Relative Potencies Derived from Hepatic Porphyrin Accumulation Following Subchronic Exposure to Polychlorinated Dibenzo-*p*-dioxins, Dibenzofurans, or Biphenyls in Female B6C3F1 Mice

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Abstract

The hepatic porphyrin accumulation was studied after subchronic dosing with single congeners of polychlorinated and polybrominated dibenzodioxins (PCDDs and PBDDs), and polychlorinated dibenzofurans and biphenyls (PCDFs and PCBs). Dose-dependent increases in hepatic porphyrin accumulation were found for all congeners tested. At lower dose levels, relative potencies of PCDDs, PCDFs, and coplanar PCBs for this effect, using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as a reference compound, were in the same range as those based on the induction of CYP1A1 and CYP1A2 enzyme activities. This suggests the involvement of an Ah receptor-mediated mechanism involving CYP1A2. However, the relative potencies of the mono-ortho substituted PCBs were higher for hepatic porphyrin accumulation than for CYP1A1 and CYP1A2 induction. Since mono-ortho substituted PCBs also induce CYP2B, as does phenobarbital, a second mechanism including an induction of δ-aminolevulinic acid synthetase may also contribute to the hepatic porphyrin accumulation.

Introduction

The porphyrias are a group of diseases associated with inherited or induced disturbances in heme biosynthesis. A specific one, porphyria cutanea tarda (PCT), occurs in humans having an inherited deficiency of hepatic uroporphyrinogen decarboxylase (URO-D), 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. Following a disastrous hexachlorobenzene poisoning in south-eastern Turkey in the 1960's, human victims displayed signs of disturbed heme synthesis, resulting in massive urinary excretion of porphyrins and hepatic accumulation of porphyrins, all consistent with PCT[1,2].

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 (ALA-S) activity, and inhibition of URO-D activity has been observed following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds[3,4]. It has been suggested that hepatic porphyrin accumulation is Ah-receptor regulated[5]. In addition, chronic exposure is necessary to establish hepatic porphyrin accumulation with TCDD-treatment[6].

The Ah receptor-mediated mechanism of action for TCDD and related compounds forms the basis of current risk assessment for mixtures of these substances. In this approach the toxic equivalency factor (TEF) concept is used with TCDD as a reference compound[7,8,9,10].
In this abstract the hepatic porphyrin accumulation is presented following subchronic dosing with individual PCDD, PBDD, PCDF, or PCB congeners. The involvement of both an Ah receptor-mediated mechanism as well as an additional pathway is discussed.

Methods

Chemicals: TCDD was purchased from Radian Corporation (Austin, TX) with purity >98% as determined by gas chromatography/mass spectrometry. 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD), 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,4 7,8-pentachlorodibenzofuran (1-PeCDF), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and octachlorodibenzofuran (OCDF) were obtained from Ultra Scientific (purity >98%). 3,3',4,4',5-Pentachlorobiphenyl (PCB 126), 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), and 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) were a generous gift of Dr. S. Safe (Texas A&M University, College Station, TX).
Animals and treatment: Female B6C3F1 mice (60 days old) were obtained from Charles River Breeding Laboratories, Raleigh, NC. The mice were held under controlled conditions of temperature (20 ± 1 °C) and lighting (12/12 light/dark cycle) and provided with water and food *ad libitum*. Mice were randomly assigned to treatment groups (5 per group) and group-housed. Mice were dosed by gavage with corn oil solutions of the test chemicals 5 days a week for 13 weeks. The doses are shown in Table 1. Three days after the last dose, animals were sacrificed. Livers were removed and prepared for acetanilide-4-hydroxylase (ACOH) measurements[11]. One part of the liver was quick-frozen in liquid N_2 and stored at -70°C until porphyrin analyses.
Porphyrin measurements. Total hepatic porphyrin content was determined according to the method of Schwartz *et al.*[12], as modified by Debets *et al.*[13], using an acid-ethanol solution of chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone) and 100 µl samples of whole liver homogenate, prepared as described before[14]. Fluorescence was measured in 96-well multiplates at λ_{ex} 409 nm and λ_{em} 645 nm using a cytofluor multiwell plate reader (Millipore Co., Bedford, MA) and coproporphyrin III as a standard.
Statistics: Data were analyzed with one-way analysis of variance (ANOVA) and the least significant difference test (LSD; $p < 0.05$). Correlations between CYP1A2 activities and hepatic porphyrin levels were tested by using Student's *t* test ($p < 0.05$).

