Synergistic Effect of 2,2′,4,4′,5,5′-Hexachlorobiphenyl and 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin on Hepatic Porphyrin Levels in the Rat

Angélique P.J.M. van Birgelen,[1,2] Kitty M. Fase,[1] Jolanda van der Kolk,[3] Hermann Poiger,[3] Abraham Brouwer,[2] Willem Seinen,[1] and Martin van den Berg[1]

[1]Research Institute of Toxicology, University of Utrecht, 3508 TD Utrecht, The Netherlands; [2]Department of Toxicology, Agricultural University, 7600 EA Wageningen, The Netherlands; [3]Institute of Toxicology, CH-8603 Schwerzenbach, Switzerland

We studied the effect of polychlorinated biphenyls (PCBs) on hepatic porphyrin accumulation in female Sprague-Dawley rats by feeding them diets containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB 156), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), or combinations of the single PCB congeners with TCDD for 13 weeks. A dose-dependent increase in hepatic porphyrin accumulation occurred after TCDD, PCB 126, or PCB 156 administration, reaching maximal levels of about twice control values. The lowest dose levels for which a significant increase in hepatic porphyrin accumulation was found were 0.7 µg TCDD/kg diet, 50 µg PCB 126/kg diet, or 6 mg PCB 156/kg diet. These doses are equivalent to 47 ng TCDD/kg/day, 3.2 µg PCB 126/kg/day, and 365 µg PCB 156/kg/day. Relative potencies for hepatic porphyrin accumulation, using TCDD as a reference, ranged from 0.015 to 0.06 for PCB 126 and from 0.0001 to 0.0003 for PCB 156. CYP1A2 activities significantly correlated with hepatic porphyrin levels, with coefficients of 0.629, 0.483, or 0.808 for TCDD, PCB 126, or PCB 156, respectively. Administration of PCB 153 alone did not result in hepatic porphyrin accumulation. Co-administration of PCB 153 and TCDD revealed a strong synergistic effect on porphyrin accumulation (about 800 times control levels). This synergistic effect was significant in rats fed diets containing any combination of PCB 153 with TCDD. Uroporphyrin III and heptacarboxylic porphyrin were accumulated in porphyrinogenic livers. These results suggest that TCDD induction of CYP1A2 may be involved, leading to oxidation of uroporphyrinogen III to uroporphyrin III, in combination with an increase in δ-aminolevulinic acid synthetase induced by PCB 153. Under porphyrinogenic conditions, an inhibitor of CYP1A2 activity may also be formed. The interactive effects on porphyrin accumulation after co-administration of dioxinlike and non–dioxinlike compounds may have significant implications for the risk assessment of these chemicals. *Key words*: Ah-receptor, chemical synergism, polychlorinated biphenyl, porphyria, porphyrins, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 104:550–557 (1996)

The porphyrias are a group of clinical symptoms associated with inherited or induced disturbances in heme biosynthesis. Porphyria cutanea tarda (PCT), the most common hepatic porphyria, occurs in humans with an inherited deficiency of hepatic uroporphyrinogen decarboxylase (UROD), an enzyme involved in the decarboxylation of uroporphyrinogen to coproporphyrinogen. The excess of hepatic uroporphyrinogen resulting from this deficiency is eventually excreted in the urine as uroporphyrin. However, alcohol ingestion is a common precipitating cause (*1–3*).

Following a disastrous hexachlorobenzene (HCB) poisoning in southeastern Turkey in the 1960s, victims displayed signs of disturbed heme synthesis, resulting in massive urinary excretion of porphyrins and hepatic accumulation of porphyrins, consistent with PCT (*4,5*). PCT was also reported in industrial workers producing the herbicides 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (*6,7*). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was found to be a contaminant in these herbicides. TCDD is a potent porphyrinogenic agent in rats and mice (*8–10*). The Seveso accident in Italy involving human exposure to TCDD revealed an increase in urinary coproporphyrin (*11*). Human exposure to a complex mixture of polychlorinated dibenzofurans (PCDFs) and biphenyls (PCBs) in the Taiwanese Yu-Cheng poisoning resulted in elevated urinary excretion of uroporphyrin and aminolevulinic acid (ALA), both precursors of heme synthesis (*12*).

Porphyrin accumulation has also been observed under field conditions and in experimental animals. Herring gulls (*Larus argentatus*) from colonies throughout the Great Lakes showed elevated hepatic porphyrin concentrations in comparison to colonies from coastal areas (*13*). The Great Lakes have been associated with a high contamination of polyhalogenated aromatic hydrocarbons such as polychlorinated dibenzo-*p*-dioxins (PCDDs), PCDFs, and PCBs.

In laboratory animals, a clear induction of PCT-like signs, i.e., accumulation of hepatic uroporphyrinogen and heptacarboxylic porphyrin, induction of δ-aminolevulinic acid synthetase (ALAS) activity, and inhibition of UROD activity, have been observed after exposure to TCDD and related compounds. Jones and Sweeney (*14*) studied the porphyrinogenic action of TCDD in genetically responsive C57BL/6 and nonresponsive DBA mice. Susceptibility to porphyria correlated with aryl hydrocarbon hydroxylase (AHH) inducibility, indicating that the aryl hydrocarbon (Ah) receptor may be involved in this response. In addition, chronic exposure of rats to TCDD caused hepatic porphyria, which could not be established after acute exposure to TCDD (*9*). Accumulation of hepatic porphyrins in mice during a 10-week feeding study was also found after exposure to 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169) or Kanechlor-500 administration (*15*). After 3 weeks of dietary exposure, porphyrins were manifest, ALAS activity was increased, and UROD activity was decreased.

In spite of a broad range of adverse human health effects observed after high-level exposure (*16,17*), the major public concern involves the protection of the general population against these compounds due to background exposure. The worldwide occurrence of PCDDs, PCDFs, and PCBs has led to concern and a need for risk assessment with regard to the general population. This need is strengthened by the adverse effects on various (neuro)developmental parameters at low-level, background exposure (*18–21*).

The mechanism of action of TCDD and related compounds involves an initial

Address correspondence to A.P.J.M. van Birgelen, U.S. Environmental Protection Agency, MD-74, Research Triangle Park, NC 27711 USA. J. van der Kolk is currently at Springborn Laboratories, PO Box 9326, Horn, Switzerland.

We are grateful to P.R. Sinclair and L.S. Birnbaum for the insightful discussions regarding porphyrin metabolism. We thank D.G. Ross for the skillful assistance with the Western blot analysis of CYP1A2. This work was supported by The Technology Foundation, The Netherlands (grant UDG 92.1869), The Swiss National Foundation, Switzerland (grant 2-77-330.91), and the Chemical Manufacturer Association. This work was presented in part at the 15th International Symposium on Chlorinated Dioxins and Related Compounds, Edmonton, Canada, August 1995.

