chemical analysis. They generally utilize mammalian cancer cell lines and primary and secondary cells in culture. The measured responses include induction of P4501A1 mRNA levels, P4501A1-dependent monooxygenase activities, porphyria and keratinization responses (Zacharewski et al., 1989; Tillit et al., 1991; Hanberg et al., 1991; Kennedy et al., 1992).

The development of other assay systems using recombinant DNA techniques should be encouraged. The benefits of bioassays have already been illustrated in wildlife toxicity studies around the Great Lakes in North America (Zacharewski et al., 1989; Tillit et al., 1991; Kennedy et al., 1992).

Environmental occurrence

PCDDs and PCDFs in abiotic samples

Background levels of PCDDs and PCDFs have been reported in a series of abiotic samples including air, water, snow, soil and sediments (Rappe and Buser, 1989). The congeneric distribution in these samples is similar to the observed patterns from combustion sources. In samples from combustion sources the profiles are generally not dominated by any particular congener group. In contrast, sample profiles from air, snow, sewage sludge and sediment are strongly dominated by the hepta- and octa-chlorinated dioxins. The reasons for these discrepancies are not completely understood. The widespread use of pentachlorophenol from 1940 to 1985 has been suggested as an explanation; however, samples collected before the commercial introduction of pentachlorophenol show a similar profile as recent environmental samples (Kjeller et al., 1991).

Atmospheric conditions can influence levels as well as patterns of PCDDs and PCDFs on airborne particulates. As part of the Swedish dioxin survey, large volumes of air samples have been collected on the island of Gotland in the Baltic Sea. After trajectory analyses, samples collected during stable weather conditions were analyzed. The levels and patterns of PCDDs and PCDFs depended on the wind direction. The greatest concentrations were detected in those air samples which originated from the more heavily industrialized regions (Egebäck et al., 1991).

PCDDs and PCDFs in biotic samples

Like other stable lipophilic pollutants the PCDDs and PCDFs bioaccumulate and biomagnify through the foodchain. In most biological samples only the 2,3,7,8-substituted PCDDs and PCDFs can be detected. The absence of the non-2,3,7,8-substituted congeners in environmental biotic samples can be explained by rapid metabolism and excretion of these congeners. In this respect the crustaceans and molluscs are exceptions; retaining PCDDs and PCDFs both with and without a 2,3,7,8-substitution pattern (Rappe and Buser, 1989;

Rappe et al., 1991). In most biological samples, other than those from humans, the concentrations of hepta- and octachlorinated compounds are quite low.

In general it can be concluded that species living in the aquatic environment contain higher levels of PCDDs and PCDFs, than those species which are exclusively terrestrial. In fish, concentrations of PCDDs and PCDFs can vary with species, tissue, age group, body mass, fat content, sex, season and location. The highest concentrations have been reported in samples from the Baltic Sea, the Great Lakes of North America, and from Newark Bay, New Jersey, USA. The concentrations of these compounds in fish caught well offshore are generally much lower than in those caught in the vicinity of the coast (Rappe and Buser, 1989).

The possible accumulation of PCDDs, PCDFs and PCBs in plants has been less well studied than in animals. At present there is no evidence for the active uptake of PCDDs and PCDFs through roots (Bush et al., 1986). Most of the analyses done on plants indicate that contamination with these compounds is associated with the leaf surface and originating from airborne particles (Rappe and Buser, 1989). PCDDs and PCDFs have been identified in vegetation such as herbage and pine needles. Deposition on the leaf surface of these compounds has been associated with a lipophilic layer, the cuticular wax, on the outer part of the leaf. This layer appears to absorb organic compounds easily (Reischl et al., 1989). Although no active uptake of PCDDs and PCDFs by roots has been detected, it has been found to occur for PCBs by the roots of purple loosestrife (*Lythrum salicaria*) (Bush et al., 1986).

Food

Based on the present information it can be concluded, that ordinary processing of food does not affect concentrations of PCDDs, PCDFs, or PCBs. However, it has been reported from Norway and Sweden that industrial processes in the food industry, such as treatment of cod liver oil, can decrease the levels of these compounds substantially.

Microbial degradation

Aerobic and anaerobic microbial degradation of these compounds have been reported from laboratory studies (Chen et al., 1988). In soil, however, microbial degradation seems insignificant or at most very slow.

Human tissue levels and dietary intake

In general, human samples contain only 2,3,7,8-substituted PCDD and PCDF congeners. The hepta- and octaCDDs are by far the most abundant in samples from the general population. By contrast, the concentration of the 2,3,7,8-CDD congeners is usually quite small in fish and other food items from the aquatic environment. They are also present at low levels in dairy products, beef and pork (Rappe and Buser, 1989). Based on an average daily food intake a bioconcentration factor between 115 and 400 was estimated for humans (Geyer et al., 1986; Webster and Cornett, 1990).

TABLE 1

Background levels of PCDDs, PCDFs and PCBs in human samples from Japan, Europe, North-America and South-East Asia (Jensen, 1989)

Compound	Blood plasma (pg/g lipid)	Human milk (pg/g lipid)	Body fat (pg/g lipid)
PCDDs total	150–1,000		
2,3,7,8-TCDD		1.0–9.7	3–10
OctaCDD		106–744	230–1,360
PCDFs total	< 25–190		
2,3,7,8-TCDF		2.5–9.4	< 2–9.1
OctaCDF		< 0.2–46	< 2–60
PCBs	650–7,700	0.32–2.79 [a]	0.56–10 [a]

[a] μg/g lipid.

Since food is the major source of human exposure to PCDDs, PCDFs and PCBs, food surveys should provide valuable information. Such surveys carried out in the Netherlands and in Germany show median daily intakes of 1–2 pg TEQ/kg body weight for PCDDs and PCDFs (Beck et al., 1989; Liem et al., 1992)

In addition analysis of human serum or milk can be used to estimate exposure to PCDDs, PCDFs and PCBs. Background levels can be routinely identified in the general population (see table 1). Evaluation of a large number of human adipose tissue and serum samples from industrialized nations indicated lipid adjusted 2,3,7,8-TCDD concentrations below 20 ng/kg, with means of 4–6 ng/kg (Patterson et al., 1989). On an individual basis PCDD and PCDF concentrations are comparable for adipose milk and blood when expressed on a lipid basis. Higher levels have been found in occupationally exposed individuals as well as in people with specific life styles, for example consuming large quantities of contaminated fish (Jensen, 1989; Svenson, 1991).

To quantify background exposure the World Health Organization (1988, 1989) has collected and compared the concentrations of PCDDs and PCDFs in human milk from many countries. The individuals were selected from urban and rural areas, but the differences between these two groups were not remarkable. Larger differences were observed between countries. The highest concentrations were detected in samples from Belgium, the Netherlands, Germany and the UK, ranging from 30 to 40 ng TEQ/kg milk fat. The concentrations in the Scandinavian countries, Poland, Japan, USA and Canada were intermediate, while the lowest were observed in samples from less industrialized

countries ranging between 5 and 12 ng TEQ/kg milk fat (The World Health Organization, 1989). It should be noted that in European countries the relative contribution of 2,3,4,7,8-pentaCDF to the total TEQ value is higher than reported from North America.

Besides the degree of industrialization, life style might also be a determining factor for background human levels of PCDDs and PCDFs. This is suggested by the comparable levels of PCDDs and PCDFs in samples from Japan and Sweden despite the fact that sources of these compounds in Japan may be up to 100 times higher than the identified sources in Sweden.

Kinetics and metabolism

Body distribution

The body distribution and metabolism of most of the toxic PCDDs and PCDFs have been well studied in the commonly used laboratory animal species (Neal et al., 1982; Safe, 1989). Quantitatively, liver and adipose tissue are the major storage sites in mammalian species, with rodents having the highest liver deposition (Brewster et al., 1987; Van den Berg et al., 1987b, 1989). Generally, the ratio between liver and adipose tissue concentration follows the order: rodents $\gg$ birds $\pm$ monkeys > humans > fish. The extraordinarily high deposition of 2,3,7,8-substituted congeners in the liver of, e.g., rat has been attributed to specific storage sites in the liver cells. Cytochrome P4501A2 has been suggested as such a specific storage site, but results differ between studies and are ambiguous (Kuroki et al., 1986; Voorman and Aust, 1989; Buckley Kedderis et al., 1991).

Limited studies of the body distribution of PCBs (in various species) show that the adipose tissue is a quantitatively more important deposition site than liver. The congener-specific body distribution of PCBs suggests that the disposition of congeners in liver and adipose tissue is dependent on chlorine substitution pattern and degree of chlorination. Liver deposition appears to be more important for non-ortho congeners with a 'dioxin-like' mechanism of action (Shain et al., 1986; Safe, 1989; De Jongh et al., 1992).

Metabolism

Metabolism of PCDDs and PCDFs in rodents involves preferential hydroxylation on the lateral (2,3,7,8) positions, thus resulting in a slower elimination of the 2,3,7,8-substituted congeners (Poiger and Buser, 1984; Poiger et al., 1984, 1986). For PCDFs the situation is slightly more complex since there is preferential oxygen insertion adjacent to the ether oxygen bond (Pluess et al., 1987). The extremely rapid elimination of higher chlorinated non-2,3,7,8-substituted PCDDs and PCDFs in rodents as well as in primates (Abraham et al., 1989; Neubert et al., 1990) remains unexplained.

Metabolic pathways have been well identified for some PCB congeners. Hydroxylation preferentially occurs on the para positions and is decreased if these positions are sterically hindered by chlorine atoms. If para positions bear chlorine substituents, positions para to other chlorine atoms are hydroxylated. Like the PCDDs and PCDFs, the presence of two vicinal unsubstituted carbon atoms greatly facilitates metabolic hydroxylation (Safe, 1989).

During metabolism of PCDDs, PCDFs and PCBs chlorine rearrangements (NIH shift) are commonly observed. Pathways and rates of metabolism of all three groups of compounds vary significantly between species (Wroblewski and Olson, 1985).

Among the species studied, humans have an extremely slow elimination of 2,3,7,8-substituted PCDDs and PCDFs with halflives ranging from 2 to 7 years, depending on the congener (Poiger and Schlatter, 1986; Pirkle et al., 1989; Ryan and Masuda, 1992).

Based on the limited available data on the kinetics of PCBs in humans the results suggest that elimination of those compounds might be somewhat faster, than the persistent PCDDs and PCDFs (Ryan and Masuda, 1992; Bühler et al., 1988).

Species differences

It can be concluded from the toxicokinetic data that the rates of metabolism and disposition of PCDDs, PCDFs and PCBs are dose-dependent as well as species-specific. In addition, many studies often used single large doses (Abraham et al., 1988; Birnbaum et al., 1980, 1988; Brewster et al., 1987; Rose et al., 1976; Van den Berg et al., 1987b, 1989; Olson et al., 1980, 1986), and the resulting data may not accurately indicate the human toxicokinetics of these compounds (Pirkle et al., 1989; Poiger and Schlatter, 1986). Careful examination of the toxicokinetic database suggests a ten-fold higher uptake in rats compared to humans (Schlatter, 1991). Allowance should be made for this factor when extrapolating results from rodents to humans.

Species differences in the distribution of PCDDs and PCDFs between liver and adipose tissue might be an important factor determining the acute toxicity of PCDDs and PCDFs. According to a recent hypothesis, the acute toxicity of PCDDs and PCDFs inversely correlates inversely with the amount of adipose tissue in a mammalian species (Geyer et al., 1990, 1991). However, it should be noted that within one species, e.g., the rat, the genetic background might also play an important role in the expression of acute toxicity (Pohjanvirta et al., 1990). The difference in distribution between liver and adipose tissue of PCDDs and PCDFs (Thoma et al., 1989) may contribute to the slow elimi-

nation rate and high bioaccumulation of these compounds in humans compared with rodents.