Results and Discussion

Cytochrome P450 activity: CYP1A2 activities have been previously reported for the individual PCDD, PBDD, PCDF, and PCB congeners[15,16]. For all compounds tested, a dose-dependent increase was found in CYP1A2 activity as measured by the 4-hydroxylation of acetanilide. The highest doses of these compounds resulted in a CYP1A2 activity ranging from 5 to 10 nmols/mg protein/min, suggesting that at these doses a comparable maximum in CYP1A2 activity had been reached. However, the highest dose of PCB 118 resulted in higher CYP1A2 activity (21 nmols/mg protein/min).
Hepatic porphyrins: All compounds tested showed a dose-dependent increase in hepatic porphyrin accumulation following 13 weeks of subchronic dosing. Figure 1A-D presents the hepatic porphyrin accumulation after administration of TCDD, 4-PeCDF, PCB 169, or PCB 118, respectively. The lowest dose levels at which a significant increase in hepatic porphyrin accumulation was found were 45 ng TCDD/kg/day, 300 ng 4-PeCDF/kg/day, 30 µg PCB 169/kg/day, and 7,500 µg PCB 118/kg/day.

Table 1
Relative potencies of dioxins, furans, and biphenyls based on hepatic porphyrin accumulation after 13-week subchronic oral exposure

Chemical	Dose (ng or μg/kg/day)	TEF (based on hepatic porphyrin accumulation)
TCDD	0, 0.15, 0.45, 1.5, 4.5, 15, 45, 150, or 450 (ng/kg/day)	1
PeCDD	0, 90, 300, 900, 3,000, or 9,000 (ng/kg/day)	0.5
TBDD	0, 30, 90, 300, 900, or 3.000 (ng/kg/day)	0.3
TCDF	0, 15, 45, 150, 450, or 1,500 (ng/kg/day)	0.3
1-PeCDF	0, 90, 300, 900, 3,000, or 9,000 (ng/kg/day)	0.005
4-PeCDF	0, 9, 30, 90, 300, or 900 (ng/kg/day)	0.16
OCDF	0, 1.5, 4.5, 15, 45, or 150 (μg/kg/day)	0.0003
PCB 126	0, 0.015, 0.045, 0.15, 0.45, or 1.5 (μg/kg/day)	0.03
PCB 169	0, 30, or 90 (μg/kg/day)	0.0005
PCB 105	0, 390, 1.300, 3,900, 13,000, or 39,000 (μg/kg/day)	0.000007
PCB 118	0, 3,000, 7,500, 15,000, or 30,000 (μg/kg/day)	0.000015
PCB 156	0, 45, 150, 450, 1,500, or 4,500 (μg/kg/day)	0.00003