Received 30 August 1995; accepted 17 January 1996.

binding to the Ah receptor (*22,23*). A clear correlation between the Ah receptor-mediated biochemical and toxic effects was observed in a number of laboratory studies (*16,17*). As a result, quantitative structure–activity relationships were obtained, subsequently leading to the development of the toxic equivalency concept for this group of compounds. In this concept, each compound is assigned a toxic equivalency factor (TEF) that reflects its relative toxic and biochemical potency to TCDD, the most potent compound, assigned a TEF value of 1. A prerequisite for the TEF concept is additive toxicity, which is supported by *in vivo* studies with mixtures of PCDDs and PCDFs (*24*), mixtures of PCDFs (*25*), mixtures of TCDD and PCBs (*26,27*), and by *in vitro* studies with mixtures of PCBs and PCDFs (*28*). The Ah receptor-mediated toxic potency of mixtures of PCDDs, PCDFs, and PCBs is therefore calculated by the summation of the toxic equivalents (TEQs), i.e., the product of the concentration and the TEF value of each individual congener.

However, antagonistic as well as synergistic effects have been found after co-administration of binary mixtures in single-dose, short-term experiments in rodents using hepatic cytochrome P450 isozymes, immunotoxicity, and teratogenicity as endpoints (*29–33*). After chronic exposure to 2,2′,4,4′,5,5′- and 3,3′,4,4′,5,5′-hexabromobiphenyl in rats, Jensen and Sleight (*34*) found synergism in hepatic tumor promotion.

By using interim TEF values (*17,35,36*), some co-planar and mono-*ortho*-substituted PCBs account for a significant contribution to the total TEQ in biotic samples (*37–39*). On this basis, and as a consequence of the nonadditive effects reported, toxicity studies involving mixture exposure using PCBs are important for risk assessment. For the PCB congeners, relevant PCBs were chosen from each PCB group (di-*ortho*-substituted, mono-*ortho*-substituted, and co-planar PCBs). This choice was based on high relative toxicity and concentration in human milk and fat tissue (*40–42*).

Additionally, a subchronic dosing regime was chosen in view of extrapolation for risk assessment of these compounds. Since risk assessment based on intake dose does not involve kinetic aspects of the compound to be evaluated, extrapolation based on intake dose will fail when large kinetic differences exist between species (*43,44*).

In the present study, subchronic effects on hepatic porphyrin accumulation were studied in rats fed diets containing 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB 156), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), or TCDD. At the same time, the single PCB congeners were co-administered with TCDD to study possible interactive effects. The concentration ratios used were comparable with those found in human milk and fat samples.

Materials and Methods

Chemicals. TCDD (purity 99%) was synthesized by Dow Chemical (Midland, Michigan). PCB 126 (purity 99%) as used in the subchronic study was obtained from Schmidt B.V. (Amsterdam). PCB 153 (purity >99.9%) as used in the subchronic study was synthesized according to Hutzinger and Safe (*45*) as described earlier (*46*). PCB 126 and PCB 153 (purities >98%) as used in the *in vitro* inhibition experiment were from Ultra Scientific. PCB 156 was synthesized according to Mullin and co-workers (*47*) as previously described (*26*). Uroporphyrinogen I, coproporphyrin III, (copro III), and a marker kit containing 2-, 4-, 5-, 6-, 7-, and 8-carboxyl porphyrin isomers I were obtained from Porphyrin Products Inc. (Logan, Utah). Acetanilide, 4-hydroxyacetanilide, 3-hydroxyacetanilide, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Methoxyresorufin was purchased from Molecular Probes Inc. (Eugene, Oregon). The other chemicals used were of analytical grade and were obtained from Merck AG (Darmstadt, Germany), BDH Chemicals Ltd. (Poole, Dorset, UK), or Sigma.

Animals and treatment. Female Sprague-Dawley rats [Iva: S/V 50 (SD)], Ivanovas (Kissley, Germany), 7 weeks old, weighing about 150 g, were kept on a standard laboratory diet (Nafag 890, Gossau, Switzerland) for 1 week before the experiment. One day before the start of the 13-week feeding experiments, the rats were randomly divided into groups of eight (first experiment) or nine (second experiment) animals with a similar mean and standard deviation in body weights. The diets, in pulverized form, were prepared according to Pluess and co-workers (*24*) and contained PCB 153, PCB 156, PCB 126, TCDD, or combinations of the PCBs with TCDD (Tables 1 and 2). Water and food were given *ad libitum*. The rats were housed three or four per cage and held under controlled conditions of temperature (20°C) and lighting (12-hr light/dark cycle).

After termination of the experiments, the animals were killed under diethylether anesthesia by taking blood of the inferior vena cava. The liver was removed, rinsed in physiological saline solution, and weighed. Parts of the liver were frozen in liquid nitrogen and stored at -70°C until cytochrome

Table 1. Dose groups, daily doses of PCB 156, PCB 126, and/or TCDD, and hepatic porphyrin levels after 13 weeks on diets containing PCB 126, PCB 156, and/or TCDD (means ± SE, $n = 8$)

Amount in diet			Daily dose[a]			
TCDD (µg/kg)	PCB 126 (µg/kg)	PCB 156 (mg/kg)	TCDD (ng/kg/day)	PCB 126 (µg/kg/day)	PCB 156 (µg/kg/day)	Porphyrins (µg/g liver)
0	0	0	0	0	0	0.494 ± 0.059[b]
0.2	0	0	14	0	0	0.494 ± 0.039
0.4	0	0	26	0	0	0.686 ± 0.066
0.7	0	0	47	0	0	0.827 ± 0.073*
5	0	0	320	0	0	0.954 ± 0.053*
20	0	0	1024	0	0	0.889 ± 0.059*
0	7	0	0	0.47	0	0.764 ± 0.117
0	50	0	0	3.18	0	0.853 ± 0.086*
0	180	0	0	10.1	0	0.841 ± 0.075*
0.4	7	0	27	0.48	0	0.694 ± 0.061
0.4	50	0	26	3.25	0	1.05 ± 0.10**
0.4	180	0	23	10.4	0	1.01 ± 0.06[c]**
5	7	0	315	0.44	0	0.899 ± 0.062
5	50	0	306	3.06	0	1.12 ± 0.10
5	180	0	269	9.68	0	0.913 ± 0.037
0	0	1.2	0	0	81	0.607 ± 0.061
0	0	6	0	0	365	0.956 ± 0.098[b]*
0	0	12	0	0	729	1.17 ± 0.11*
5	0	1.2	317	0	76	1.14 ± 0.09†
5	0	6	305	0	366	1.19 ± 0.08
5	0	12	290	0	696	1.18 ± 0.15[b]

[a]Daily doses are estimated average values based on estimated food consumption, dietary level of compound, and average body weight over the 13-week feeding period.
[b]$n = 7$.
[c]$n = 6$.
*Significant from control (least significant difference test, $p < 0.05$).
**Significant from 0.4 µg TCDD/kg alone (least significant difference test, $p < 0.05$).
†Significant from 1.2 mg PCB 156/kg alone (least significant difference test, $p < 0.05$).