Toxicity of metabolites

The toxicities of PCDDs and PCDFs are primarily caused by the parent compounds. Like the PCDDs and PCDFs, the Ah receptor-mediated effects of PCBs are also assumed to be induced by the parent compound. Metabolites are several orders of magnitude less active with respect to Ah receptor-mediated biochemical and toxicological effects (Mason and Safe, 1986; Weber et al., 1982).

For some lower chlorinated PCBs, in vivo effects on vitamin A and thyroid hormone metabolism and transport, are caused by interactions of hydroxylated metabolites at the receptor site (Brouwer and Van den Berg, 1986; Brouwer, 1991). To what extent metabolites of the higher chlorinated penta- and hexachlorinated PCBs can cause a direct influence on thyroid hormone and vitamin A levels in vivo should be further investigated.

At present, there is no in vivo evidence which shows that hydroxylated metabolites of PCDDs and PCDFs are involved in alterations in vitamin A and thyroid hormone levels.

In addition, methylsulphone derivatives have been detected in mammalian tissues, including those from heavily exposed Yusho victims (Safe, 1989). It should be noted, that these methylsulphone metabolites can bind irreversibly to lung and adrenal cells (Brandt and Wachtmeister, 1987), however their toxicological significance is still unclear.

Definition of dioxin-like activity for risk management

Several compounds, that are chemically different from chlorinated dibenzo-p-dioxins, e.g., chloro and bromo derivatives of dibenzofurans and biphenyls, are included, or considered for inclusion in the TEF approach for risk management on the basis of their dioxin-like mode of action (Safe, 1990). In addition, several other classes of chemicals exhibit at least part of the dioxin-induced biochemical responses, but may not elicit dioxin-specific toxic responses. It is therefore essential, for risk management purposes, to define what criteria should be met by a certain congener, or group of chemicals for inclusion in the TEF concept.

The following criteria should be met by a compound: (1) it should bind to the Ah receptor, (2) elicit dioxin-specific biochemical and toxic responses in short-term or semi-chronic animal experiments, and (3) be persistent and accumulate in the food chain.

These criteria are met by PCDDs, PCDFs and coplanar PCBs, that are persistent in the environment, in wildlife and in human tissues (Safe, 1990). The toxic effects elicited by these compounds are presumably initiated by persistent occupation of the Ah receptor and the induction of specific gene products. The responses in animal species include chloracne and related dermal lesions, thymic atrophy and immunotoxicity, hepatotoxicity and porphyria, reproductive and developmental toxicity, endocrine responses, tissue-specific hypo- and hyperplastic responses and carcinogenesis (Poland and Knutson, 1982). These effects are also age-, sex-, species- and strain-dependent.

Several diverse structural classes of compounds, in addition to PCDD/Fs and PCBs are Ah receptor agonists, i.e., they bind competitively to the Ah receptor and elicit induction of specific cytochrome P450 gene expression (CYP1A1 and CYP1A2) and associated enzyme activities, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD). These include polynuclear aromatic hydrocarbons, substituted flavones and coumarins, photolysed tryptophan products and several other polycyclic aromatic natural products (Safe, 1984, 1986; Piskorska-Pliszczynska et al., 1986; Helferich and Denison, 1991; Bjeldanes et al., 1991; Rannug et al., 1987). However, most of these chemicals do not meet criteria 2 and 3, namely, they do not elicit the 'dioxin-like' toxicities and do not persist in tissues due to rapid metabolism. Therefore, although they possess part of the dioxin-like mode of action, these chemicals need not be considered for inclusion in the TEF concept.

Development and application of the TEF concept

The concept of toxic equivalency factors (TEFs) was developed for risk management, to interpret the complex database derived from the analysis of samples containing mixtures of PCDDs and PCDFs. Risk assessment was initially focused on 2,3,7,8-TCDD, but because of the lack of data on the other environmentally relevant congeners, several agencies have adopted the TEF approach as an interim process for risk management for these complex mixtures (Bellin and Barnes, 1989; Kutz et al., 1990; Ahlborg, 1989; Van Zorge et al., 1988).

In laboratory animals and in mammalian cells in culture, 2,3,7,8-TCDD and related PCDDs and PCDFs elicit a common spectrum of sex-, age-, strain- and species-specific biochemical and toxic responses, as described under 'Definition of dioxin-like activity for risk management' (Whitlock, 1990; Poland and Knutson, 1982; Safe, 1990; Goldstein and Safe, 1989). Some of these responses including chloracne and related dermal lesions, P4501A1 and P4501A2 induction are also observed in humans, and human cells in culture. Genetic studies in inbred mice (Poland and Knutson, 1982), structure-activity relationships (Safe, 1986, 1990)

TABLE 2

Proposed toxic equivalency factors for PCDDs and PCDFs

Congener	TEFs		
	Safe [a]	Int./EPA	Nordic
2,3,7,8-tetraCDD	1.0	1.0	1.0
1,2,3,7,8-pentaCDD	0.5	0.5	0.5
1,2,3,6,7,8-hexaCDD	0.1	0.1	0.1
1,2,3,7,8,9-hexaCDD	0.1	0.1	0.1
1,2,3,4,7,8-hexaCDD	0.1	0.1	0.1
1,2,3,4,6,7,8-heptaCDD	0.01	0.01	0.01
OctaCDD	0.001	0.001	0.001
2,3,7,8-tetraCDF	0.1	0.1	0.1
2,3,4,7,8-pentaCDF	0.5	0.5	0.5
1,2,3,7,8-pentaCDF	0.1	0.05	0.01
1,2,3,4,7,8-hexaCDF	0.1	0.1	0.1
2,3,4,6,7,8-hexaCDF	0.1	0.1	0.1
1,2,3,6,7,8-hexaCDF	0.1	0.1	0.1
1,2,3,7,8,9-hexaCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-heptaCDF	0.1	0.01	0.01
1,2,3,4,7,8,9-heptaCDF	0.1	0.01	0.01
OctaCDF	0.001		

[a] Safe, 1990.

and molecular biology studies (Whitlock, 1990) support the hypothesis that PCDDs and PCDFs elicit most of their biochemical and toxic responses through initial binding to the aryl hydrocarbon (Ah) receptor in the target cells or organs. However, the subsequent development of late toxic responses, such as, reproductive, developmental and carcinogenic responses may require many other cellular and physiological alterations.

The relative potencies of PCDDs and PCDFs as Ah receptor agonists are comparable for most receptor-mediated responses, although some quantitative differences in potency exist between species and between responses, because of several factors, including the multiple physiological differences between species. Safe (1990) has summarized the relative potencies of the environmentally relevant 2,3,7,8,-substituted PCDDs and PCDFs compared to 2,3,7,8-TCDD. Several regulatory agencies went through a similar excercise and have selected a single value from the range of TEFs observed for each congener, for risk management of complex mixtures (table 2). In general more weight is given to TEF values based on semi-chronic/chronic studies rather than derived from short-term toxicity studies or in vitro bioassays.

The TEF approach can be used to transform analytical results into toxic equivalents (TEQs) where

$$\Sigma([\text{Congener}] \times \text{TEF})_{\text{PCDD/F}} = \text{TEQ} \quad (1)$$

The simple relationship in (1) has been used to calculate the toxic potency of various complex mixtures, expressed by a single value, the TEQ value. The point is stressed that this summation is only valid when the individual congeners exhibit an additive interactive response. The same mixtures have also been tested for their toxic potency in laboratory animals. The results indicate that the calculated TEF-derived TEQs correlated well with the experimentally determined TEQs for several different Ah receptor-mediated responses, e.g., AHH/EROD induction, LD_{50}, hepatotoxic effects, body weight loss and thymic atrophy (Safe, 1990; Eadon et al., 1986; USEPA, 1991). These data support the application of the TEF concept for risk management of toxic PCDD and PCDF mixtures.

Risk management of PCBs using the TEF concept

Structure-Ah receptor binding and structure-toxicity relationships have also been investigated for the coplanar-non-ortho PCB congeners, 3,4,4',5-tetraCB, 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB and their mono-ortho analogs (Safe, 1984; Safe et al., 1982; Parkinson and Safe, 1987). For most responses, there was a good correlation between their structure-binding and structure-activity/toxicity relationships. In addition, these coplanar non-ortho and mono-ortho PCBs are persistent in the environment and compared to the 2,3,7,8-PCDD/Fs relatively high levels of the mono-ortho PCBs have been detected in biota. Therefore it has been proposed that the TEF approach may be useful for PCBs (Safe, 1990). Like the PCDD and PCDF congeners, the TEFs for these coplanar PCBs range in value. Safe (1990) has suggested TEFs of 0.1, 0.05 and 0.01 for 3,3',4,4',5-pentaCB, 3,3',4,4',5,5'-hexaCB and 3,3',4,4'-tetraCB, respectively, and 0.001 for their mono-ortho analogs.

It should be noted, however that there is no international agreement yet on the TEFs for coplanar, non-ortho and mono-ortho PCBs. Two working groups of the World Health Organization, WHO/EURO and WHO/IPCS, have adopted a programme to discuss available data and will recommend TEF values for PCDDs, PCDFs and dioxin-like PCBs during 1992 and 1993.

One of the earliest applications of the TEF approach was to determine the TEQs of environmental extracts containing PCDDs, PCDFs and PCBs (Kannan et al., 1988, 1989; Dewailly et al., 1991). Based on the TEFs derived from in vivo experiments as noted above or TEFs derived from in vitro AHH induction potencies in rat hepatoma H-4-II E cells, it was estimated that the PCBs contributed > 50% of the total TEQs in these samples. A comparison of calculated TEQs for different PCB mixtures using the TEF approach significantly overestimated the observed induction and immunotoxicity potencies of several commercial PCBs (USEPA, 1991; Davis and Safe, 1989).

These results point out one of the limitations of the present TEF approach, namely that it only takes into account additive but not synergistic or antagonistic

effects of PCBs (alone) and PCBs in combination with PCDDs and PCDFs. The problems associated with interactive effects will be discussed in more detail in 'Role of kinetics in the TEF concept'.

Another limitation, in terms of risk assessment of PCDD/F/PCB mixtures, is the fact that only Ah receptor-mediated effects are taken into consideration in the TEF approach. In particular the nonplanar, di-ortho PCB congeners elicit a diverse spectrum of non-Ah receptor-mediated responses (see also paragraph 'Non-Ah receptor-mediated effects') in experimental animals, including the induction of specific P450 isozymes, hypovitaminosis A, hypothyroidea, neurotoxicity and carcinogenicity (Seegal et al., 1990a,b,c; Shain et al., 1991; Scheutz et al., 1986; Denomme et al., 1983; Buchmann et al., 1986; Robertson et al., 1991; Norback and Weltman, 1985; Brouwer and Van den Berg, 1986). The TEF approach cannot be used for these responses and is valid only for those toxicities that are mediated through the Ah receptor. Furthermore, the TEF approach may considerably underestimate the risk for development of those toxic responses, following mixed PCDD/F/PCB exposure, that are affected by both Ah-mediated (PCDD/Fs, coplanar, non-/mono-ortho PCBs) and non-Ah-mediated (nonplanar, di-ortho PCBs) pathways, such as carcinogenicity and endocrine alterations. Therefore, the TEFs for PCBs will not be predictive for all toxic responses associated with these compounds in experimental animals, wildlife species and man.

Quantitative approaches for the risk management of PCB congeners which do not act through the Ah receptor are currently not available and requires additional research.