At the highest dose level of each of the administered compounds, differences in the highest level of porphyrin accumulation were observed which appeared to be compound-dependent. In general, high doses of PCDDs resulted in greater porphyrin accumulation than did high doses of PCDFs and coplanar PCBs. For example, the highest dose of TCDD resulted in a porphyrin accumulation of 37 μg/g liver, whereas the highest doses of 4-PeCDF and PCB 169 gave only 1.4 μg/g and 1.2 μg total hepatic porphyrins/g, respectively. However, high doses of the mono-ortho substituted PCBs resulted in the highest hepatic porphyrin levels of all, e.g., up to 540 μg total porphyrins/g liver for PCB 118.
Using TCDD as a reference compound and hepatic porphyrin accumulation as a marker, the order of relative potencies is TCDD > PeCDD > TBDD > 4-PeCDF > TCDF > PCB 126 > 1-PeCDF > OCDF ≈ PCB 169 > PCB 156 > PCB 118 > PCB 105 (Table 1). The relative potencies for the PCDDs, PCDFs, and coplanar PCBs are in the same range as those reported using CYP1A1 and CYP1A2 activities as markers[15,16]. This suggests the involvement of an Ah receptor-mediated mechanism in hepatic porphyrin accumulation. Hepatic porphyrin accumulation concurred with aryl hydrocarbon hydroxylase activity after TCDD exposure in Ah-responsive C57BL/6 and Ah-non-responsive DBA mice[5]. This also suggests the involvement of the Ah-receptor in hepatic porphyrin accumulation. The involvement of the Ah-receptor is strengthened by the absence of hepatic porphyrin accumulation after subchronic dosing with di-ortho substituted PCBs, such as 2,2'4,4',5,5'-hexachlorobiphenyl (PCB 153) or 2,2',3',4,4',5,5'-heptachlorobiphenyl (PCB 180), in rats for 13 weeks or 24 months,

respectively[17,18]. The Ah receptor-mediated mechanism may involve the induction of CYP1A2. Strong evidence for a CYP1A2-related mechanism in the oxidation of uroporphyrinogen III to uroporphyrin III has been previously reported by Lambrecht and co-workers using purified CYP1A2[19]. In our study, hepatic CYP1A2 activities correlated significantly ($p < 0.05$) with hepatic porphyrin accumulation for all compounds (data not shown).

In contrast to what was observed for the dioxins, furans, and coplanar PCBs, the mono-ortho substituted PCBs (PCB 105, PCB 118, and PCB 156) demonstrated at low doses relative potencies for hepatic porphyrin accumulation one order of magnitude greater than their relative potencies for induction of CYP1A1 and CYP1A2 activities[16]. In addition, the greater hepatic porphyrin accumulation at high dose levels with these congeners suggests an additional mechanism besides the involvement of CYP1A2.

One possibility is an effect on the rate limiting enzyme in heme synthesis, ALA-S[17]. Phenobarbital (PB) has been reported to induce ALA-S mRNA and activity in rat hepatocytes[20]. Since mono-ortho substituted PCBs are known CYP2B inducing compound, as is PB, a similar ALA-S induction may have occurred. This, together with the dioxin-like effect of mono-ortho substituted PCBs, may have had an interactive effect, resulting in more porphyrin accumulation than either the ortho- or dioxin-like effect alone. This synergism has been reported previously for 2,4,5,2',4',5'- and 3,4,5,3',4',5'-hexabromobiphenyls and PB and TCDD in cultured chick hepatocytes[21,22]. In rats, synergistic porphyrin accumulation was found after 13 weeks of daily dosing with TCDD in co-administration with 2,2',4,4',5,5'-hexachlorobiphenyl[17]. These apparently interactive effects on porphyrin metabolism after co-administration of dioxin-like and non-dioxin-like compounds have important implications for the risk assessment of these chemicals.

[This abstract does not necessarily represent USEPA policy].

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Literature

1) Böger, A., G. Koss, W. Koransky, R. Naumann, and H. Frenzel (1979) : Rat liver alterations after chronic treatment with hexachlorobenzene. Virchows Arch. A. Patol. Anat. Histol. 382, 127-137
2) Peters, H.A., A. Gockmen, D.J. Cripps, G.T. Bryan, and I. Dogramaci (1982) : Epidemiology of hexachlorobenzene-induced porphyria in Turkey. Arch. Neurol. 39, 744-749
3) Marks, G.S. (1985) : Exposure to toxic agents: The heme biosynthetic pathway and hemoproteins as indicator. CRC Crit. Rev. Toxicol. 15, 151-179
4) Smith, A.G., and F. De Matteis (1990) : Oxidative injury mediated by the hepatic cytochrome P-450 system in conjunction with cellular iron. Effects on the pathway of haem biosynthesis. Xenobiotica 20, 865-877
5) Jones, K.G., and G.D. Sweeney (1980) : 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
6) Goldstein, J.A., P. Linko, and H. Bergman (1982) : Induction of porphyria in the rat by chronic versus acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem. Pharmacol. 31, 1607-1613
7) Safe, S. (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). CRC Crit. Rev. Toxicol. 21, 51-88