Table 2. Dose groups, daily doses of PCB 153 and/or TCDD, and hepatic porphyrin levels after 13 weeks on diets containing PCB 153 and/or TCDD (means ± SE, $n = 9$)

Amount in diet		Daily dose[a]		
TCDD (μg/kg)	PCB 153 (mg/kg)	TCDD (ng/kg/day)	PCB 153 (mg/kg/day)	Porphyrins (μg/g liver)
0	0	0	0	1.9 ± 0.5
0	10	0	0.72	2.8 ± 1.6
0	30	0	2.07	1.4 ± 0.3[b]
0	100	0	6.61	3.0 ± 0.9
0.5	0	33.4	0	1.9 ± 0.5
0.5	10	33.9	0.68	300 ± 203*
0.5	30	32.6	1.95	969 ± 270*
0.5	100	32.0	6.40	1223 ± 326*
5	0	320	0	2.5 ± 0.3
5	10	318	0.64	22.0 ± 6.4*
5	30	301	1.81	1527 ± 480*
5	100	293	5.85	1094 ± 393*

[a]Estimated average value based on estimated food consumption, dietary level of compound, and average body weight over the 13-week feeding period.
[b]$n = 8$.
*Significant from PCB 153 or TCDD at corresponding dose alone (least significant difference test, $p < 0.05$).

P450 activity measurements, or at -20°C until porphyrin analyses. The same part of the liver was used for the same type of analysis for all animals.

Determination of porphyrins. Total hepatic porphyrin content was determined according to the method of Schwartz et al. (*48*), as modified by Debets et al. (*49*), using an acid–ethanol solution of chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone) and 100-μl samples of whole total-liver homogenate, prepared as described earlier (*26*). Fluorescence was measured in 96-well multiplates at λ_{ex} 409 nm and λ_{em} 645 nm using a cytofluor multiwell plate reader (Millipore B.V., Etten-Leur, the Netherlands) and coproporphyrin III as a standard.

The pattern of porphyrins accumulated in the livers were analyzed as described earlier (*50*), after a porphyrin extraction-step of liver homogenates (*51*) using reverse-phase HPLC (LKB-Produkter, Bromma, Sweden).

Cytochrome P450 measurements. Liver microsomes were prepared according to the method of Burke and Mayer (*52*). Microsomal CYP1A2 activities in the first experiment (PCB 126/PCB 156/TCDD) were measured by the 4-hydroxylation of acetanilide (4-OH-AA) according to Liu et al. (*53*) as described earlier (*26*). Microsomal CYP1A2 activities in the second experiment (PCB 153/TCDD) were measured by the demethylation of methoxyresorufin (MROD), using the same protocol as previously described for ethoxyrosorufin-*O*-deethylase (*54*). The substrate concentration used was 1.5 nM methoxyresorufin. Western blot analysis of CYP1A2 protein was determined in selected samples of the second experiment as described previously (*55*), with the exception that data are expressed as optical densities/μg protein. Protein levels were spectrophotometrically measured according to Bradford (*56*) by using a Bio-Rad Model 3550 microplate reader and BSA as a standard.

In vitro inhibition of CYP1A2 activity. Liver microsomes of rats treated with 100 mg PCB 153/kg diet in co-administration with 5 μg TCDD/kg diet were used for the *in vitro* inhibition of CYP1A2 measurement using MROD as a marker. To test the effect of substrate on possible competition with an unknown binding inhibitor *in vitro*, the concentration of methoxyresorufin ranged from 1.5 nM to 30 nM using microsomes of a responder and a nonresponder. To test the effect of PCB 126 and PCB 153 on *in vitro* inhibition of CYP1A2 activity, the methoxyresorufin concentration used was 1.5 nM. PCB 153 or PCB 126 was dissolved in dimethyl sulfoxide (DMSO) to a final concentration ranging from 1.3 ng/ml to 13 μg/ml. Samples were preincubated with 6 μl DMSO or 6 μl of the tested PCB in DMSO for 4 min. Reaction was started with NADPH and followed for 2–3 min. All measurements were performed in duplicate. Data are expressed as a percentage of CYP1A2 activity using 6 μl of DMSO.

Statistics. Data were analyzed for differences from controls with one-way analysis of variance and the least significant difference test ($p < 0.05$ for groups exposed to one compound). Groups treated with mixtures of two compounds were compared to the corresponding compounds alone. Two-way analysis of variance was used to determine possible interactive effects ($p < 0.05$). Correlations between CYP1A2 activities and hepatic porphyrin levels were examined by using Student's *t*-test ($p < 0.05$).

Results

Total hepatic porphyrin levels are summarized in Table 1 for groups fed diets containing PCB 126 or PCB 156, with or without co-administration of TCDD (first experiment). Table 2 shows the hepatic porphyrin levels in rats fed on diets containing PCB 153 and/or TCDD (second experiment).

TCDD showed a dose-dependent increase in hepatic porphyrin levels after 13 weeks of dietary exposure in the first experiment. This increase was also found for PCB 126 or PCB 156, reaching maximum levels about twice the control values (Table 1). However, it should be noted that the control values of the first experiment were lower than the control values of the second experiment. The lowest dietary dose levels for which a significant increase in hepatic porphyrin accumulation was found were 0.7 μg TCDD/kg, 50 μg PCB 126/kg, and 6 mg PCB 156/kg. In addition, co-administration of 50 or 180 μg PCB 126/kg and 0.4 μg TCDD/kg resulted in a significant increase in porphyrin accumulation as compared to TCDD alone. Similarly, co-administration of 1.2 mg PCB 156/kg and 5 μg TCDD/kg in the diet resulted in an additional accumulation (1.9-fold) compared to 1.2 mg PCB 156 alone.

In the second experiment, both PCB 153 and TCDD, when administered alone, did not alter hepatic porphyrin levels compared to controls up to levels of 100 mg PCB 153/kg or 5 μg TCDD/kg, respectively. Co-administration of any dose of PCB 153 and TCDD resulted in a strong accumulation of hepatic porphyrins, which was statistically different from control groups and from the corresponding PCB or TCDD group alone. The highest hepatic porphyrin accumulation was 1500 μg/g liver (i.e., about 800 times control levels). At the highest dose groups (i.e., 100 mg PCB 153/kg in co-administration with 5 μg TCDD/kg), four rats showed hardly any porphyrin accumulation (84 ± 2 μg/g liver), whereas in six rats a porphyrin level was found which was nearly 1200 times above control levels, i.e., 2215 ± 640 μg/g liver. This extreme individual variation was only observed in the experimental group fed on diets containing 100 mg PCB 153/kg co-administered with 5 μg TCDD/kg.