Non-Ah receptor-mediated effects

Traditional toxicological responses of PCDDs, PCDFs and coplanar PCBs thought to be mediated by the Ah receptor are listed under 'Definition of dioxin-like effects for risk management'. However, exposure to mono- and di-ortho PCB congeners and metabolites may elicit several other important toxic responses in experimental animals, including neurobehavioural (Bowman and Heironimus, 1981; Schantz et al., 1989, 1991), neurochemical (Fingerman and Russell, 1980; Seegal et al., 1990a,b,c; Seegal, 1992), carcinogenic (Silberhorn et al., 1990) and endocrinological (Brouwer et al., 1990; Brouwer, 1991) changes.

The direction and magnitude of change in neurological function by PCBs depends on the developmental status of the animal at the time of exposure (Agrawal et al., 1981; Seegal et al., 1991; Seegal and Shain, 1992; Seegal, 1992). Thus perinatal exposure of the non-human primate to Aroclor 1016 (Schantz et al., 1989, 1991; Levin et al., 1988; Jacobson et al., 1985a) leads to long-term dysfunctions in behaviour that may be similar to the observed defects in cognitive function in children of mothers who consumed PCB contaminated fish (Jacobson et al., 1985b, 1990a,b; Schantz et al., 1990; Tilson et al., 1990). In addition, perinatal exposure of rats to Aroclor 1016 induces persistent increases in biogenic amine concentrations (Seegal, 1992). In contrast, the effects of di-ortho-PCBs on brain dopamine (DA) concentrations in rats following adult exposure are opposite in direction to those seen with perinatal exposure (Agrawal et al., 1981; Tilson et al., 1990). The mechanisms for these changes are not yet known but may include alterations in steroidal and thyroid hormone function (Tuomisto and Männistö, 1985; Morse et al., 1992).

Carcinogenic effects of PCBs in experimental animals are primarily localized in the liver and they include preneoplastic lesions, neoplastic nodules and hepatocellular carcinomas. Although PCBs are considered as complete, non-genotoxic carcinogens in rats and mice, they are particular efficacious tumour promoters. Coplanar non-ortho and mono-ortho PCBs, as well as nonplanar mono- and di-ortho PCBs are tumour promoters in rats, following an initiating dose of diethylnitrosamine (Silberhorn et al., 1990; Buchmann et al., 1991; Sargent et al., 1992). Thus the carcinogenic effects of PCBs can follow a structure-toxicity relationship which is different from the Ah-mediated toxic effects. This does not exclude that, Ah receptor-mediated processes may play a role early in the development of liver tumours, especially in mixed exposure situations with chemicals other than PCB/PCDD/Fs; for example, PCB-induced activation of polycyclic aromatic hydrocarbons, procarcinogens or in the synergistic effects of iron on PCB-induced hepatocellular carcinomas in C57BL/10ScSn mice (Smith et al., 1990).

In addition to the effects noted above the parent coplanar and nonplanar PCBs and their metabolites can cause marked endocrine changes in laboratory animals and these include decreased plasma thyroid hormone and vitamin A levels (Brouwer and Van den Berg, 1986; Brouwer, 1991; Van Birgelen et al., 1992). In addition, their hydroxy PCB metabolites interact specifically and competitively with plasma transthyretin (TTR), inhibit hepatic thyroxine 5'-deiodinase activity and uncouple mitochondrial oxidative phosphorylation (Lans et al., 1990, 1991; Adams et al., 1990; Brouwer et al., 1990). These alterations in vitamin A and thyroid hormone concentrations may significantly modulate tumour promotion and developmental and adult neurobehavioural changes (Wearn et al., 1991; Morse et al., 1992).

Finally, changes in vitamin K-dependent blood coagulation have been observed in rats following exposure to either 2,2',4,4',5,5'-HxCB and 2,3,7,8-TCDD (Bouw-

man et al., 1990), and this may play a role in PCB or PCDD-induced haemorrhages.

Unlike the Ah-mediated toxic events, the neurobehavioural, neurochemical, carcinogenic and endocrine changes induced in experimental animals following exposure to mono- and di-ortho PCBs may be elicited by both the parent compounds and their metabolites and involve multiple mechanisms of action. These mechanisms need to be elucidated and structure-activity/toxicity relationships should be determined in order to develop an alternate TEF approach for these non-Ah-mediated toxic responses.

Role of kinetics in the TEF concept

Short-term experiments

At present the TEQ values for 2,3,7,8-substituted congeners are either derived from short-term, or semichronic in vivo experiments or from in vitro bioassays. The large differences in elimination rates found for the 2,3,7,8-substituted PCDDs and PCDFs (Birnbaum et al., 1980; Brewster and Birnbaum, 1987), suggest that inclusion of toxicokinetics is limited in most experiments. Even in in vivo experiments up to 3 months in duration, steady state tissue concentrations for some 2,3,7,8-substituted congeners may not yet be established (Pluess et al., 1988; Van der Kolk et al., 1992). Therefore, a comparison of the toxicity of the individual congeners based on 3 month studies may be limited from a toxicokinetic point of view (Van den Berg and Poiger, 1990).

To determine TEQ values with a more realistic toxicokinetic approach, the use of a loading and maintaining dose regime is recommended for semichronic experiments (Krowke et al., 1989; Wearn et al., 1991).

The arguments stated above are likely to apply also for PCBs. Toxicokinetic information is extremely scarce for PCBs, as only a limited number of experiments has been carried out with individual congeners. Therefore, the determination of TEQ values of 'dioxin-like' PCBs relies even more on short-term tests and in vitro assays (Safe 1989).

Bioassays

The role of toxicokinetics is also minimal in most short-term bioassays used for PCDDs, PCDFs and PCBs. For example, in cell lines or primary cell cultures compound metabolism is usually lower than observed in vivo. In addition, the viability of primary cell cultures is restricted to several days, again limiting the expression of toxicokinetics with these biopersistent compounds.

Nevertheless, good correlations have been obtained between induction of cytochrome P450 1A related activities in some cell cultures, and several short-term toxic effects including thymic atrophy and body weight loss in vivo (Safe, 1989). Moreover, TEF values derived from semichronic studies with some highly biopersistent PCDDs, PCDFs and PCBs were in the same range as those reported from bioassays using cell lines (Pluess et al., 1988; Van Birgelen et al., 1992). The similarities observed between semichronic in vivo experiments and in vitro assays indicate the utility of the low cost bioassays to determine TEF and TEQ values. However, when these data are used for, e.g., human risk assessment by computer modelling, they should always be used in combination with kinetic data (Van den Berg and Poiger, 1990).

Interactive effects

Interactions have been reported for toxic and biochemical effects when mixtures of PCDDs/PCDFs and PCBs were administered. Many in vivo and in vitro studies support the hypothesis, that combinations of PCDDs and PCDFs are additive (Pluess et al., 1988; Bol et al., 1989; Safe, 1989). However, combinations of PCDDs or PCDFs and PCBs resulted in significant antagonistic as well as some synergistic effects which were response- and species-dependent (Bannister and Safe, 1987; Bol et al., 1989; Davis and Safe, 1990).

There is some data, which suggests that some non-additive effects may have a toxicokinetic basis. For example, it was shown that PCBs and PCDDs or PCDFs can modulate each other's body distribution (Van der Kolk et al., 1992; De Jongh et al., 1992).

The exclusion of non-additive mixture interactions in the present TEQ concept can be justified by the following arguments: (a) the antagonistic or synergistic effects are observed at only very high dose levels and the magnitude of these interactions are smaller than the uncertainties already present in the TEF values; (b) the observed non-additive effects are highly species-, response- and dose-dependent and their relevance might be of minimal importance; and (c) the mechanisms responsible for these non-additive effects are unknown.

Adverse human health effects

Accidental / occupational exposure

Data on the human toxicology of PCDDs, PCDFs and PCBs is primarily derived from the results of accidental or occupational exposure. Less is known about possible adverse health effects from the chronic, low background levels present in human populations.

One well recognized effect of relatively high exposure to PCDDs, PCDFs and PCBs in some individuals is chloracne. In the Seveso incident and in cases of occupational exposure, where chloracne was used as an indicator of exposure, symptoms of peripheral nerve

dysfunction were observed (Filippini, 1981; Jirasek, 1974; Moses et al., 1984). However, heavily exposed chemical workers (Sweeney, 1990) and less heavily exposed US Air Force Ranch Hand personnel showed no chronic effects on the peripheral nervous system in studies conducted more than 15 years after removal from exposure. In these studies no effects were observed on liver or gastrointestinal disease, porphyria, or pulmonary function (Calvert, 1992a,b; Wolfe, 1991). In the Ranch Hand study in which serum TCDD measurements were used to estimate exposure; diabetes, glucose intolerance, cholesterol and HDL increased significantly with increasing serum levels of TCDD (Wolfe et al., 1991).

Some evidence that TCDD is a carcinogen in heavily exposed humans was provided by three studies of chemical production workers, where serum TCDD levels were used as measures of exposure (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990). Statistically significant excesses of total cancer were found in the high exposure groups of all three studies, and a significant excess of respiratory cancer and soft tissue sarcoma was associated with high exposure in one study. Exposure to chlorophenols or phenoxy acids without simultaneous exposure to TCDD also correlates with soft tissue sarcoma or non-Hodgkins lymphoma in other studies; this complicates the interpretation of the data. There have been few studies on carcinogenicity of PCBs in humans and these provide inconclusive results (Silberhorn et al., 1990).

In Japan and Taiwan, accidental consumption of cooking oil contaminated with PCDFs and PCBs (Yusho and Yucheng accidents) resulted in a diverse spectrum of toxic lesions, including ocular, neurological, endocrine, hepatotoxic, immunotoxic and respiratory effects. Children born to exposed women several years after exposure were smaller at birth, with dermal lesions and poorer performance on standardized intelligence tests administered during childhood (Higuchi, 1976; Wong and Hwang, 1981; Rogan et al., 1988). The long-term implications of these findings are not yet clear.

Non-accidental / non-occupational exposure

Background environmental exposure of the human population to PCDDs, PCDFs and PCBs is mainly via dietary intake and has resulted in the accumulations of low levels in blood plasma and higher concentrations in body fat and human milk (see table 1). These levels have not been associated with any overt signs of toxicity such as chloracne or hepatotoxicity.

Prenatal exposure to PCBs is associated with cognitive deficits in human infants and young children (Jacobson et al., 1985b, 1990a, 1992; Rogan et al., 1986). These effects have been noted particularly in short-term memory and cognitive processing efficiency. The possible long-term implications for school performance and achievement are presently unclear. These effects are dose-dependent and subclinical. Prenatally exposed children are smaller at birth (Fein et al., 1984; Jacobson et al., 1990b; Yamashita and Hayashi, 1985). The birth size deficit is comparable in magnitude to that associated with smoking, but unlike the smoking deficit persists through early childhood. Both the size and cognitive deficits are specifically correlated to levels of prenatal exposure to PCBs. Although substantially higher levels of PCBs are transmitted to the infant via lactation than to the foetus in utero, none of the physical growth or cognitive deficits are associated with postnatal exposure from breast-feeding (Jacobson et al., 1985b, 1990a,b, 1992; Gladen et al., 1988).

Human risk management and the TEF concept

As indicated earlier, TEQs are useful for assessing toxicological risks associated with mixtures of PCDDs, PCDFs, non-ortho and mono-ortho coplanar PCBs in animals; to that extent they may be useful for assessing toxicity to humans.

A number of toxicological effects associated with PCBs may also be due to other mechanisms. Such risks cannot be estimated by TEQs. For example, congeners such as 2,4,4′-tri-CB, that accumulate preferentially in monkey brain and cause alterations in dopamine levels, are not Ah receptor agonists, but are involved in neurotoxic effects.

The mechanisms of action involved in these non-Ah-mediated effects are not well understood; therefore no method analogous to TEQs exists for estimating the relative toxicities of the non-dioxin-like congeners on the basis of structure-activity relationships. Total PCB levels in cord serum and maternal milk correlate with physical growth and cognitive deficits in infants and children and may provide the best summary measure of potential toxicity currently available.