8) Safe, S.H. (1994) : Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit. Rev. Toxicol. 24, 87-149
9) Ahlborg, U.G., A. Brouwer, M.A. Fingerhut, J.L. Jacobson, S.W. Jacobson, S.W. Kennedy, A.A.F. Kettrup, J.H. Koeman, H. Poiger, C. Rappe, S.H. Safe, R.F. Seegal, J. Tuomisto, and M. Van den Berg (1992) : 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
10) Birnbaum, L.S., and M.J. DeVito (1995) : Use of toxic equivalency factors for risk assessment for dioxins and related compounds. Toxicology, in press
11) DeVito, M.J., W.E. Maier, J.J. Diliberto and L.S. Birnbaum, (1993) : Comparative 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
12) Schwartz, S., P. Edmondson, B. Stephenson, D. Sarker, and H. Freyholtz (1976) : Direct spectrofluorimetric determination of porphyrin in diluted urine. Ann. Clin. Res. 8, 156-167
13) Debets, F.M.H., J.H. Reinders, A.J.M. Debets, T.G. Lössbroek, J.J.T.W.A. Strik, and G. Koss (1981) : Biotransformation and porphyrinogenic action of hexachlorobenzene and its metabolites in a primary liver cell culture. Toxicology 19, 185-196
14) Van Birgelen, A.P.J.M., J. Van der Kolk, K.M. Fase, I. Bol, H. Poiger, M. Van den Berg, and A. Brouwer (1994) : 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
15) Birnbaum, L.S., D.G. Ross, and M.J. DeVito (1993) : Dose response relationships for EROD induction in liver, lung and skin for dioxins and dibenzofurans. In Dioxin '93. 13th International Symposium on Chlorinated Dioxins and Related Compounds. Organohalogen compounds, volume 13, Human exposure, Toxicology, Epidemiology pp. 141-144
16) Ross, D.G., M.J. DeVito, and L.S. Birnbaum (1994) : Relative induction potency of polychlorinated biphenyls (PCBs) and TCDD in mouse liver, lung and skin. The Toxicologist 14, 278
17) Van Birgelen, A.P.J.M., K.M. Fase, J. van der Kolk, H. Poiger, A. Brouwer, W. Seinen, and M. van den Berg (1994) : Highly 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. In: Toxic and biochemical effects of polychlorinated biphenyls (PCBs) relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat, Ph.D. thesis, Utrecht University
18) Koss, G., D. Meyer-Rogge, S. Seubert, A. Seubert, and M. Losekam (1993) : 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
19) Lambrecht, R.W., P.R. Sinclair, N. Gorman, and J.F. Sinclair (1992) : Uroporphyrinogen oxydation catalyzed by reconstituted cytochrome P4501A2. Arch. Biochem. Biophys. 294, 504-510
20) Sinclair, P.R., E.G. Schuetz, W.J. Bement, S.A. Haugen, J.F. Sinclair, B.K. May, D. Li, and P.S. Guzelian (1990) : 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. 282, 386-392
21) Sinclair, P., J. Bement, J. Sinclair, and H. Bonkowsky (1981) : Porphyrogenic effects of PBB in chick embryo hepatocytes in culture. The Toxicologist 1, 71
22) De Verneuil, H., S. Sassa, and A. Kappas (1983) : Effects of polychlorinated biphenyl compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, phenobarbital and iron on hepatic uroporphyrinogen decarboxylase. Biochem. J. 214, 145-151