A qualitative HPLC analysis revealed uroporphyrin III and heptacarboxylic porphyrin as the major accumulated porphyrins in porphyrinogenic livers (data not shown).

Dose-dependent induction of 4-hydroxylation of acetanilide (4-OH-AA) by TCDD, PCB 126, and PCB 156 has been

reported before (*26,27,57*). Figure 1 shows significant correlations between hepatic CYP1A2 activities, measured as 4-OH-AA, and hepatic porphyrin levels after TCDD (Fig. 1A), PCB 126 (Fig. 1B), or PCB 156 (Fig. 1C) administration. The correlation coefficients were 0.629 (p <0.001), 0.483 (p <0.01), and 0.808 (p <0.001) for TCDD, PCB 126, and PCB 156, respectively.

CYP1A2 activity, measured as MROD, was increased 10-fold after TCDD (5 µg/kg diet) exposure in experiment 2 (Table 3). PCB 153 (100 mg/kg diet) gave no increase in MROD activity compared to controls. Co-administration of 0.5 or 5 µg TCDD/kg with 30 or 100 mg PCB 153/kg resulted in a decrease in MROD activity compared to the corresponding TCDD dose alone (Table 3). The porphyrinogenic rats exposed to 5 µg TCDD/kg co-administered with 100 mg PCB 153/kg had CYP1A2 activities of 516 ±57 nmol/mg/min (mean ± SE). The non-responding rats in this combined treatment group had CYP1A2 activities of 1410 ±171 nmol/mg/min.

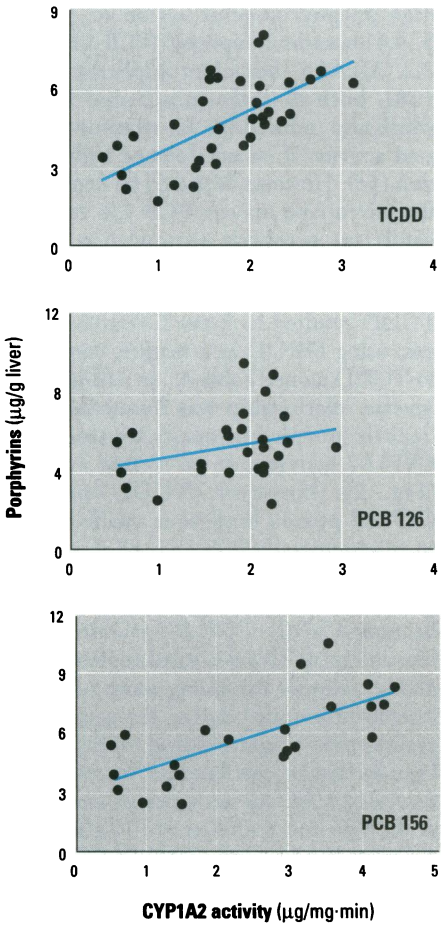

Figure 1. Correlations between hepatic microsomal CYP1A2 activities, measured as 4-hydroxylation of acetanilide and hepatic porphyrins after 13 weeks on diets containing (A) TCDD, (B) PCB 126, or (C) PCB 156. Correlation coefficients were 0.629 (p <0.001), 0.483 (p <0.01), and 0.808 (p<0.001) for TCDD, PCB 126, and PCB 156, respectively.

Figure 2 shows the relationship between hepatic porphyrin accumulation and CYP1A2 activities, using MROD as a marker, in TCDD, PCB 153, and co-exposed rats. CYP1A2 activities and hepatic porphyrin levels in the co-exposed animals were significantly correlated (p <0.05; r = -0.792).

A tight band of CYP1A2 protein was observed in all samples. However, in porphyrinogenic rats, a smear of protein staining throughout the gel was present in addition to the tight CYP1A2 band. Measurements of CYP1A2 protein levels in selected samples in this experiment gave the same optical density for TCDD-treated rats as for rats co-exposed with PCB 153 (Table 3). No difference in CYP1A2 protein levels was observed between the porphyrinogenic rats (responders) and the nonporphyrinogenic rats (nonresponders) after co-treatment with the highest dose levels of TCDD and PCB 153.

Using MROD as a marker and microsomes of a nonresponding and responding (porphyrinogenic) rat in the highest dose group in experiment 2 (TCDD/PCB 153), increasing substrate concentrations of methoxyresorufin up to 30 nM did not restore MROD activities *in vitro*. In the *in vitro* inhibition experiment with PCB 153 and PCB 126 using microsomes of a nonresponding and responding (porphyrinogenic) rat, both PCB 153 and PCB 126 were able to inhibit MROD activity. PCB 153 inhibited MROD activity by 50% in both samples at a final concentration of 13 µg/ml. At a final concentration of 13 ng/ml, reductions of 50% and 25% were observed in the responding and nonresponding rat. A final concentration of 1.3 ng PCB 126/ml resulted in 25% and 15% reduction in MROD activity in the responding and nonresponding rat, respectively.

Discussion

The work described in this study clearly shows that porphyrins accumulate in the liver after subchronic treatment with TCDD, PCB 126, or PCB 156 in rats. However, co-treatment of TCDD with PCB 153 resulted in enhanced hepatic porphyrin levels, which was not observed in the PCB 126/PCB 156 groups when co-treated with TCDD. The reason for the differences between the control groups in

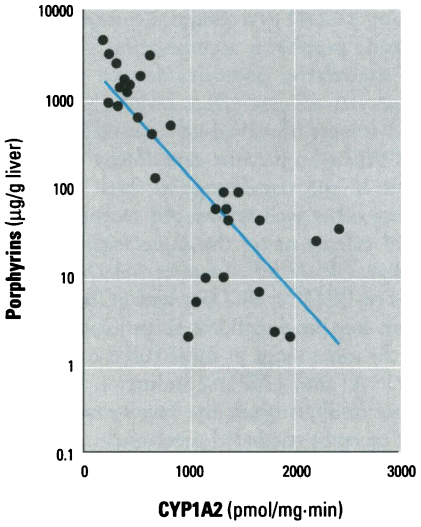

Figure 2. Correlation between microsomal CYP1A2 activities, measured as methoxyresorufin-*O*-demethylase, and hepatic porphyrin accumulation after 13 weeks on diets containing TCDD and PCB 153.