The human infant seems particularly sensitive to PCB neurotoxicity when exposure occurs in utero. Although much larger quantitites of PCBs are transferred to the infant postnatally via breast-feeding, only minimal effects have been linked to moderate levels of exposure of this kind. A WHO/EURO expert group recommended in 1988 that given its well known benefits to developing infants, breast feeding should be continued and promoted despite the occurrence of PCDDs, PCDFs, PCBs and other chlorinated compounds in mothers' milk. Data available today do not call for a revision of this recommendation.

In cases of accidental or occupational exposure, or where PCB-contaminated fish is a primary food source for women of child bearing age, potential risks of pre- and post-natal exposure to the developing infant may be considerably elevated (Hara, 1985).

Adverse effects on wildlife and domestic species

Adverse effects in several wildlife species have been correlated with environmental exposure to PCBs, PCDDs and PCDFs (Hoffman et al., 1987; Kubiak et al., 1989; Bellward et al., 1990). The effects observed in field studies are consistent with those found in laboratory animals, but the possible contribution of other chemicals to these effects is difficult to rule out (Gilbertson, 1989).

Studies in North America and Western Europe have concentrated on the possible effects on fish-eating birds, marine and freshwater mammals and fish. The biochemical parameters used so far in wildlife toxicity studies are cytochrome P4501A1 related enzyme activities, porphyria (Kennedy et al., 1990; Fox et al., 1988), plasma thyroid hormone and vitamin A levels in plasma and hepatic tissue (Brouwer et al., 1989; Brouwer, 1991).

Cell culture bioassays based upon the induction of cytochrome P4501A1 related activities and porphyria have been successfully applied to estimate TEQs in extracts from fish and wild bird eggs (Zacharewski et al., 1989; Kennedy et al., 1990, 1992; Tillit et al., 1991). Based on this information the TEF concept seems to be applicable in wildlife toxicity studies.

Fish-eating birds

The most detailed information yet available is from bird species. Studies with fish-eating birds around the Great Lakes and in the Netherlands indicate a correlation between tissue or egg concentrations and reproductive or developmental impairment and death (Koeman et al., 1973; Kubiak et al., 1989; Tillit et al., 1992; Van den Berg et al., 1992; Fox et al., 1991).

Extracts from tissues of double crested cormorants *Phalacrocorax auritus* containing PCBs and related compounds showed a dose-dependent EROD inducing potency. These induction potencies were negatively correlated with the hatching success of this species in different colonies around the Great Lakes (Tillit et al., 1992). Similar correlations have been observed in young hatchling cormorants *Phalacrocorax carbo* for hepatic EROD activity, yolksac concentrations and developmental impairment (Van den Berg et al., 1992).

Several reports have focused on the relative contribution of PCDDs, PCDFs and PCBs as causal agents in the above observed effects (Tanabe et al., 1987). Using a TEF or bioassay approach the non- or mono-ortho planar PCBs seem to play a major role in the Ah receptor-mediated effects in wild bird species. The relative contributions of the dioxin-like PCBs, especially 2,3′,4′4′,5-PnCB (#118) and 3,3′,4,4′,5-PnCB (#126) were estimated to be more than 50% of the observed TEQs (Kubiak et al., 1989; Tillit et al., 1992).

Marine and freshwater mammals

The possible role of PCBs and related compounds in the decline of some populations of marine mammals, such as seals in northern and western Europe has been studied intensively since the early 1970s. The pathological findings in seals resemble effects associated with PCBs and related compounds (Gilbertson, 1989). Studies with harbour seals fed with fish diets with different levels of pollutants, especially PCBs, indicate that these compounds impair reproductive success (Reijnders et al., 1986). Moreover, in this study plasma thyroid hormone and vitamin A levels were significantly decreased in the heavily exposed group of seals, suggesting an impairment of normal physiology (Brouwer et al., 1989).

In laboratory experiments some freshwater mammals, like mink and otter, were found to be highly sensitive to PCB toxicity. Field observations on mink and otter populations around the Great Lakes and in the Nordic countries indicated an association between relatively high PCB concentrations in fat and decline in populations. PCB concentrations in wild mink around Lake Ontario are similar to those which affect reproduction in controlled studies (Gilbertson 1989).

Fish

Correlations between tissue concentrations of PCBs and related compounds in fish and the induction of hepatic cytochrome P4501A1 related activities have been reported. This has been observed for several fish species including flounder, *Platichthys flesus*, rattail, *Coryphaenoides armatus* and lake trout, *Onochorhynchus mykiss* (Binder and Lech, 1984; Stegeman et al., 1988). Although the biological significance of this cytochrome P4501A1 induction in fish is unclear, its elevation might play a role in the activation of procarcinogenic polycyclic aromatic hydrocarbons present in the environment (Stegeman and Lech, 1991).

PCBs and related compounds may also have an adverse effect on the reproduction in fish (Spics and Rice, 1988), while developmental impairment has been observed in the early life stage (Helder, 1981). Laboratory studies have shown that some species such as trout, are extremely sensitive to 2,3,7,8-TCDD and related compounds in their early life stage (Helder, 1981; Bol et al., 1989). It is unclear if PCBs and related compounds have a role as etiologic agents in the failure of early life stages of lake trout larvae to survive in the Great Lakes of North America (Gilbertson, 1989).

Domestic animals

Accidental contaminations of chickens, cows, and other farm animals with halogenated biphenyls or related compounds have caused significant health effects, as well as making them unsuitable for use as sources of food for humans (Anderson, 1989). To date, no adverse

health effects have been reported for domestic animal species following background environmental exposure to these compounds.

Conclusions

The main conclusions of this scientific evaluation are as follows:

(1) industrial and other anthropogenic sources of PCDDs, PCDFs and PCBs greatly exceed natural sources;
(2) dietary intake is the main source of environmental exposure to PCDDs, PCDFs and PCBs for humans, marine and freshwater mammals and fish-eating birds;
(3) for risk management purposes, analysis of PCDDs, PCDFs and PCBs in biological samples should be congener specific and conform to internationally accepted standardized procedures;
(4) in vitro bioassays for Ah receptor-mediated toxic responses are useful for estimating the toxic potencies of extracts of biological samples;
(5) the TEF concept may be useful for risk management, i.e., quantitative estimation of Ah-mediated toxic potential, of mixtures of PCDDs, PCDFs and the coplanar non-ortho and mono-ortho PCBs;
(6) any other class of compounds considered for inclusion in the TEF concept should meet specific criteria, namely, to exhibit Ah receptor agonist activity, elicit dioxin-specific toxic responses in short-term or semi-chronic animal experiments, and bioaccumulate in the food chain;
(7) many of the potentially adverse, e.g., neurobehavioural and carcinogenic effects of PCBs are also mediated by mechanisms which do not involve the Ah receptor. For such responses, the TEF concept is not applicable;
(8) neurobehavioural responses in young children following prenatal exposure to PCBs are among the most sensitive responses in humans;
(9) TCDD and PCBs have been shown to be carcinogenic in animals. In addition some recent occupational studies indicate that TCDD may be a carcinogen in heavily exposed humans. There are no studies providing solid evidence of PCBs causing cancer in humans.

Acknowledgements

The authors wish to acknowledge the valuable contributions of the following persons: John Herrman, International Programme on Chemical Safety (IPCS), Geneva, Switzerland; Jan W. Huismans, International Register of Potentially Toxic Chemicals (IRPTC), Geneva, Switzerland; Robert Kroes, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands; John V. Lake, European Environmental Research Organisation (EERO), Wageningen, Netherlands; Robertus M.C. Theelen, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands; Ludo W.A. Vischer, Ministry of Agriculture, Nature Management and Fisheries, The Hague, Netherlands; Erkki Yrjänheikki, World Health Organization, Copenhagen, Denmark; Jacob A. van Zorge, Ministry of Housing, Physical Planning and Environment, Leidschendam, Netherlands. This paper is the outcome of a meeting held from May 6–8, 1992 in Rixensart, Belgium, under auspices of the European Environmental Research Organisation (EERO), Wageningen, Netherlands.

Comments

The EERO invited three external referees to give their opinion on the outcome of the assessment. Their comments are presented below.

Comments by Dr. Andrew G. Smith, MRC Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK

Polyhalogenated aromatic chemicals such as the chlorinated biphenyls (PCBs) and the polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), including the 'prototype' molecule 2,3,7,8-tetrachloro-p-dioxin (TCDD), have been recognized as potential major toxicological risks to the environment and humans for at least 30 years. This is especially the result of their poor metabolism and associated bioaccumulation.

Evaluation of the risks is greatly complicated not only by the huge number of compounds involved with greatly differing potencies (orders of magnitude) but also by widely differing susceptibilities of species. It is often presumed (not always justifiably) that the common mechanism of action of the PCBs, PCDDs, PCDFs and related chemicals depend on their chronic binding to the aryl hydrocarbon (Ah) receptor instigating a cascade of subsequent changes in gene expression.

From knowledge of the affinities of individual chemical for the Ah receptor or the inducibility of the associated gene for cytochrome P4501A1, the toxic equivalency factor (TEF) approach has been developed. This concept allows complex mixtures in biological samples to be given toxic equivalency values (TEQ) to assess their toxic potencies.

In this article, some of the leading workers in the field of polyhalogenated aromatic chemicals have reviewed the origins, bioaccumulation, metabolism and the toxicological impact of the PCBs, PCDDs and PCDFs, especially in the context of the TEF approach.

Although many reviews, reports and books have been written in the last decade on the subject of PCBs, PCDDs and PCDFs, this review is a concise summary of our knowledge and will be useful to anyone wishing a brief introduction to this highly complex and confusing field of toxicology. Some aspects are treated more lightly than others and in a number of instances I was left with the desire for just a little more information. For instance, why do sulphur metabolites of PCBs particularly bind covalently to lung and adrenals? Is there more known of the influence of TCDD in vitamin K-dependent blood coagulation? However, I presume that too much extra information was thought not desirable considering the anticipated brevity of the article.

Perhaps more pertinently than a summary of our knowledge, does this review contribute in an original way to the assessment of the toxicological impact of PCBs, PCDDs and PCDFs? My opinion is that the authors outline the TEF concept well, and how this can be applied to assessment of complex mixtures, including biological samples, but at the same time they quite rightly point to the drawbacks in this approach. Anyone who is experienced in studying the chronic effects of PCBs and TCDD will be familiar with the problems of equating inducibility of cytochrome P4501A1 for example, with the variable toxicological effects in vivo. Binding to the Ah receptor is very probably involved in say the hepatic toxic effects of PCBs and TCDD, but other genes are undoubtedly implicated and may well be dominant with chemicals that bind to the Ah receptor with low affinity. As the authors demonstrate, although the TEF concept depends on additive effects of individual PCB congeners, etc., synergistic and antagonistic influences are also observed. It was good to see some discussion of bioassays. Because of the power and driving force of modern analytical chemistry, there is a tendency to assume that any level of these chemicals is biologically significant. The authors could have speculated on the future of modern biological techniques.

There is also the problem of toxic effects of PCBs and PCDD which are not apparently mediated by the Ah receptor and thus difficult to allow for using TEFs. I was pleased to see the authors discuss this problem and this to me is the most important point in the review. However, I am a little concerned as to the criteria used to put particular toxic effects into the non-Ah-mediated category. The discussions of carcinogenicity, for example, are a little mixed-up and the authors are in danger (as are many other research workers) with confusing promotion of diethylnitrosamine-initiated carcinogenesis with the carcinogenity of PCBs and TCDD per se. Again binding to the Ah receptor may well be involved but the systems are multifactorial and have not yet been dissected rigorously. I suspect eventually it will be difficult to totally separate toxicities into Ah- and non-Ah-mediated effects.

