Synergistic Effect of 2,2',4,4',5,5'-Hexachlorobiphenyl and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on Hepatic Porphyrin Levels in Female Sprague-Dawley Rats

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Abstract

The hepatic porphyrin accumulation was studied after subchronic dosing with 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153; 10, 30, and 100 mg PCB 153/kg diet), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 0.5 and 5 µg TCDD/kg diet), or all possible combinations of both compounds. Both PCB 153 and TCDD, when administered as single congeners, did not alter hepatic porphyrin levels compared to controls up to levels of 100 mg PCB 153/kg or 5 µg TCDD/kg. Co-administration of any dose of PCB 153 and TCDD resulted in a strong accumulation of hepatic porphyrins. A qualitative HPLC analysis showed uroporphyrin III (URO III) and heptacarboxylic porphyrin as the accumulated porphyrins present in porphyrinogenic livers. The highest hepatic porphyrin accumulation was found to be 1000 µg/g liver, about 800 times control levels. Although TCDD exposure alone did not result in hepatic porphyrin accumulation, the involvement of an Ah-receptor mediated mechanism has been suggested, possibly regulated by CYP1A2. CYP1A2 is involved in the oxidation of uroporphyrinogen III to URO III. Since PCB 153 induce CYP2B, as does phenobarbital, a second mechanism such as an additional induction of δ-aminolevulinic acid synthetase may have also been involved.

Introduction

Polyhalogenated aromatic compounds, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), can increase hepatic uroporphyrin accumulation in rodents[1,2]. This accumulation of hepatic uroporphyrinogen and heptacarboxylic porphyrin, concurs with the induction of δ-aminolevulinic acid synthetase (ALA-S) activity, and inhibition of uroporphyrinogen decarboxylase (URO-D) activity. It has been suggested that hepatic porphyrin accumulation is Ah-receptor regulated, since hepatic porphyrin accumulation concurred with aryl hydrocarbon hydroxylase activity in the Ah-responsive C57B6/6 and Ah-non-responsive DBA

mice[3]. In addition, chronic exposure is necessary to establish hepatic porphyrin accumulation by TCDD[4].
The common mechanism of action of TCDD and related compounds forms the basis for the risk assessment of mixtures of these substances. In this approach the toxic equivalency factor (TEF) concept is used with TCDD as a reference compound[5,6,7,8]. A prerequisite for the TEF concept emanates from additive toxicity which is supported by *in vivo* studies with mixtures of PCDDs and PCDFs, mixtures of PCDDs, PCDFs, or PCBs, and mixtures of PCBs and TCDD[9,10,11,12].
In this extended abstract the hepatic porphyrin accumulation is presented after subchronic dosing with 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), TCDD, or after co-administration. The involvement of both an Ah receptor-mediated mechanism as well as an additional pathway are discussed.

Methods

Chemicals: TCDD (purity 99%) was synthesized by Dow Chemical (MI, USA). PCB 153 was synthesized according to Hutzinger and Safe[13] as described before (purity > 99.9%)[14]. Uroporphyrinogen I (URO-gen I), uroporphyrin III (URO III), coproporphyrin III (COPRO III), and a markerkit containing 2-, 4-, 5-, 6-, 7-, and 8-carboxyl porphyrin isomers I were obtained from Porphyrin products Inc. (Logan, Utah, USA).
Animals and treatment: Female Sprague-Dawley rats [Iva: S/V 50 (SD)], Ivanovas (Kissley, Germany), 7 weeks old, starting weight of about 150 g, were kept on a standard laboratory diet (Nafag 890, Giossau, Switzerland) starting 1 week before the experiment. One day before the start of the 13-week feeding experiment, the rats were randomly divided into groups of nine animals with a similar mean and standard deviation in body weights. The diets, in pulverized form, were prepared according to Pluess *et al.*[9] and contained PCB 153, TCDD, or combinations of both compounds (Table 1). 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 (12/12 light/dark cycle). After termination of the experiments, the animals were sacrificed. The liver was removed, rinsed in a physiological saline solution and weighed.
Parts of the liver were first frozen in liquid N_2 and stored at -20°C until porphyrin analyses.
Porphyrin measurements. Total hepatic porphyrin content was determined according to the method of Schwartz *et al.*[15], as modified by Debets *et al.*[16], using an acid-ethanol solution of chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone) and 100 µl samples of whole liver homogenate, and prepared as described before[11]. Fluorescence was measured in 96-well multiplates at λ_{ex} 409 nm and λ_{em} 645 nm by using a cytofluor multiwell plate reader (Millipore B.V., Etten-Leur, the Netherlands) and COPRO III as a standard. The pattern of porphyrins accumulated in the livers were analyzed using HPLC as described before[17], after a porphyrin extraction-step of liver homogenates[18].
Statistics: Data were analyzed with one-way analysis of variance (ANOVA) and the least significant difference test (LSD; $p < 0.05$) for differences to controls (for groups exposed to one compound). Groups co-administered with two compounds were compared to the corresponding compounds alone. Two-way analysis of variance was used to determine possible interactive effects ($p < 0.05$).