Table 3. Rat hepatic CYP1A2 activity, measured as methoxyresorufin-*O*-demethylase, and protein levels after 13 weeks on diets containing PCB 153 and/or TCDD (means ± SE)

Amount in diet		CYP1A2 activity	CYP1A2 protein
TCDD (µg/kg)	PCB 153 (mg/kg)	(pmol/mg/min)[a]	(optical density/µg protein)
0	0	185 ± 5	
0	10	183 ± 18	
0	30	156 ± 23	
0	100	123 ± 19	0.013 ± 0.004[b]
0.5	0	1195 ± 45*	
0.5	10	1258 ± 59	
0.5	30	628 ± 142**	
0.5	100	441 ± 100**	
5	0	1864 ± 50*	1.27 ± 0.16[c]
5	10	2021 ± 135	
5	30	706 ± 392**	1.20 ± 0.29[c]
5	100	888 ± 221**	1.32 ± 0.26 (responders)[c] 1.43 ± 0.10 (nonresponders)[b]

[a] n = 4 or 5.
[b] n = 2.
[c] n = 3.
*Significant from control (least significant difference test, p <0.05); **significant from corresponding TCDD dose alone (least significant difference test, p <0.05).

the two experiments, or in general between the two experiments (TCDD effect/lack of effect) is not known. This difference was not due to variability in the porphyrin assay since all porphyrin samples were analyzed (both experiment 1 and 2) at the same time using the same reagent solutions, standards, and (positive) control samples. In addition, the animals in the 5 µg TCDD/kg groups ate the same amount of food in both experiments, so the doses were comparable (Tables 1 and 2).

The porphyrinogenic activity of polyhalogenated aromatic hydrocarbons is well known (*8–10,14,58*). Jones and Sweeney (*14*) postulated the involvement of an Ah receptor-mediated mechanism in the accumulation of hepatic porphyrins by TCDD. Using TCDD as a reference compound and hepatic porphyrin accumulation as a marker, the relative potencies derived from no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) in the first experiment (Table 4) were in the same range as those reported using other well-known Ah receptor-mediated effects using the same experimental design (*26,27,59*). In mice, relative potencies of PCDDs, PCDFs, and PCBs derived from hepatic porphyrin accumulation were in the same range as those based on hepatic CYP1A1 and CYP1A2 induction (*60*). All these results suggest the involvement of an Ah receptor-mediated mechanism in hepatic porphyrin accumulation. Moreover, this is strengthened by the absence of hepatic porphyrin accumulation after subchronic dosing with di-*ortho*-substituted PCBs, such as PCB 153 (Table 1) (*58*) or 2,2′,3′,4,4′,5,5′-heptachlorobiphenyl (PCB 180) (*61*). However, Stonard and Greig (*58*) also found hepatic porphyrin accumulation by subchronic administration of 2,2′,3,3′,4,4′-hexachlorobiphenyl (PCB 128) and 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB 138) in rats.

It can be suggested that an Ah receptor-mediated mechanism in hepatic porphyrin accumulation involves the induction of CYP1A2. Strong evidence for a CYP1A2-related mechanism in the oxidation of uroporphyrinogen III to uroporphyrin III has been reported by Lambrecht and co-workers using hepatic rat microsomes and purified mouse CYP1A2 (*62,63*). In our study, CYP1A2 activities were correlated with hepatic porphyrin levels after administration of TCDD, PCB 126, or PCB 156 (Fig. 2). At the highest dose levels of these compounds, CYP1A2 activities were maximally induced as reported earlier (*26,27,57*). The lower correlation for PCB 126 might be a consequence of inhibition of the catalytic activity of CYP1A2 by PCB 126, which has been suggested to be a competitive high-affinity binding inhibitor (*27,44,64,65*). In addition, hepatic porphyrin levels were slightly but not significantly decreased in rats treated with 20 µg TCDD/kg diet compared to 5 µg TCDD/kg diet. TCDD (5 µg/kg diet) co-administered with 180 µg PCB 126/kg diet resulted in slightly lower hepatic porphyrin levels compared to co-administration with 50 µg PCB 126/kg. The same trend was observed in these specific groups for CYP1A2 activities using 4-OH-AA as a marker (*26,27*). Additionally, MROD activity was inhibited by PCB 126 and PCB 153 *in vitro* in the presented study. However, PCB 126 was about 1000 times more potent than PCB 153 for this effect *in vitro*. The results of this study, combined with the results from the literature, suggest that the relative potencies of the single PCB congeners for hepatic porphyrin accumulation might be based on an Ah receptor CYP1A2-mediated oxidation of uroporphyrinogen III to uroporphyrin III.

A strong synergistic porphyrin accumulation occurred after co-administration of PCB 153 and TCDD, leading to hepatic porphyrin levels as high as 800-fold the level of control animals (second experiment). The accumulated hepatic porphyrins were uroporphyrin III and heptacarboxylic porphyrin, which indicate PCT-like effects (*11,66*). In contrast, co-administration of PCB 126 or PCB 156 with TCDD yielded no further hepatic porphyrin accumulation compared to the highest single dose of PCB or TCDD congeners (first experiment).

The synergism on porphyrin accumulation in our study has been reported previously after co-administration of phenobarbital-type and 3-methylcholanthrene-type inducing compounds. Co-administration of 2,2′,4,4′,5,5′- and 3,3′,4,4′,5,5′-hexabromobiphenyls or phenobarbital and TCDD in cultured chick embryo hepatocytes resulted in synergistic porphyrin accumulation (*67,68*). Additionally, induction of ALAS activity and inhibition of UROD activity was synergistically affected after co-administration of TCDD and phenobarbital in these chick hepatocytes (*68*). In female Sprague-Dawley rats, a synergistic response on hepatic porphyrin accumulation was reported after 20-week co-exposure of the 2,2′,4,4′,5,5′- and 3,3′,4,4′,5,5′-hexabromobiphenyls (*69*).

It can be speculated that the cause for the synergistic response after co-administration with PCB 153 may be found in an effect on the rate-limiting enzyme in heme synthesis, ALAS. Phenobarbital has been reported to induce both ALAS mRNA and enzyme activity in rat hepatocytes (*70–73*). As PCB 153 and phenobarbital are well-known CYP2B-inducing compounds, a similar ALAS induction may have occurred in our study. Both mechanisms, i.e., induction of ALAS by PCB 153 and enhanced oxidation of uroporphyrinogen III to uroporphyrin III by CYP1A2, might have led to the strong synergistic effect observed after co-administration of the compounds. This dual mechanism might explain the high porphyrinogenic action of 2,2′,3,3′,4,4′-hexachlorobiphenyl (PCB 128) and 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB 138), both di-*ortho*-substituted PCBs, which also induced the Ah receptor-associated activity of benzo[*a*]pyrene hydroxylation (*58*). However, it should be noted that the mixed-type inducer PCB 156 revealed hardly any porphyrin accumulation in the liver after 13 weeks of exposure.

