TOXICITY AND BIOCHEMICAL POTENCIES OF POLYCHLORINATED BIPHENYL CONGENERS RELATIVE TO 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN THREE MONTHS FEEDING STUDIES IN THE RAT

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ABSTRACT

Groups of rats were fed diets containing several concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,2',4,4',5,5'-hexa- (PCB 153), 2,3,3',4,4',5-hexa- (PCB 156), or 3,3',4,4',5-penta-chlorobiphenyl (PCB 126) in ratios found in human fat tissue, for 13 weeks.

No observed effect levels (NOELs) and lowest observed adverse effect levels (LOAELs) were based on plasma thyroid hormone levels, 7-ethoxyresorufin-O-deethylation (EROD) induction, 7-pentoxyresorufin-O-depentylation (PROD) induction, relative liver- and thymusweights, and bodyweight gain.

The NOEL for PCB 153 was in the range of $<6*10^2$-$60*10^2$ µg/kg/day, for PCB 156 <70-70 µg/kg/day and for TCDD <0.012-0.030 µg/kg/day. The NOEL for PCB 126, based on thyroid hormone levels and bodyweights after 4 weeks, was found to be <0.5-0.5 µg/kg/day.

The toxicity relative to TCDD based on the NOELs and LOAELs was 0.000005->0.00005 for PCB 153, <0.0002-0.005 for PCB 156 and <0.06-0.6 for PCB 126.

We suggest that NOELs for PCB congeners might be derived from the NOEL for TCDD using toxic equivalency factors from short term experiments.

INTRODUCTION

Polyhalogenated compounds, such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are industrial chemicals or byproducts which are widely distributed in the environment.

Most of the effects of these compounds are mediated by a common mechanism of action involving a single receptor protein, the Ah-receptor. This has been confirmed by several studies and has led to the development of toxic equivalency factors (TEFs)[1]. Risk assessment of polyhalogenated compounds is based on these TEFs.

However, the greater part of the studies to obtain these TEFs are acute experiments and in vitro experiments. This paper reports on the toxic and biochemical potency of several PCB congeners relative to TCDD observed in subchronic feeding studies with rats.

The PCBs used in our studies are 2,2',4,4',5,5'-HxCB (PCB 153), 3,3',4,4',5-PnCB (PCB 126) and 2,3,3',4,4',5-HxCB (PCB 156), which induce isoenzymes of cytochrome P450 2B, 1A or 1A + 2B, respectively. EROD and PROD were used as markers for cytochrome P 450 1A and 2B activity. The administered compounds were investigated in ratios that are usually found in human adipose tissue.

To make an estimate about the toxicity of PCB 126 a pilot, single dose experiment was done.

METHODS

2,2',4,4',5,5'-Hexa-chlorobiphenyl (PCB 153) was prepared as described[2]. 2,3,3',4,4',5-Hexa-chlorobiphenyl (PCB 156) was synthesized via Cadogan coupling of 3,4-dichloroaniline and 1,2,3,4-tetrachlorobenzene, and purified by column chromatography according to Mullin et al[3]. The synthesized PCBs were >97% pure as determined by HRGC-LRMS analysis. 3,3',4,4',5-Penta-chlorobiphenyl (PCB 126) was from Schmidt B.V. (Amsterdam, The Netherlands). TCDD originated from Dow Chemical (Midland, Mi USA).

Female Sprague-Dawley (Iva: S/V 50 (SD)) rats, 8 or 9 per group, starting weight about 150 gram, were fed experimental diets for 13 weeks. The diets, pulverized feed (Nafag 890), contained 0, 0.2, 0.5, 5, or 20 ppb TCDD, 10, 30, or 100 ppm PCB 153, 1.2, 6, or 12 ppm PCB 156, and 7, 50, or 180 ppb PCB 126.

Food consumption and bodyweights were recorded twice a week. Blood samples were taken at 4, 8, and 13 weeks and were analysed for plasma total (TT4) and free (FT4) thyroxin levels by Chemoluminescence immuno assays using standard Amerlite kits (Amersham, U.K.). 7-Ethoxyresorufin-O-deethylation (EROD) and 7-pentoxyresorufin-O-depentylation (PROD) were measured using the method of Burke et al.[4] Terminal body-, liver-, and thymusweights were determined. No observed effect levels (NOELs) and lowest observed adverse effect levels (LOAELs) were based on the mentioned parameters at the end of the study. For PCB 126 plasma thyroid hormone levels and bodyweight gain were determined after 4 weeks.