In summary, this is a useful short review of the toxicity of polyhalogenated aromatic chemicals and how assessment of the risks they pose to the environment and human health can be simplified using the TEF approach. At the same time it illustrates the problems with this method of risk assessment. It also reinforced my conviction that for true appreciation of risk as much effort as possible should also be focussed on the absolute mechanisms of action of this pernicious group of chemicals.

Comments by Dr. Ross J. Norstrom, Environment Canada, Canadian Wildlife Service, Hull, Quebec, Canada

The document is correct in its cautious acceptance of the concept of toxic equivalents. The merits and limitations of the approach are adequately discussed. Particularly important messages are (1) that only part of the toxicity of these classes of compounds is described by a TEQ approach, (2) that synergism/antagonism may make the approach unworkable in some species/chemical combinations, and (3) that in vivo kinetics is an important factor in the expression of potential toxicity as measured by bioassays (whether the toxicity is Ah receptor-mediated or not).

Given the large literature base relative to toxic equivalents, not to speak of the other aspects of sources, occurrence, environmental chemistry and analysis of this class of compounds, the document can not be considered to be an exhaustive review. The balance in the depth to which the various topics are covered is highly variable and selective. The document could have

been strengthened if this, the last stage of the review, had been used to alter the final draft, rather than publishing the reviewers comments! Better use should have been made of existing review papers and books. Very sparse and selective reference support is used from the beginning of the document up to 'Kinetics and metabolism'. Considerable use could have been made of individual chapters in the IARC book (Rappe et al., 1991 under 'Listing of national and international assessment reports'), in which sources, exposure, human tissue levels and analysis are quite thoroughly treated. The various analytical methods are presented in great detail in the IARC book. This is germane to recommendation (2) of this document, in which standardized analytical procedures are recommended. There are only two highly specific references to sources of PCBs. The section 'Environmental occurrence' contains seven references, and the section 'Human tissue levels and dietary intake' contains eight references. Only one of these references is in the nature of the review. It would have been better to have left these sections out than treat them so trivially. Why is there no section on occurrence and levels of PCBs? At least the non-ortho PCB literature should have been covered. As a consequence of the above, conclusions (1) and (2) are absolutely not supported by the document, in spite of the fact they are true.

A strong emphasis has been placed on experimental evidence gathered for purposes of human toxicology and risk assessment. This is unfortunate, because much interesting research on development of TEFs and the validity of TEQs, comes from fish and wildlife studies. These studies surely provide as useful information as those derived from laboratory rodents. Although some of the more important wildlife research has not been published in complete form, such as the Swedish PCB-mink toxicity study, most panel members are fully aware of the results. This study demonstrates synergistic effects in reproductive toxicity of a fractionated commercial PCB mixture. Primary toxicity was associated with those PCBs to have a high Ah receptor-mediated toxicity, but this toxicity was enhanced by addition of non-Ah active fractions (Kihlström et al., in press). A significant omission is lack of reference to the findings of Walker and Petersen (Aquat. Toxicol. (1991) 21, 219–238) on the toxicity of PCDD and PCB congeners to rainbow trout. The embryotoxicity of PCDDs and PCBs did not follow the usual pattern of rank order of TEFs in mammals and birds: 2,3,7,8-substituted PnCDD, HxCDD and PnCDF had TEFs > 0.3 while the normally highly toxic PCB-126 had a TEF of only 0.005. These findings are the most definitive that we have to date of the potential problems in the assumption of universal TEFs.

I do not believe enough emphasis is placed on the rapidly expanding knowledge on the toxicity of metabolites, especially PCB metabolites. Although quite a lot is known about the toxic effects of 3,4,3′,4′-TeCB, this compound is relatively minor in nature. Recent research in Sweden at the Wallenberg Laboratory has shown that similar metabolites of major PCB compounds, such as PCB-118 and PCB-105 are present in blood of humans and wildlife. In the case of methylsulfone PCBs, we know that there are a number of specific associations with receptor proteins (other than the Ah receptor) causing highly unusual tissue distributions and that some of these compounds are remarkably persistent. The chapter is certainly not closed on metabolites. Incidentally, methylsulfone metabolites do not bind irreversibly to lung, and it is methylsulfone-DDE that does so in adrenal cells.

Comments by Dr. Heidelore Fiedler and Prof. Dr. Otto Hutzinger, Chair of Ecological Chemistry and Geochemistry, University of Bayreuth, P.O. Box 10 12 51, W-8580 Bayreuth, Germany

The consensus paper of the EERO assessment 'The impact of PCDD, PCDF and PCB on human and environmental health with special implications of the TEF concept' is published under the authorship of 14 scientists from the United States, Canada, and several European countries. It gives a summary of the actual status of knowledge on sources, analytical methods, environmental occurrence, human tissue levels and dietary intake, biochemical effects, development and application of the TEF concept for risk management in various areas. The following comment will mainly refer to the chapters related to environmental chemistry.

The strength is that in a relative short document the most important information is made available to those engaged in risk assessment for mixtures of polychlorinated aromatic compounds. Especially chapter 'Analytical methods' is good and essential. Moreover, intralaboratory control should be included, too. For PCDD/PCDF these analytical standard procedures have been developed and evaluated whereas relatively little has been done for isomer-specific PCB analysis.

Several unclear definitions appear in the document, e.g., 'biota' does not only refer to higher organisms which exclusively only contain 2,3,7,8-substituted PCDD/PCDF. Samples of plant origin, invertebrates, etc., do contain the whole spectrum of dioxins and

furans (including non-toxic PCDD/PCDF).

The term 'secondary source' was used in a very unusual sense: food, drinking water, inhalation, skin absorption are not secondary sources but the result of contamination/concentration in environmental compartments.

The process of phototransformation should be classified into gas phase solution and absorbed state reactions since behaviour is different.

Although PCDD/PCDF and PCBs represent an international problem and scientists from all over the world are involved, not all information available has been taken into consideration, e.g.: the results of the research programme on chloroaromatics by the state of Northrhine-Westphalia (Germany) give details on the fate of chlorinated organic compounds, especially root uptake of PCDD/PCDF from soil has been studied and published in detail. In general the results showed that soil levels are of minor importance. A transfer factor < 0.1 for soil-root uptake has been determined. The international literature data from Hagenmaier (Dioxin '90) showed that the consumption of cold smoked meat is calculated to about 3% of the total average daily uptake of PCDD/PCDF via food.

The international TEFs for PCDD/PCDF(I-TEFs) were developed within the NATO-CCMS study on dioxins and related compounds and have been adapted afterwards by several countries such as the United States (and EPA), Canada, the Netherlands, Germany and others. The EPA-TEFs (developed in 1987) are not identical with the I-TEFs. The first TEFs for PCBs were developed by Safe.

In table 2 Safe's TEFs for PCDD/PCDF are not official. For EPA-TEF and I-TEF, see above. The TEFs for octaCDF in the international and Nordic scheme are 0.001 and not zero.

Listing of national and international assessment reports

Ahlborg, U.G., H. Håkansson, F. Waern and A. Hanberg, 1988, Nordisk dioxinriskbedömning. Rapport fran en nordisk expertgrupp, Nordisk Ministerrådet, Copenhagen, Miljorapport, no. Nord. 49.

Bellin, J.S. and D.G. Barnes, 1989, Interim Procedures for Estimating Risk Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans (CDDs and CDFs), 2nd edn. (USEPA, Washington, DC).

Gallo, M.A., R.J. Scheuplein and C.A. van der Heijden (eds.), 1991, Biological Basis for Risk Assessment of Dioxins and Related Compounds, Banbury Report 35 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

NATO/CCMS, 1988, North Atlantic Treaty (NATO)/Committee on the Challenges of Modern Society (CCMS), Report Number 176.

Pollution Paper no. 27, 1989, Report of an Interdepartmental Working Group on polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), Dioxins in the Environment (Department of the Environment, Central Directorate of Environmental Protection, London, UK).

Rappe, C., H.R. Buser, B. Bodet and I.K. O'Neill, eds., 1991, Environmental carcinogens: methods of analysis and exposure measurement, IARC Sci. Publ. no. 108, Vol. 11, Polychlorinated Dioxins and Dibenzofurans (IARC, Lyon).

USEPA, 1991, Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners, Risk Assessment Forum, USEPA, EPA/625/3-91/020.

WHO-IPCS, 1989, Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, WHO Environmental Health Criteria 88 (World Health Organization, Geneva).

Literature references

Abraham, K., R. Krowke and D. Neubert, 1988, Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 1. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin-O-deethylase in rats following a single injection, Arch. Toxicol. 62, 359.

Abraham, K., T. Wiesmuller, H. Brunner, R. Krowke, H. Hagenmaier and D. Neubert, 1989, Elimination of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in rat faeces, Arch. Toxicol. 63, 75.

Adams, C., M.C. Lans, E. Klasson-Wehler, J.G.M. van Engelen, T.J. Visser and A. Brouwer, 1990, Hepatic thyroid hormone 5'-deiodinase, another target protein for monohydroxy metabolites of 3,3',4,4'-tetrachlorobiphenyl, in: Organohalogen Compounds, Vol. 1, eds., O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 51.

Agrawal, A.K., H.A. Tilson and S.C. Bondy, 1981, 3,4,3',4'-Tetrachlorobiphenyl given to mice prenatally produces long term decreases in striatal dopamine and receptor binding sites in the caudate nucleus, Toxicol. Lett. 7, 417.

Ahlborg, U.G., 1989, Nordic risk assessment of PCDDs and PCDFs, Chemosphere, 19, 603.

Anderson, H.A., 1989, General population exposure to environmental concentrations of halogenated biphenyls, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 325.

Ballschmiter, K., C. Rappe and H.R. Buser, 1989, Chemical properties, analytical methods and environmental levels of PCBs, PCTs, PCNs and PBBs, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 47.

Bannister, R. and S. Safe, 1987, Synergistic interactions of 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexachlorobiphenyl in C57BL/6J and DBA/2J mice: role of the Ah-receptor, Toxicology 44, 159.

Beck, H., K. Eckhart, W. Mathar and R. Wittkowski, 1989, PCDD and PCDF body burden from food intake in the Federal Republic of Germany, Chemosphere 18, 417.

Bellin, J.S. and D.G. Barnes, 1989, Interim Procedures for Estimating Risk Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans (CDDs and CDFs), 2nd edn. (USEPA, Washington, DC).

Bellward, G.D., R.J. Norstrom, P.E. Whitehead, J.E. Elliott, S.M.

Bandiera, C. Dworschak, T. Chang, S. Forbes, B. Cadario and L.E. Hart, 1990, Comparison of polychlorinated dibenzodioxin levels with hepatic mixed-function oxidase induction in great blue herons, J. Toxicol. Environ. Health 30, 33.

Binder, R.L. and J.J. Lech, 1984, Xenobiotics in gametes of Lake Michigan lake trout (*Salvelinus namaycush*) induce hepatic monooxygenase activity in their offspring, Fund. Appl. Pharmacol. 4, 1042.

Birnbaum, L.S. and L.A. Couture, 1988, Disposition of octachlorodibenzo-p-dioxin (OCDD) in male rats, Toxicol. Appl. Pharmacol. 30, 22.

Birnbaum, L.S., G.M. Decad and H.B. Matthews, 1980, Disposition and excretion of 2,3,7,8-tetrachlorodibenzofuran in the rat, Toxicol. Appl. Pharmacol. 55, 342.

Bjeldanes, L.F., J.Y. Kim, K.R. Grose, J.C. Bartholomew and C.A. Bradfield, 1991, Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo-comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin, Proc. Natl. Acad. Sci. USA 88, 9543.

Bol, J., M. van den Berg and W. Seinen, 1989, Interactive effects of PCDDs, PCDFs and PCBs as assessed by the E.L.S.-Bioassay, Chemosphere 19, 899.