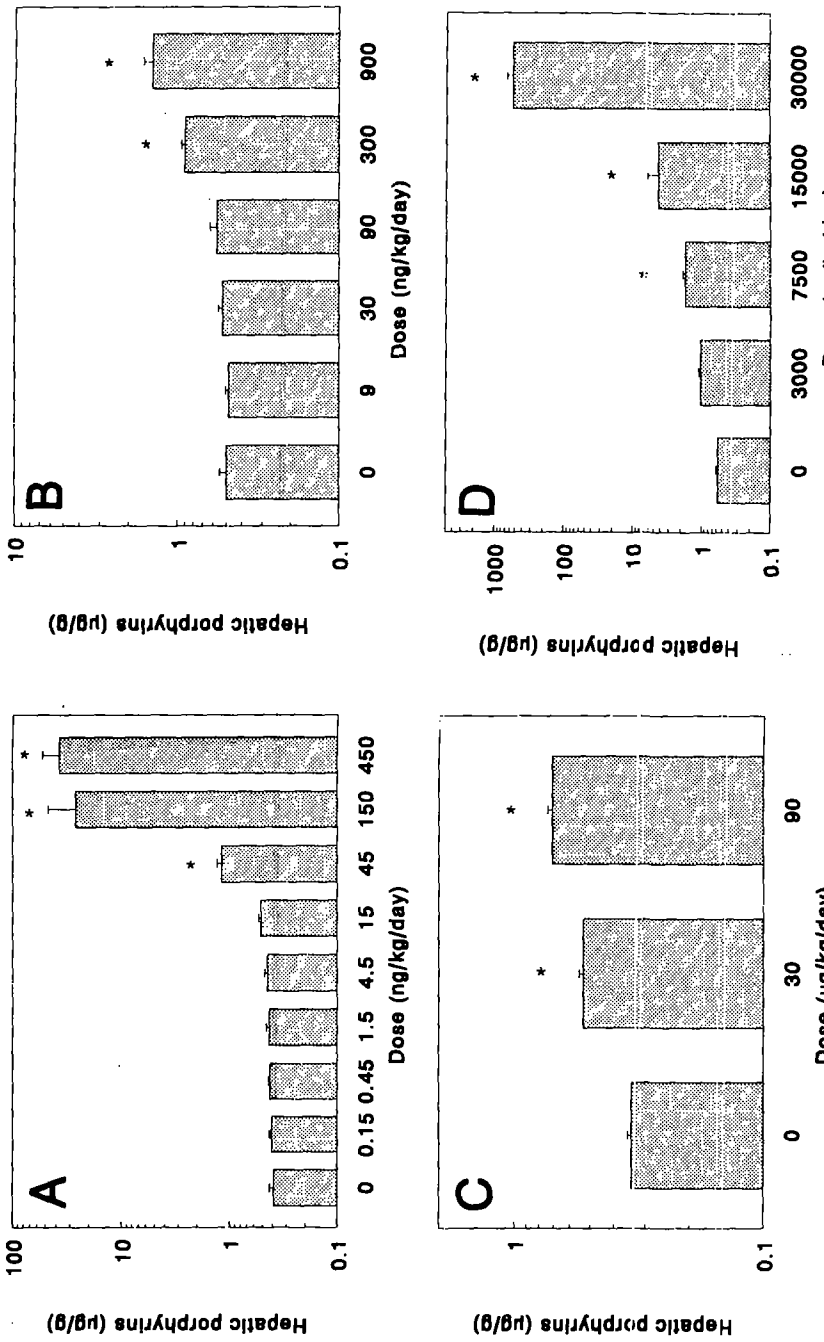

Fig. 1. Hepatic porphyrin accumulation after 13 weeks subchronic dosing with TCDD (**A**), 4-PeCDF (**B**), PCB 169 (**C**), or PCB 118 (**D**). * Significantly different from control ($p < 0.05$).