Table 4. No-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) for porphyrin accumulation after 13-week exposure to TCDD, PCB 126, or PCB 156 and estimated relative potencies

	(µg/kg/day)		Estimated relative potency	
Compound	NOAEL	LOAEL	NOAEL	LOAEL
TCDD	0.026	0.047	1	1
PCB 126	0.47	3.18	0.06	0.014
PCB 156	81	365	0.0003	0.0001

Co-administration of PCB 153 and TCDD resulted in lower CYP1A2 activities, using MROD as a marker, compared to TCDD alone (Table 3). In addition, an inverse relationship was found between hepatic porphyrin accumulation and CYP1A2 activities in co-treated animals (Fig. 2). However, TCDD-induced CYP1A2 protein levels were unaffected by co-treatment with PCB 153. All this information suggests that under porphyrinogenic conditions, CYP1A2 activity is decreased while CYP1A2 protein levels remain intact. This decreased activity could not be restored by adding more substrate (methoxyresorufin) during the assay, suggesting that a tight-binding inhibitor has been formed *in vivo*. Because free PCB 153 decreased CYP1A2 activities *in vitro* only at the high concentration of 13 µg/ml, it is unlikely that microsomal-bound PCB 153 from co-treated rats was responsible for the inhibition of CYP1A2 (Table 2).

Whether PCB 153 was left over in these microsomes at all, since PCB 153 has more affinity for fat tissue than for liver, is an unanswered question. It has been reported that under porphyrinogenic conditions, a tight-binding inhibitor of UROD is formed (*74,75*). Based on our

results, it can be speculated that under the same conditions, a tight-binding inhibitor of CYP1A2 activity is also formed. This suggestion is strengthened by the dose-dependent inhibition in chicken hepatocyte CYP1A activity, using EROD as a marker, after treatment with PCDDs and PCBs (*76–78*). This dose-dependent inhibition in CYP1A activity occurred at dose levels that caused an increase in porphyrin levels (*76,78*). Nevertheless, it remains unclear why a large degree of variability was observed in the PCB 153/TCDD co-treatment group at the high dose levels of both compounds. However, it can be excluded that this was due to variability in PCB 153 or TCDD concentrations in hepatic tissue (*46*).

In summary, an Ah receptor-mediated mechanism most likely plays a role in porphyrin accumulation after single-congener exposure, as relative potencies of PCB 126 and PCB 156 are in the same range as other well-known Ah receptor-mediated effects. However, this porphyrin accumulation is enhanced by the combined effect of PCB 153 and TCDD, leading to an exceptionally high hepatic porphyrin accumulation of rats subchronically exposed to combinations of these compounds. We postulate that in this synergistic process a dioxin-like induced CYP1A2 mechanism is involved, leading to oxidation of uroporphyrinogen III to uroporphyrin III, together with induction of ALAS by PCB 153. In addition, it can be speculated that high levels of TCDD of PCB 126 decrease hepatic porphyrin accumulation by tightly binding to CYP1A2. We suggest that under porphyrinogenic conditions, a binding inhibitor of CYP1A2 is formed. The interactive effect on porphyrin accumulation after co-administration of dioxinlike and non-dioxinlike compounds may have significant implications for the risk assessment of these chemicals.

References

1. Doss M. Normal ranges of porphyrins and precursors in human tissue, urine and feces. In: Chemical porphyria in man (Strik JJTWA, Koeman JH, eds). Amsterdam:North-Holland Biomedial Press, 1979;221.
2. Doss MO. Porphyrinurias and occupational disease. Ann NY Acad Sci 514:204–218 (1987).
3. Marks GS. Exposure to toxic agents: the heme biosynthetic pathway and hemoproteins as indicator. CRC Crit Rev Toxicol 15:151–179 (1985).
4. Böger A, Koss G, Koransky W, Naumann R, Frenzel H. Rat liver alterations after chronic treatment with hexachlorobenzene. Virchows Arch A Pathol Anat Histol 382:127–137 (1979).
5. Peters HA, Gockmen A, Cripss DJ, Bryan GT, Dogramaci I. Epidemiology of hexachlorobenzene-induced porphyria in Turkey. Arch Neurol 39:744–749 (1982).
6. Bleiberg J, Wallen M, Brodkin R, Applebaum IL. Industrially acquired porphyria. Arch Dermatol 89:793–797 (1964).
7. Poland AP, Smith D, Metter G, Possick P. A health survey of workers in 2,4-D and 2,4,5-T plant. Arch Environ Health 22:316–327 (1971).
8. Goldstein JA, Hickman P, Vos JG. Hepatic porphyria induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the mouse. Res Commun Chem Pathol Pharmacol 6:919 (1973).
9. Goldstein JA, Linko P, Bergman H. Induction of porphyria in the rat by chronic versus acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem Pharmacol 31:1607–1613 (1982).
10. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnard SD, Hummel RA, Humiston CG. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. Toxicol Appl Pharmacol 46:279–303 (1978).
11. Strik JJTWA. Porphyrins in urine as an indication of exposure to chlorinated hydrocarbons. Ann NY Acad Sci 320:308–310 (1979).
12. Chang K-J, Lu F-J, Tung T-C, Lee T-P. Studies on patients with polychlorinated biphenyl poisoning. 2. Determination of urinary coproporphyrin, uroporphyrin, delta-aminolevulinic acid and porphobilinogen. Res Commun Chem Pathol Pharmacol 30:547–554 (1980).
13. Fox GA, Kennedy SW, Norstrom RJ, Wigfield DC. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lake food chains. Environ Toxicol Chem 7:831–839 (1988).
14. Jones KG, Sweeney GD. Dependence of the porphyrinogenic effect of 2,3,7,8-tetrachlorodibenzo(*p*)dioxin upon inheritance of aryl hydrocarbon hydroxylase responsiveness. Toxicol Appl Pharmacol 53:42–49 (1980).
15. Seki Y, Kawanishi S, Sano S. Role of inhibition of uroporphyrinogen decarboxylase in PCB-induced porphyria in mice. Toxicol Appl Pharmacol 90:116–125 (1987).
16. Safe S. 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). CRC Crit Rev Toxicol 21:51–88 (1990).
17. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit Rev Toxicol 24:87–149 (1994).
18. Fein GG, Jacobson JL, Jacobson SW, Schwartz PM, Dowler JK. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. J Pediatr 105:315–320 (1984).
19. Jacobson JL, Jacobson SW, Humphrey HEB. Effects of *in utero* exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 116:38–45 (1990).
20. Jacobson JL, Jacobson SW, Humphrey HEB. Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol 12:319–326 (1990).
21. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, Van der pauw CG, Tuinstra LGMT, Weisglas-Kuperus N, Sauer PJJ Touwen BCL, Boersma ER. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 41:111–127 (1995).
22. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 22:517–554 (1982).
23. Safe SH. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Annu Rev Pharmacol Toxicol 26:371–399 (1986).
24. Pluess N, Poiger H, Hohbach C, Schlatter C. Subchronic toxicity of some chlorinated dibenzofurans (PCDFs) and a mixture of PCDFs and chlorinated dibenzodioxins (PCDDs) in rats. Chemosphere 17:973–984 (1988).
25. Birnbaum LS, Harris MW, Crawford DD, Morrissey RE. Teratogenic effects of polychlorinated dibenzofurans in combination in C57BL/6N mice. Toxicol Appl Pharmacol 91:246–255 (1987).
26. Van Birgelen APJM, van der Kolk J, Fase KM, Bol I, Poiger H, van den Berg M, Brouwer A. Toxic potency of 2,3,3′,4,4′,5-hexachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. Toxicol Appl Pharmacol 126:202–213 (1994).
27. Van Birgelen APJM, van der Kolk J, Fase KM, Bol I, Poiger H, Brouwer A, van den Berg M. Toxic potency of 3,3′,4,4′,5-pentachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. Toxicol Appl Pharmacol 127:209–221 (1994).
28. Sawyer TW, Safe S. In vitro AHH induction by polychlorinated biphenyl and dibenzofuran mixtures: additive effects. Chemosphere 14:79–84 (1985).
29. Bannister R, Safe S. 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 44:159–169 (1987).
30. De Jongh J, DeVito MJ, Nieboer R, Birnbaum LS, van den Berg M. Induction of cytochrome P450 isozymes after toxicokinetic interactions between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HxCB) in the liver of the mouse. Fundam Appl Pharmacol 25:264–270 (1995).
31. Leece B, Denomme MA, Towner R, Li A, Landers J, Safe S. Nonadditive interactive effects of polychlorinated biphenyl congeners in rats: role of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin receptor. Can J Physiol Pharmacol 65:1908–1912 (1987).
32. Biegel L, Harris M, Davis D, Rosengren R, Safe L, Safe S. 2,2′,4,4′,5,5′-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6J mice. Toxicol Appl Pharmacol 97:561–571 (1989).
33. Morrissey RE, Harris MW, Diliberto JJ, Birnbaum LS. Limited PCB antagonism of TCDD-induced malformations in mice. Toxicol Lett 60:19–25 (1992).
34. Jensen RK, Sleight SD. Sequential study on the synergistic effects of 2,2′,4,4′,5,5′-hexabromobiphenyl and 3,3′,4,4′,5,5′-hexabromobiphenyl on hepatic tumor promotion. Carcinogenesis 7:1771–1774 (1986).
35. Ahlborg UG, Brouwer A, Fingerhut MA,