Bouwman, C.A., W. Seinen, J.G. Koppe and M. van den Berg, 1990, Effects of 2,3,7,8-TCDD or 2,2′,4,4′,5,5′-HxCB on vitamin K-dependent blood coagulation factors in male and female germfree WAG/Ry rats, in: Organohalogen Compounds, Vol. 1, eds., O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 59.

Bowman, R.E. and M.P. Heironimus, 1981, Hypoactivity in adolescent monkeys perinatally exposed to PCBs and hyperactive as juveniles, Neurobehav. Toxicol. Teratol. 3, 15.

Brandt, I. and C.A. Wachtmeister, 1987, PCB methyl sulphones and related compounds: identification of target cells and tissues in different species, Chemosphere 16, 1671.

Brewster, D.W. and L.S. Birnbaum, 1987, Disposition and excretion of 2,3,7,8-penta-chlorodibenzofuran in the rat, Toxicol. Appl. Pharmacol. 15, 243.

Brouwer, A. and K.J. van den Berg, 1986, Binding of a metabolite of 3,3′,4,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex, carrying both retinol and thyroxin, Toxicol. Appl. Pharmacol. 85, 301.

Brouwer, A., 1991, Role of biotransformation in PCB-induced alterations in vitamin A and thyroid hormone metabolism in laboratory and wildlife species, Biochem. Soc. Transact. 19,731.

Brouwer, A., P.J.A. Reijnders and J.H. Koeman, 1989, Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*), Aquat. Toxicol. 15, 99.

Brouwer, A., E. Klasson-Wehler, M. Bokdam, D.C. Morse and W.A. Traag, 1990, Competitive inhibition of thyroxine binding to transthyretin by monohydroxy metabolites of 3,3′,4,4′-tetrachlorobiphenyl, Chemosphere 20, 1257.

Buchmann, A., W. Kunz, C.R. Wolf, F. Oesch and L.E. Robertson, 1986, Polychlorinated biphenyls, classified as either phenobarbital- or 3-methylcholanthrene-type (PB, or 3-MC) inducers of cytochrome P-450, are both hepatic tumor promoters in diethylnitrosamine-initiated rats, Cancer Lett. 32, 243.

Buchmann, A., S. Ziegler, A. Wolf, L.W. Robertson, S.K. Durham and M. Schwarz, 1991, Effects of polychlorinated biphenyls in rat liver: correlation between primary subcellular effects and promoting activity, Toxicol. Appl. Pharmacol. 111, 454.

Buckley Kedderis, L., J.J. Diliberto, P. Linko, J.A. Goldstein and L.S. Birnbaum, 1991, Disposition of 2,3,7,8-tetrabromodibenzo-p-dioxin in the rat: Biliary excretion and induction of cytochromes CYP1A1 and CYP1A2, Toxicol. Appl. Pharmacol. 111, 163.

Bühler, F., P. Schmid and Ch. Schlatter, 1988, Kinetics of PCB elimination in man, Chemosphere 17, 1717.

Bush, B., L.A. Shane, L.R. Wilson, E.L. Barnard and D. Barnes, 1986, Uptake of polychlorobiphenyl congeners by purple loosestrife (*Lythrum salicaria*) on the banks of the Hudson River, Arch. Environ. Contam. Toxicol. 15, 285.

Calvert, G.M., R.W. Hornung, M.H. Sweeney, M.A. Fingerhut, W.E. Halperin, 1992a, Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin, J. Am. Med. Assoc. 267, 2209.

Calvert, G.M., M.H. Sweeney, J.A. Morris, M.A. Fingerhut, R.W. Hornung, W.E. Halperin, 1992b, Evaluation of chronic bronchitis, chronic obstructive pulmonary disease, and ventilatory function among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin, Am. Rev. Respir. Dis. 144, 1302.

Chen, M., C.S. Hong, B. Bush and G.Y. Rhee, 1988, Anaerobic biodegradation of polychlorinated biphenyls by bacteria from Hudson River sediments, Ecotoxicol. Environ. Saf. 16, 95.

Clement, R. and C. Tashiro, 1991, Forest fires as a source of PCDDs and PCDFs, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991.

Czuczwa, J.M. and R.A. Hites, 1984, Environmental fate of combustion-generated polychlorinated dioxins and furans, Environ. Sci. Technol. 18, 444.

Davis, D. and S. Safe, 1989, Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin, Toxicol. Lett. 48, 35.

Davis, D. and S. Safe, 1990, Immunosuppressive activities of polychlorinated biphenyls in C57BL/6N mice: structure-activity relationships as Ah receptor agonists and partial antagonists, Toxicology 63, 97.

De Jongh, J., F. Wondergem, W. Seinen and M. van den Berg, 1992, Toxicokinetic interactions in the liver of the C57Bl/6J mouse after administration of a single oral dose of a binary mixture of some PCBs, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, September 23–27, 1991, Research Triangle Park, North Carolina, USA, Chemosphere, in press.

Denomme, M.A., S. Bandiera, I. Lambert, L. Copp, L. Safe and S. Safe, 1983, Polychlorinated biphenyls as phenobarbitone-type inducers of microsomal enzymes – structure activity relationships for a series of 2,4-dichloro-substituted congeners, Biochem. Pharmacol. 32, 2955.

Dewailly, E., J.P. Weber, S. Gingras and C. Laliberté, 1991, Coplanar PCBs in human milk in the province of Quebec, Canada: are they more toxic than dioxin for breast fed infants?, Bull. Environ. Contam. Toxicol. 47, 491.

Duinker, J.C., M.T.J. Hillebrand, T. Zeinstra and J.P. Boon, 1989, Individual chlorinated biphenyls and pesticides in tissues of some cetacean species from the North Sea and the Atlantic Ocean: tissue distribution and biotransformation, Aquat. Mammals 15, 95.

Eadon, G., L. Kaminsky, J. Silkworth, K. Aldous, D. Hilker, P. O'Keefe, R. Smith, J.F. Gierthy, J. Hawley, N. Kim and A. DeCapro, 1986, Calculation of 2,3,7,8-TCDD equivalent concentrations of complex environmental contaminant mixtures, Environ. Health Perspect. 70, 221.

Egebäck, A.L., B. Jansson, U. Järnberg, K. Lexén, M. Strandell, C. de Wit, U. Wideqvist, L.O. Kjeller and C. Rappe, 1991, Levels of PCDD, PCDF and PCB in ambient air samples collected in Sweden, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991.

Fein, G.G., J.L. Jacobson, S.W. Jacobson, P.M. Schwartz and J.K.

Dowler, 1984, Prenatal exposure to polychlorinated biphenyls: Effects on birth size and gestational age, J. Pediatr. 105, 315.

Filippini, G., B. Bordo, P. Crenna, N. Massetto, M. Musicco and R. Boeri, 1981, Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-tetrachlorodibenzo-dioxin, Scand. J. Work Environ. Health 7, 257.

Fingerhut, M.A., W.E. Halperin, D.A. Marlow, L.A. Piacitelli, P.A. Honchar, M.H. Sweeney, A.L. Greife, P.A. Dill, K. Steenland and A.J. Suruda, 1991, Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin, New Engl. J. Med. 324, 212.

Fingerman, S.W. and L.C. Russel, 1980, Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the Gulf killifish, *Fundus grandis*, Bull. Environ. Contam. Toxicol. 25, 682.

Fox, G.A., S.W. Kennedy, R.J. Norstrom and D.C. Wigfield, 1988, Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains, Environ. Toxicol. Chem. 7, 831.

Fox, G., A. Collins, E. Hayakawa, D.V. Weseloh and J.P. Ludwig, 1991, Reproductive outcomes in colonial fish-eating birds: A biomarker for developmental toxins in Great Lakes food chains, J. Great Lakes Res. 17, 158.

Gallo, M.A., R.J. Scheuplein and C.A. van der Heijden (eds.), 1991, Biological Basis for Risk Assessment of Dioxins and Related Compounds, Banbury Report 35 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Geyer, H.J., I. Scheunert and F. Korte, 1986, Bioconcentration potential of organic environmental chemicals in humans, Reg. Toxicol. Pharmacol. 6, 313.

Geyer, H.J., I. Scheunert, K. Rapp, A. Kettrup, F. Korte, H. Greim and K. Rozman, 1990, Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and total body fat content in mammals, Toxicology 65, 97.

Geyer, H.J., I. Scheunert and A. Kettrup, 1991, A new approach in estimating the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to humans, Poster presentation at the 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991.

Gilbertson, M., 1989, Effects on fish and wildlife populations, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 103.

Gladen, B.C., W.J. Rogan, P. Hardy, J. Thulen, J. Tingelstad and M. Tully, 1988, Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk, J. Pediatr. 113, 991.

Goldstein, J.A. and S.H. Safe, 1989, Mechanisms of action and structure-activity relationship for the chlorinated dibenzo-p-dioxins and related compounds, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 239.

Hanberg, A., M. Stahlberg, A. Georgellis, C. de Wit and U.G. Ahlborg, 1991, Swedish dioxin survey: Evaluation of the H4IIE bioassay for screening environmental samples for dioxin-like enzyme induction, Pharmacol. Toxicol. 69, 442.

Hara, I., 1985, Health status and PCBs in blood of workers exposed to PCBs and of their children, Environ. Health Perspect. 59, 85.

Helder, Th., 1981, Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on early life stages of rainbow trout (*Salmo gairdneri*, Richardson), Toxicology 19, 101.

Helferich, W.G. and M.S. Denison, 1991, Ultraviolet photoproducts of tryptophan can act as dioxin agonists, Mol. Pharmacol. 40, 674.

Higuchi, K., 1976, PCB Poisoning and Pollution, New York Academic Press, New York.

Hoffman, D.J., B.A. Rattner, L. Sileo, D. Docherty and T.J. Kubiak, 1987, Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's tern on Green Bay, Lake Michigan, Environ. Res. 42, 176.

Jacobson, S.W., G.G. Fein, J.L. Jacobson, P.M. Schwartz and J.K. Dowler, 1985a, The effect of intrauterine PCB exposure in monkeys, Arch. Toxicol. 62, 267.

Jacobson, S.W., G.G. Fein, J.L. Jacobson, P.M. Schwartz and J.K. Dowler, 1985b, The effect of intrauterine PCB exposure on visual recognition memory, Child Dev. 56, 853.

Jacobson, J.L., S.W. Jacobson and H.E.B. Humphrey, 1990a, Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children, J. Pediatr. 116, 38.

Jacobson, J.L., S.W. Jacobson and H.E.B. Humphrey, 1990b, Effects of exposure to PCBs and related compounds on growth and activity in children, Neurotox. Teratol. 12, 319.

Jacobson, J.L., S.W. Jacobson, P.J. Padgett, G.A. Brumitt and R.L. Billings, 1992, Effects of prenatal PCB exposure on cognitive processing efficiency and sustained attention, Dev. Psychol. 28, 297.

Jensen, A.A., 1989, Background levels in humans, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 345.

Jirasek, L., K. Kalensky, K. Kubec, J. Pazderova and E. Lukas, 1974, Chronic poisoning by 2,3,7,8-tetrachlorodibenzo-p-dioxin, Cesk. Dermatol. 49, 145.

Kannan, N., S. Tanabe and R. Tatsukawa, 1988, Potentially hazardous residues of non-ortho chlorine substituted coplanar PCBs in human adipose tissue, Arch. Environ. Health 43, 11.

Kannan, N., S. Tanabe, M. Ono and R. Tatsukawa, 1989, Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: increasing impact of non-ortho and mono-ortho coplanar polychlorinated biphenyls from land to ocean, Arch. Environ. Contam. Toxicol. 18, 850.

Kennedy, S.W. and G.A. Fox, 1990, Highly carboxylated porphyrins as a biomarker of polyhalogenated aromatic hydrocarbon exposure in wildlife: confirmation of their presence in Great Lakes herring gull chicks in the early 1970s and important methodological details, Chemosphere 21, 407.