Results and Discussion

Figure 1 shows the hepatic porphyrin concentration after 13 weeks of subchronic dosing with PCB 153, TCDD, or combinations of both compounds. Both PCB 153 and TCDD, when administered as single congeners, did not alter hepatic porphyrin levels compared to controls up to levels of 100 mg PCB 153/kg or 5 µg TCDD/kg. The absence of porphyrin accumulation by PCB 153 alone is in agreement with Koss *et al.*[19], who found no alterations in porphyrin metabolism after 6 months in female Wistar rats dosed every other day with 7 mg 2,2',3',4,4',5,5'-heptachlorobiphenyl (PCB 180)/kg, which is also a di-*ortho* substituted PCB.
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, or the corresponding PCB or TCDD group alone. The highest hepatic porphyrin accumulation was found to be 1000 μg/g liver, 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 (0.54 ± 0.014 μg/g liver) whereas in six rats a porphyrin level was found which was nearly 1200 times above control levels, i.e., 14.2 ± 4.1 μg/g liver. This extreme individual variation was only observed in the experimental group fed on diets containing 100 mg PCB 153/kg in co-administration with 5 μg TCDD/kg.

A qualitative HPLC analysis revealed uroporphyrin III (URO III) and heptacarboxylic porphyrin as the accumulated porphyrins present in porphyrinogenic livers.

Synergism on porphyrin accumulation has been reported previously after co-administration of phenobarbital-type and 3-methylcholanthrene-type inducing compounds. The co-administration of 2,4,5,2',4',5'- and 3,4,5,3',4',5'-hexabromobiphenyls or phenobarbital (PB) and TCDD in cultured chick hepatocytes resulted in a synergistic porphyrin accumulation[20,21]. In female Sprague-Dawley rats a similar synergistic response on hepatic porphyrin accumulation was reported after 20-week co-exposure of the 2,4,5,2',4',5'- and 3,4,5,3',4',5'-hexabromobiphenyls[22].

Although TCDD exposure alone did not result in hepatic porphyrin accumulation in our study, the involvement of an Ah-receptor mediated mechanism has been suggested. Hepatic porphyrin accumulation concurred with aryl hydrocarbon hydroxylase activity after TCDD exposure in the Ah-responsive C57B6/6 and Ah-non-responsive DBA mice[3]. Recent 90-day studies after exposure to single polychlorinated or polybrominated dioxins, furans, and planar biphenyls gave similar relative potencies for hepatic porphyrin accumulation, using TCDD as a reference compound, compared to those obtained for CYP1A1 and CYP1A2 activities[23].

The role of the Ah-receptor is strengthened by the absence of hepatic porphyrin accumulation after subchronic dosing of the di-ortho substituted PCB 2,2',3',4,4',5,5'-heptachlorobiphenyl (PCB 180) to rats after 24 months[19].

This Ah-receptor mediated mechanism may involve the induction of CYP1A2. Strong evidence for a CYP1A2 related mechanism in the oxidation of uroporphyrinogen III to URO III has been reported before by Lambrecht and co-workers using purified CYP1A2[24]. In addition, after subchronic dosing of rats with the planar PCB 3,3',4,4',5-pentachlorobiphenyl (PCB 126), TCDD, or 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) significant correlations were found between hepatic porphyrin accumulation and CYP1A2 activities[25].