Jacobson JL, Jacobson SW, Kennedy SW, Kettrup AAF, Koeman JH, Poiger H, Rappe C, Safe SH, Seegal RF, Tuomisto J, van den Berg M. 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. Eur J Pharmacol 228:179–199 (1992).

36. Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, Safe SH, Schlatter C, Wærn F, Younes M, Yrjänheikki E. Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28:1049–1067 (1994).
37. Safe S, Safe L, Mullin M. Polychlorinated biphenyls: congeners-specific analysis of a commercial mixture and a human milk extract. J Agric Food Chem 33:24–29 (1985).
38. Tanabe S, Kannan, N, Subramanian A, Watanabe S, Tatsukawa R. Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ Pollut 47:147–173 (1987).
39. Bosveld ATC, Gradener J, Murk AJ, Brouwer A, van Kampen M, Evers EHG, van den Berg M. Effects of PCDDs, PCDFs and PCBs in common tern (Sterna hirunda) breeding in estuarine and coastal colonies in the Netherlands and Belgium. Environ Toxicol Chem 14:99–115 (1995).
40. Leece B, Denomme MA, Towner R, Li SMA, Safe S. Polychlorinated biphenyls: correlation between in vivo and in vitro quantitative structure-activity relationships (QSARs). J Toxicol Environ Health 16:379–388 (1985).
41. Norén K, Lundén Å. Trend studies of polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans in human milk. Chemosphere 23:1895–1901 (1991).
42. Dewailly E, Weber J-P, Gingras S, Laliberté C. Coplanar PCBs in human milk in the province of Québec, Canada: are they more toxic than dioxin for breast fed infants? Bull Environ Contam Toxicol 47:491–498 (1991).
43. Neubert D, Abraham K, Golor G, Krowke R, Krüger N, Nagao T, Neubert R, Schulz-Schalge T, Stahlman R, Wiesmüller T, Hagenmaier H. Comparison of the effects of PCDDs and PCDFs on different species taking kinetic variables into account. In: Biological basis for risk assessment of dioxins and related compounds, Banbury report 35 (Gallo MA, Scheuplein RJ, van der Heijden KA, eds). New York:Cold Spring Harbor Laboratory Press, 1991;27–44.
44. Van den Berg M, De Jongh J, Poiger H, Olson JR. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxin (PCDDs) and dibenzofurans (PCDFs), and their relevance for toxicity. CRC Crit Rev Toxicol 24:1–74 (1994).
45. Hutzinger O, Safe S. A general method for the preparation of tritiated polychlorobiphenyls of high specific activity: 2,2′,5,5′-tetrachlorobiphenyl-(^{3}H) and 2,2′,4,4′,5,5′-hexachlorobiphenyl-(^{3}H). Bull Environ Contam Toxicol 7:374–375 (1972).
46. Van der Kolk J, van Birgelen APJM, Poiger H, Schlatter C. Interactions of 2,2′,4,4′, 5,5′-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. Chemosphere 25:2023-2027 (1992).
47. Mullin MD, Pochini CM, McCrindle S, Romkes M, Safe SH, Safe LM. High-resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners. Environ Sci Technol 18:468–476 (1984).
48. Schwartz S, Edmondson P, Stephenson B, Sarker D, Freyholtz H. Direct spectrofluorimetric determination of porphyrin in diluted urine. Ann Clin Res 8:156–167 (1976).
49. Debets FMH, Reinders JH, Debets AJM, Lössbroek TG, Strik JJTWA, Koss G. Biotransformation and porphyrinogenic action of hexachlorobenzene and its metabolites in a primary liver cell culture. Toxicology 19:185–196 (1981).
50. Kennedy SW, Wigfield DC, Fox GA. Tissue porphyrin pattern determination by high-speed high-performance liquid chromatography. Anal Biochem 157:1–7 (1986).
51. Kennedy SW, Maslen AL. Separation of porphyrin isomers by high-performance liquid chromatography. J Chromatogr 493:53–62 (1989).
52. Burke MD, Mayer RT. Ethoxyresorufin: Direct fluorometric assay of a microsomal O-dealkylation1 which is preferentially inducible by 3-methylcholanthrene. Drug Metab Dispos 2:583–588 (1974).
53. Liu G, Gelboin HV, Myers MJ. Role of cytochrome P450 1A2 in acetanilide 4-hydroxylation as determined with cDNA expression and monoclonal antibodies. Arch Biochem Biophys 284:400–406 (1991).
54. DeVito MJ, Maier WE, Diliberto JJ, Birnbaum LS. Comparitive ability of various PCBs, PCDFs, and TCDD to induce cytochrome P450 1A1 and 1A2 activity following 4 weeks of treatment. Fundam Appl Toxicol 20:125–130 (1993).
55. Santostefano MJ, Little KM, DeVito MJ, Diliberto JJ, Birnbaum LS. The role of CYP1A2 in localization of TCDD in subcellular fractions of rat and mouse tissues. In: Dioxin '95: 15th International Symposium on Chlorinated Dioxins and Related Compounds. Organohalogen compounds, volume 25. Toxicology, ecotoxicology, mechanism of action, metabolism. Edmonton, Alberta, Canada:Dioxin '95 Secretariat, 1995; 203–206.
56. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254 (1976).