Kennedy, S.W., A. Lorenzen, C.A. James and R.J. Norstrom, 1992, Ethoxyresorufin-O-deethylase (EROD) and Porphyria induction in chicken embryo hepatocyte cultures – a new bioassay of PCB, PCDD and related chemical contamination in wildlife, Chemosphere 25, 193.

Kimbrough, R.D., 1987, Human health effects of polychlorinated biphenyls and polybrominated biphenyls (PBBs), Ann. Rev. Pharmacol. Toxicol. 27, 87.

Kjeller, L.O., K.C. Jones, A.E. Johnston and C. Rappe, 1991, Increases in the polychlorinated dibenzo-p-dioxin and -furan content of soils and vegetation since the 1840s, Environ. Sci. Technol. 25, 1619.

Koeman, J.H., H.C.W. van Velzen-Blad, R. de Vries and J.G. Vos, 1973, Effects of PCB and DDE in Cormorants and evaluation of PCB residues from an experimental study, J. Reprod. Fert. 19 (suppl.), 353.

Krowke, R., I. Chahoud, I. Baumann-Wilschke and D. Neubert, 1989, Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2. Pharmacokinetics in rats using a loading-dose/maintenance-dose regime with high doses, Arch. Toxicol. 63, 356.

Kubiak, T.J., H.J. Harris, L.M. Smith, T.R. Schwartz, D.L. Stalling, J.A. Trick, L. Sileo, D. Docherty and T.C. Erdmann, 1989, Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan, Arch. Environ. Contam. Toxicol. 18, 706.

Kuroki, J., N. Koga and H. Yoshimura, 1986, High affinity of 2,3,4,7,8-pentachloro-dibenzofuran to cytochrome P-450 in the hepatic microsomes of rats, Chemosphere, 15, 731.

Kutz, F.W., D.G. Barnes, E.W. Bretthauer, D.P. Bottimore and H. Greim, 1990, The international toxicity equivalence factor (I-TEF) method for estimating risks associated with exposures to complex mixtures of dioxins and related compounds, Toxicol. Environ. Chem. 26, 99.

Lans, M.C., G. Kubiczak, J. Douwes and A. Brouwer, 1990, In vitro effects of 3,3',4,4',-tetrachlorobiphenyl and its hydroxy-metabolites on mitochondrial function, in: Organohalogen Compounds, Vol. 1, eds. O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 103.

Lans, M.C., P. de Winden, S. Safe and A. Brouwer, 1991, Effects of hydroxy-dioxins on thyroid hormone binding to transthyretin and thyroid hormone type 1 deiodinase activity in vitro and in vivo [abstract], 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991.

Levin, E.D., S.L. Schantz and R.E. Bowman, 1988, Delayed spatial alteration deficits resulting from perinatal PCB exposure in monkeys, Arch. Toxicol. 62, 267.

Liem, A.K.D., A.P.J.M. De Jong, R.M.C. Theelen, J.H. van Wijnen, P.C. Beijen, H.A. van der Schee, H.A.M.G. Vaessen, G. Kleter and J.A. van Zorge, 1992, Occurrence of dioxins and related compounds in Dutch foodstuffs, Part I: Sampling strategy and analytical results, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991, Chemosphere, in press.

Manz, A., J. Berger, J.H. Dwyer, D. Flesh-Janys, S. Nagel and H. Waltsgott, 1991, Cancer mortality among workers in chemical plant contaminated with dioxin, Lancet, 338 (8773), 959.

Mason, G. and S. Safe, 1986, Synthesis, biologic and toxic effects of the major 2,3,7,8-tetrachlorodibenzo-p-dioxin metabolites in the rat, Toxicology 41, 153.

McConnell, E.E., 1989, Acute and chronic toxicity and carcinogenesis in animals, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 161.

Morse, D.C., H.B.W.M. Koëter, A.E. Smits van Prooijen and A. Brouwer, 1992, Interference of polychlorinated biphenyls in thyroid hormone metabolism: possible neurotoxic consequences in fetal and neonatal rats, Chemosphere, in press.

Moses, M., R. Lilis, K.D. Crow, J. Thorton, Fischbein, H.A. Anderson and I.J. Selikoff, 1984, Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichloro-phenoxyacetic acid: Comparison of findings with and without chloracne, Am. J. Ind. Med. 5, 161.

Neal, R.A., J.R. Olson, T.A. Gasiewicz and L.E. Geiger, 1982, The toxicokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian systems, Drug Metab. Rev. 13, 355.

Neubert, D., T. Wiesmuller, K. Abraham, R. Krowke, H. Hagenmaier, 1990, Persistence of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in hepatic and adipose tissue of marmoset monkeys, Arch. Toxicol. 64, 431.

Norback, D.H. and R.H. Weltman, 1985, Polychlorinated biphenyl induction of hepatocellular carcinoma in the Sprague-Dawley rat, Environ. Health Perspect. 60, 97.

Norstrom, R.J., M. Simon, D.C.G. Muir and R.E. Schweinberg, 1988, Organochlorine contaminants in arctic food chains: Identification, geographical distribution, and temporal trends in polar bears, Environ. Sci. Technol. 22, 1063.

Norstrom, R.J., M. Simon and D.C.G. Muir, 1990, Polychlorinated dibenzo-p-dioxins and dibenzofurans in marine mammals in the Canadian North, Environ. Pollut. 66, 1.

Öberg, L.G., R. Andersson and C. Rappe, 1992, De novo formation of hepta- and octachlorodibenzo-p-dioxins from pentachlorophenol in municipal sewage sludge (Institute of Environmental Chemistry, University of Umeå, Sweden).

Olson J.R., 1986, Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin in guinea pigs, Toxicol. Appl. Pharmacol. 85, 263.

Olson J.R., T.A. Gasiewicz and R.A. Neal, 1980, Tissue distribution, excretion and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the golden Syrian hamster, Toxicol. Appl. Pharmacol. 56, 78.

Parkinson, A. and S. Safe, 1987, Mammalian biologic and toxic effects of PCBs, in: Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology. Environmental Toxin Series, Vol. I, eds. S. Safe and O. Hutzinger (Springer-Verlag, Heidelberg) p. 49.

Patterson, Jr., D.G., M.A. Fingerhut, D.W. Roberts, M.H. Sweeney, D.A. Marlow, J.S. Andrews, Jr. and W.E. Halperin, 1989, Levels of polychlorinated dibenzo-p-dioxin (PCDDs) and dibenzofurans (PCDFs) in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin, Am. J. Ind. Med. 16, 135.

Pirkle, J.L., W.H. Wolfe, D.G. Patterson, L.L. Needham, J.E. Michalek, J.C. Miner, M.R. Peterson and D.L. Phillips, 1989, Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Vietnam veterans of operation Ranch Hand, J. Toxicol. Environ. Health 27, 165.

Piskorska-Pliszczynska, J., B. Keys, S. Safe and M.S. Newman, 1986, The cytosolic receptor binding affinities and AHH induction potencies of 29 polynuclear aromatic hydrocarbons, Toxicol. Lett. 34, 67.

Pluess, N., H. Poiger, C. Schlatter and H.R. Buser, 1987, The metabolism of some pentachlorodibenzofurans in the rat, Xenobiotica 17, 209.

Pluess, N., H. Poiger, C. Hohbach and C. Schlatter, 1988, Subchronic toxicity of some chlorinated dibenzofurans (PCDFs) and a mixture of PCDFs and chlorinated dibenzodioxins (PCDDs) in rats, Chemosphere 17, 973.

Pohjanvirta R., T. Vartiainen, A. Uusi-Rauva, J. Monkkonen and J. Tuomisto, 1990, Tissue distribution, metabolism and excretion of ^{14}C-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain, Pharmacol. Toxicol. 66, 93.

Poiger, H. and H.R. Buser, 1984, The metabolism of TCDD in the dog and rat, Banbury Report 18: Biological Mechanisms of Dioxin Action, 39.

Poiger, H. and C. Schlatter, 1986, Pharmacokinetics of 2,3,7,8-TCDD in man, Chemosphere, 15, 1489.

Poiger H., H.R. Buser and C. Schlatter, 1984, The metabolism of 2,3,7,8-tetrachlorodibenzofuran in the rat, Chemosphere 13, 351.

Poland, A. and J.C. Knutson, 1982, 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity, Ann. Rev. Pharmacol. Toxicol. 22, 517.

Rannug, A., U. Rannug, H.S. Rosenkranz, L. Winqvist, R. Westerholm, E. Agurell and A-K. Grafström, 1987, Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances, J. Biol. Chem. 262, 15422.

Rappe, C., 1991, Sources of and human exposure to PCDDs and PCDFs, Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and Related Compounds (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) p. 121.

Rappe, C. and H.R. Buser, 1989, Chemical and physical properties, analytical methods, sources and environmental levels of halogenated dibenzodioxins and dibenzofurans, in: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam) p. 71.

Rappe, C., L.O. Kjeller, S.E. Kulp, C. de Wit, I. Hasselsten and O. Palm, 1991, Levels, profile and pattern of PCDDs and PCDFs in

samples related to the production and use of chlorine, Chemosphere, 23, 1629.

Reischl, A., M. Reissinger, H. Thoma and O. Hutzinger, 1989, Accumulation of organic air constituents by plant surfaces: Part IV, Chemosphere 18, 561.

Reijnders, P.J.A., 1986, Reproductive failure in common seals feeding on fish from polluted coastal waters, Nature 324, 456.

Robertson, L.W., E.M. Silberhorn and H.P. Glauert, 1991, Do structure-activity relationships for the acute toxicity of PCBs and PBBs also apply for induction of hepatocellular carcinoma?, Environ. Toxicol. Chem. 10, 715.

Rogan, W.J., B.C. Gladen, J.D. McKinney, et al. 1986, Neonatal effects of transplacental exposure to PCBs and DDE, J. Pediatr. 109, 335.

Rogan, W.J., B.C. Gladen, K. Hung, S. Koong, L. Shih, J.S. Taylor, Y. Wu, D. Yang, N.B. Ragan and C. Hsu, 1988, Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan, Science 241, 334.

Rose, J.Q., J.C. Ramsey, T.H. Wentzler, R.A. Hummel and P.J. Gehring, 1976, The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat, Toxicol. Appl. Pharmacol. 36, 209.

Ryan, J.J. and Y. Masuda, 1992, Elimination of polychlorinated dibenzofurans (PCDFs) in humans from the Yusho and Yucheng rice oil poisonings, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991, Chemosphere, in press.

Safe, S.H., 1984, Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action, CRC Crit. Rev. Toxicol. 12, 319.

Safe, S.H., 1986, Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. Ann. Rev. Pharmacol. Toxicol. 26, 371.

Safe, S.H., 1989, Polyhalogenated aromatics: uptake, disposition and metabolism. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd edn., eds. R.D. Kimbrough and A.A. Jensen (Elsevier, Amsterdam), 131.

Safe, S.H., 1990, Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs), Crit. Rev. Toxicol. 21, 51.

Safe, S.H., L.W. Robertson, L. Safe, A. Parkinson, S. Bandiera, T. Sawyer and M.A. Campbell, 1982, Halogenated biphenyls: molecular toxicology, Can. J. Physiol. Pharmacol. 60, 1057.

Sargent, L.M., G.L. Sattler, B. Roloff, Y.H. Xu, C.A. Sattler, L. Meisner and H.C. Pitot, 1992, Ploidy and specific karyotypic changes during promotion with phenobarbital, 2,2′,5,5′-tetrachlorobiphenyl and/or 3,3′4,4′-tetrachlorobiphenyl in rat liver, Cancer Res. 52, 955.