The synergistic response after co-administration with PCB 153 may also be found in an effect on the rate limiting enzyme in heme synthesis, ALA-S. PB has been reported to induce ALA-S mRNA and activity in rat hepatocytes[26]. Since PCB 153 is a well-known CYP2B inducing compound, as is PB, a similar ALA-S induction may have occurred. The latter mechanism in combination with the Ah-receptor mediated effect of TCDD, may have caused this synergistic effect, resulting in a high porphyrin accumulation.

The synergistic effect on porphyrin accumulation after co-administration of dioxin-like and non-dioxin-like compounds have important implications for risk assessment of these compounds. To evaluate the extent of this, further research at lower dose levels is clearly required.

Table 1

Daily doses of PCB 153 and TCDD in the 13-week feeding experiment

Level of TCDD in diet (μg/kg)	Level of PCB 153 in diet (mg/kg)	Dose TCDD (ng/kg/day)	Dose PCB 153 (mg/kg/day)
0	0	0	0
0	10	0	0.72
0	30	0	2.07
0	100	0	6.61
0.5	0	33.4	0
0.5	10	33.9	0.68
0.5	30	32.6	1.95
0.5	100	32	6.40
5	0	320	0
5	10	318	0.64
5	30	301	1.81
5	100	293	5.85

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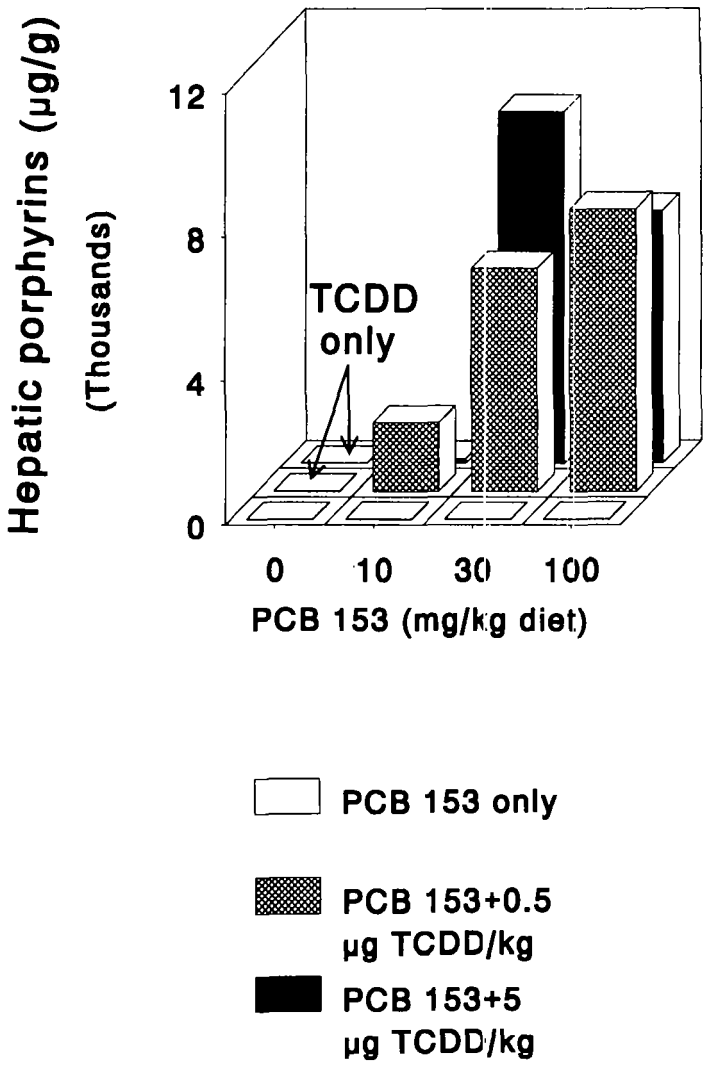

Fig. 1. Hepatic porphyrin accumulation after 13 weeks subchronic dosing with TCDD and/or PCB 153.