57. Van Birgelen APJM, van der Kolk J, Fase KM, Bol I, Poiger H, Brouwer A, van den Berg M. Subchronic dose-response study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague-Dawley rats. Toxicol Appl Pharmacol 132:1–13 (1995).
58. Stonard MD, Greig JB. Different patterns of hepatic microsomal enzyme activity produced by administration of pure hexachlorobiphenyl isomers and hexachlorobenzene. Chem-Biol Interact 15:365–379 (1976).
59. Van Birgelen APJM, Smit EA, Kampen IM, Groeneveld CN, Fase KM, van der Kolk J, Poiger H, van den Berg M, Koeman JH, Brouwer A. Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. Eur J Pharmacol 293:77–85 (1995).
60. Van Birgelen APJM, DeVito MJ, Akins JM, Ross DG, Diliberto JJ, Birnbaum LS. Relative potencies of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls derived from hepatic porphyrin accumulation in mice. Toxicol Appl Pharmacol 137 (in press).
61. Koss G, Meyer-Rogge D, Seubert S, Seubert A, Losekam M. 2,2′,3′,4,4′,5,5′-Heptachlorobiphenyl (PCB 180)—on its toxicokinetics, biotransformation and porphyrinogenic action in female rats. Arch Toxicol 67:651–654 (1993).
62. Lambrecht RW, Sinclair PR, Gorman N, Sinclair JF. Uroporphyrinogen oxidation catalyzed by reconstituted cytochrome P4501A2. Arch Biochem Biophys 294:504–510 (1992).
63. Jacobs JM, Sinclair PR, Bement WJ, Lambrecht RW, Sinclair JF, Goldstein JA. Oxidation of uroporphyrinogen by methylcholanthrene-induced cytochrome *P*-450. Essential role of cytochrome *P*-450*d*. Biochem J 258:247–253 (1989).
64. Kuroki J, Koga N, Yoshimura H. High affinity of 2,3,4,7,8-pentachlorodibenzofuran to cytochrome P-450 in the hepatic microsomes of rats. Chemosphere 15:731–738 (1986).
65. Voorman R, Aust SD. Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome *P*-450d to the cytochrome and inhibition of its estradiol 2-hydroxylase activity. Toxicol Appl Pharmacol 90:69–78 (1987).
66. Jacob K, Doss MO. Composition of urinary coproporphyrin isomers I-IV in human porphyrias. Eur J Clin Chem Clin Biochem 31:617–624 (1993).
67. Sinclair P, Bement J, Sinclair J, Bonkowsky H. Porphyrogenic effects of PBB in chick embryo hepatocytes in culture. Toxicologist 1:71 (1981).
68. De Verneuil H, Sassa S, Kappas A. Effects of polychlorinated biphenyl compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, phenobarbital and iron on hepatic uroporphyrinogen decarboxylase. Biochem J 214:145–151 (1983).
69. Voorman R, Jensen RK, Sleight SD, Aust SD. Induction of porphyria in rats by PBB. Toxicologist 3:98 (1983).
70. May BK, Borthwick IA, Srivastava G, Pirola B, Elliott WH. Control of 5-aminolevulinate synthase in animals. Curr Top Cell Regul 28:233–262 (1986).
71. Srivastava G, Borthwick IA, Maguire DJ, Elferink CJ, Bawden MJ, Mercer JFB, May KB. Regulation of 5-aminolevulinate synthase mRNA in different rat tissues. J Biol Chem 263:5202–5209 (1988).
72. Srivastava G, Hansen AJ, Bawden AJ, May BK. Hemin administration to rats reduces levels of hepatic mRNAs for phenobarbitone-inducible enzymes. Mol Pharmacol 38:486–493 (1990).
73. Sinclair PR, Schuetz EG, Bement WJ, Haugen SA, Sinclair JF, May BK, Li D, Guzelian PS. Role of heme in phenobarbital induction of cytochromes P450 and 5-aminolevulinate synthase in cultured rat hepatocytes maintained on an extracellular matrix. Arch Biochem Biophys 386:386–392 (1990).
74. Cantoni L, Dal Fiume D, Rizzardini M, Ruggieri R. In vitro inhibitory effect on porphyrinogen carboxylase of liver extracts from TCDD treated mice. Toxicol Lett 20:211–217 (1984).
75. Smith AG, Francis JE. Chemically-induced formation of an inhibitor of hepatic uroporphyrinogen decarboxylase in inbred mice with iron overload. Biochem J 246:221–226 (1987).
76. Kennedy SW, Jones SP, Bastien LJ. Efficient analysis of cytochrome P4501A catalytic activity, porphyrins, and total proteins in chicken embryo hepatocyte cultures with a fluorescence plate reader. Anal Biochem 226:362–370 (1995).
77. Bosveld ATC, Verhallen E, Seinen W, van den Berg M. Mixture interactions in the *in vitro*

CYP1A1 induction bioassay using chicken embryo hepatocytes. In: Dioxin '95. 15th International Symposium on Chlorinated Dioxins and Related Compounds. Organohalogen compounds, volume 25, Toxicology, ecotoxicology, mechanism of action, metabolism. Edmonton, Alberta, Canada:Dioxin '95 Secretariat, 1995; 309–312.

78. Tysklind M, Bosveld B, Andersson P, Verhallen E, Sinnige T, Seinen W, Rappe C, van den Berg M. Interaction between the heme biosynthesis and induction of EROD activity in chicken embryo hepatocytes exposed to 2,3,7,8-TCDD and PCBs. In: Dioxin '95. 15th International Symposium on Chlorinated Dioxins and Related Compounds. Organohalogen compounds, vol 25. Toxicology, ecotoxicology, mechanism of action, metabolism. Edmonton, Alberta, Canada:Dioxin '95 Secretariat, 1995; 49–52.