Schantz, S.L., E.D. Levin, R.E. Bowman, M.P. Heironimus and N.K. Laughlin, 1989, Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys, Neurotoxicol. Teratol. 11, 243.

Schantz, S.L., J.L. Jacobson, S.W. Jacobson and H.E.B. Humphrey, 1990, Behavioural correlates of polychlorinated biphenyl (PCB) body burden in school-aged children, Toxicologist 10, 303.

Schantz, S.L., E.D. Levin and R.E. Bowman, 1991, Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys, Environ. Toxicol. Chem. 10, 747.

Scheutz, E.G., S.A. Wrighton, S. Safe and P.S. Guzelian, 1986, Regulation of cytochrome P-450p by phenobarbital and phenobarbital-like inducers in adult rat hepatocytes in primary monolayer culture, In Vivo Biochem. 25, 1124.

Schlatter, C., 1991, Data on kinetics of PCDDs and PCDFs as a prerequisite for human risk assessment in: Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and related compounds, eds. M.A. Gallo, R.J. Scheuplein and K.A. van der Heijden (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) p. 215.

Seegal, R.F., 1992, Perinatal exposure to Aroclor 1016 elevates brain dopamine concentrations in the rat, Toxicologist 12, 320.

Seegal, R.F. and W. Shain, 1992, Neurotoxicity of polychlorinated biphenyls: The role of ortho-substituted congeners in altering neurochemical function, in: The Vulnerable Brain and Environmental Risks, Vol. 2, Toxins in Food, eds., R.L. Isaacson and K.F. Jensen (New York, Plenum Press), p. 169.

Seegal, R.F., B. Bush and W. Shain, 1990a, Lightly chlorinated ortho-substituted PCB congener decrease dopamine in nonhuman primate brain and in tissue culture, Toxicol. Appl. Pharmacol. 106, 136.

Seegal, R.F., B. Bush and W. Shain, 1990b, Neurotoxicology of PCBs: a novel structure-activity relationship, in: Organohalogen Compounds vol. 1, eds. O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 157.

Seegal, R.F., B. Bush and W. Shain, 1990c, PCB neurotoxicity is associated with non-planar ortho-substituted congeners, Toxicologist 10, 252.

Seegal, R.F., B. Bush and K.O. Brosch, 1991, Comparison of effects of Aroclors 1016 and 1260 on nonhuman primate catecholamine function, Toxicology 66, 145.

Shain, W., S.R. Overmann, L.R. Wilson, J. Kostas and B. Bush, 1986, A congener analysis of polychlorinated biphenyls accumulating in rat pups after perinatal exposure, Arch. Environ. Contam. Toxicol. 15, 687.

Shain, W., B. Bush and R.F. Seegal, 1991, Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners, Toxicol. Appl. Pharmacol. 111, 33.

Silberhorn, E.M., H.P. Glauert and L.W. Robertson, 1990, Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs, Crit. Rev. Toxicol. 20, 439.

Smith, L.M., D.L. Stalling and J.L. Johnson, 1984, Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples, Anal. Chem. 56, 1830.

Smith, A.G., J.E. Francis and P. Carthew, 1990, Iron as a synergist for hepatocellular carcinoma induced by polychlorinated biphenyls in Ah-responsive C57BL/10ScSn mice, Carcinogenesis 11, 437.

Spics, R.B. and D.W. Rice, 1988, Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory, Mar. Biol. 98, 191.

Stegeman, J.J. and J.J. Lech, 1991, Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure, Environ. Health Perspect. 90, 101.

Stegeman, J.J., B.R. Woodin and A. Goksoyr, 1988, Apparent cytochrome P-450 induction and as an indicator of exposure to environmental chemicals in the flounder *Platichthys flesus*, Mar. Ecol. Prog. Ser. 46, 55.

Stephens, R.D., C. Rappe, D. Hayward, M. Nygren, J. Startin, P. Christensen and E. Yrjänheikki, 1992, WHO International interlaboratory calibration on PCDD and PCDFs in human blood and milk, analysis of results of laboratories, analytical methods and data quality for the seventeen toxic congeners, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991, Chemosphere, in press.

Svenson, B.G., A. Nilsson, M. Hansson, C. Rappe, B. Åkesson and S. Kerfving, 1991, Exposure to Dioxins and Dibenzo furans through the consumption of fish. New Engl. J. Med., January issue, 8.

Swanson, S.E, 1988, Dioxins in the bleach plant (Institute of Environmental Chemistry, University of Umeå, Sweden).

Sweeney, M.H., 1990, Evaluation of the peripheral nervous system among workers employed in the production of chemicals contam-

inated with 2,3,7,8-tetrachloro-dibenzo-p-dioxin [dissertation] (Ann Arbor (MI): University of Michigan).

Tanabe, S., N. Kannan, A. Subramanian, S. Watannabe and R. Tatsukawa, 1987, Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans, Environ. Pollut. 47, 147.

Thoma, H., W. Mucke, E. Kretschemer, 1989, Concentrations of PCDD and PCDF in human fat and liver samples, Chemosphere 18, 491.

Tillit, D.E., J.P. Giesy and G.T. Ankley, 1991, Characterization of the H4IIE rat hepatoma cell bioassay as a tool for assessing toxic potency of planar halogenated hydrocarbons in environmental samples, Environ. Sci. Technol. 25, 87.

Tillit, D.E., G.T. Ankley, J.P. Giesy, J.P. Ludwig, H. Kurita-Matsuba, D.V. Weseloh, P.S. Ross, C. Bishop, L. Sileo, K.L. Stromberg, J. Larson and T.J. Kubiak, 1992, Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes, Environ. Toxicol. Chem., in press.

Tilson, H.A., J.L. Jacobson and W.J. Rogan, 1990, Polychlorinated biphenyls and developing nervous system: cross-species comparisons, Neurotoxicol. Teratol. 12, 239.

Tuomisto, J. and P. Männistö, 1985, Neurotransmitter regulation of anterior pituitary hormones, Pharmacol. Rev. 37, 249.

Tysklind, M. and C. Rappe, 1991, Photolytic transformation of polychlorinated dioxins and dibenzofurans in complex mixtures in: Organohalogen compounds, Vol. 1, eds. O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 385.

USEPA, 1991, Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners, Risk Assessment Forum, USEPA, EPA/625/3–91/020.

Van Bavel, B., I. Fängmark, S. Marklund, G. Söderström, K. Ljung and C. Rappe, 1992, Quantitative analysis or organic trace compounds by isotope dilution: formation of the non-ortho PCBs 77, 126, 169 during incineration. Paper presented at Dioxin '92, August 24–28, 1992, Tampere, Finland.

Van Birgelen, A.P.M.J., J. van der Kolk, H. Poiger, M. van den Berg and A. Brouwer, 1992, Interactive effects of 2,2′,4,4′,5,5′-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin on thyroid hormone, vitamin A and vitamin K metabolism in the rat, Chemosphere, in press.

Van den Berg, M., F. Blank, C. Heeremans and K. Olie, 1987a, The presence of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in fish-eating birds and fish from the Netherlands, Arch. Environ. Contam. Toxicol. 16, 149.

Van den Berg, M., C. Heeremans, E. Veenhoven and K. Olie, 1987b, Transfer of poly-chlorinated dibenzo-p-dioxins and dibenzofurans to fetal and neonatal rats, Fundam. Appl. Toxicol. 9, 635.

Van den Berg, M., J. de Jongh, P. Eckhart and F.W. van der Wielen, 1989, Disposition and elimination of three polychlorinated dibenzofurans in the liver of the rat, Fundam. Appl. Toxicol. 12, 738.

Van den Berg, M. and H. Poiger, 1990, Toxicokinetic limitations in the present Toxic Equivalency (TEQ) concept, in: Organohalogen Compounds, Vol 1, eds. O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 333.

Van den Berg, M., L.H.J. Craane, T. Sinnige, I.J. Lutke-Schipholt, B. Spenkelink and A. Brouwer, 1992, The use of biochemical parameters in comparative toxicological studies with fish-eating birds in the Netherlands, Chemosphere, in press.

Van der Kolk, J., A. van Birgelen and H. Poiger, 1992, Interactions of 2,2′,4,4′,5,5′-HxCB and 2,3,7,8-TCDD in a subchronic feeding study in rats, 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin '91, Research Triangle Park, NC, USA, September 23–27, 1991, Chemosphere, in press.

Van Zorge, J.A., J.H. van Wijnen, R.C. Theelen, K. Olie and M. van den Berg, 1988, Assessment of the toxicity of halogenated dibenzo-p-dioxins and dibenzofurans by use of toxicity equivalent factors (TEFs), Chemosphere, 12, 1881.

Voorman, R. and S.D. Aust, 1989, TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a tight binding inhibitor of cytochrome P-450d, J. Biochem. Toxicol. 4, 105.

Wearn, F., S. Flodström, L. Busk, T. Kronevi, I. Nordgren and U.G. Ahlborg, 1991, Relative liver tumor promoting activity and toxicity of some polychlorinated dibenzo-p-dioxin- and dibenzofuran-congeners in female Sprague-Dawley rats, Pharmacol. Toxicol. 69, 450.

Weber, H., H. Poiger and C. Schlatter, 1982, Acute oral toxicity of TCDD-metabolites in male guinea pigs, Toxicol. Lett. 14, 117.

Webster, T. and P. Connet, 1990, Estimating bioconcentration factors and halflives in humans using physiologically based pharmacokinetic modelling, part I: 2,3,7,8-TCDD. In: Dioxin '90 Epri-Seminar Short Papers, Toxicology-Environment, Food, Exposure-Risk, in: Organohalogen Compounds, Vol. 1, eds., O. Hutzinger and H. Fiedler (Eco Informa Press, Bayreuth, Germany) p. 611.

Whitlock, Jr., J.P., 1990, Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. Ann. Rev. Pharmacol. Toxicol. 30, 251.

Wolfe, W.H., J.E. Michalek and J.C. Miner, 1991, An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides, in: Air Force Health Study Vols. I–IX, Analysis of 1987 Examination Results, Contract No. F41689-85-D-0010, SAIC Project No. 1-813-X4-195/254/437/011/942/943.

Wong, K.C. and M.Y. Hwang, 1981, Children born to PCB poisoned mothers, Clin. Med. (Taipei) 7, 83.

World Health Organization – Regional Office for Europe, 1988, Assessment of Health Risks in Infants Associated with Exposure to PCBs, PCDDs and PCDFs in Breast Milk, Environmental Health Series no. 29, eds. P. Granjean, S. Tarkowski, R. Kimbrough, E.J. Yrjänheikki and J.H. Rantanen (WHO, Geneva).

World Health Organization – Regional Office for Europe, 1989, Levels of PCBs, PCDDs, and PCDFs in Breast Milk, Environmental Health Series no. 34, ed. E.J. Yrjänheikki (WHO, Geneva).

Wroblewski, V.J. and J.R. Olson, 1985, Hepatic metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat and guinea pig. Toxicol. Appl. Pharmacol. 81, 231.

Yamashita, F. and M. Hayashi, 1985, Fetal PCB syndrome: Clinical features, intrauterine growth retardation and possible alteration in calcium metabolism, Environ. Health Perspect. 59, 41.

Zacharewski, T.L., L. Safe, S. Safe, B. Chittim, D. DeVaultu, K. Wilberg, P.-A. Bergqvist and C. Rappe, 1989, Comparative analysis of polychlorinated dibenzo-p-dioxin and dibenzofuran congeners in Great Lakes fish extracts by gas chromatography-mass spectrometry and in vitro enzyme induction activities, Environ. Sci. Technol. 23, 730.

Zober, A., P. Messerer and P. Huber, 1990, Thirty-four-year mortality follow-up of BAsf employees exposed to 2,3,7,8-TCDD after the 1953 accident, Int. Arch. Occup. Environ. Health 62, 139.