In the pilot experiment with PCB 126, ED_{10}, ED_{25}, and ED_{50} values for a decrease in bodyweight gain, thymic atrophy and liver enlargement were obtained as described essentially by Leece et al.[5] Female Sprague-Dawley (Iva: S/V 50 (SD)) rats were administered PCB 126 in doses ranging from 0 to 2 µmol/kg. Bodyweights were determined every two days. After 14 days the thymia and livers were weighed. The results were used to determine the dosage for the semichronic study with PCB 126.

RESULTS AND DISCUSSION

The ED_{10}, ED_{25} and ED_{50} values for a decrease in bodyweight gain, thymic atrophy and liver enlargement are given in table 1. The obtained ED_{10} and ED_{25} values for bodyweight gain and thymic atrophy, respectively, are approximately the same as those obtained by Leece et al.[5], who found ED_{25} values of 2.0 and 0.50 µmol/kg, respectively, in male Wistar rats.

The no observed effect levels (NOELs) and lowest observed adverse effect levels (LOAELs) for the administered compounds are given in table 2. These values are based on the food consumption during the whole experiment (for PCB 126 up to 4 weeks). At the beginning of the study these values will be higher than at the end of the study because of a higher food consumption per kilogram bodyweight.

PCB 153 had no effect on relative thymusweight and reduction in bodyweight gain below 6 mg/kg/day. Relative liverweight was found to be a more sensitive morphological parameter with a LOAEL of 6 mg/kg/day. This liver enlargement might be related to the induction of cytochrome P450 2B (PROD activity), which was found from 0.7 mg/kg/day on. Plasma thyroxin levels were reduced by PCB 153. The NOEL was about the same as those observed for the gross pathology parameters.

Table 1. ED_{10}, ED_{25}, and ED_{50} values for PCB 126 (µmol/kg) in the acute, single dose experiment.

Parameter	ED_{10}	ED_{25}	ED_{50}
Relative thymusweight	0.3	0.9	1.7
Relative liverweight	0.7	1.8	
Bodyweight	1.3		

Table 2. NOELs and LOAELs for PCB 153, PCB 156, PCB 126, and TCDD (µg/kg bodyweight/day)@.

Compound	NOEL						LOAEL					
	FT4 and TT4	EROD	PROD	Relative liverwght.	Relative thymuswght.	Bodyweight	FT4 and TT4	EROD	PROD	Relative liverwght.	Relative thymuswght.	Bodyweight
PCB 153	$18*10^2$	---	$<6*10^2$	$18*10^2$	$60*10^2$	$60*10^2$	$60*10^2$	---	$6*10^2$	$60*10^2$	$>60*10^2$	$>60*10^2$
PCB 156	70	<70	<70	70	<70	<70	$36*10^2$	70	70	$36*10^2$	70	70
PCB 126	0.51$	#	#	#	#	<0.51$	3.6$	#	#	#	#	0.51$
TCDD	0.030	<0.012	---	0.030	0.030	0.030	0.32	0.012	---	0.32	0.32	0.32

@ = based on food consumption during the whole experiment and bodyweight at the end of the study
# = not measured yet
$ = based on data after 4 weeks

Table 3. TEFs based on NOELs and LOAELs.

Compound	NOEL	LOAEL
PCB 153	0.000005-0.00002	0.00005->0.00005
PCB 156	<0.0002-0.0004	0.0002-0.005
PCB 126	<0.06-0.06	0.09-0.6

The toxicity of and changes in biochemical parameters by PCB 156 were higher than those observed for PCB 153. The LOAELs for cytochrome P 450 1A induction (EROD activity), thymic atrophy and reduction in bodyweight gain were the same, approximately 70 µg/kg/day.

Results from the study with PCB 126 are not completely available yet, but data from the short term and the ongoing semichronic experiment indicate a toxicity of 0.1 relative to TCDD.

For TCDD the LOAEL we found is in the same range as reported by Kociba et al. in a two year chronic feeding study with the same rat strain.[6] In this study a LOAEL of 0.01-0.1 µg/kg/day was determined using the development of carcinomas and some biochemical parameters (like urinary excretion of porphyrins, serum activities of alkaline phosphatase and glutamic-pyruvic transaminase) as endpoints. A LOAEL of 0.01 µg/kg/day was found in a 13-week oral toxicity study with TCDD, using gross pathological and biochemical effects as endpoints[7].

From our studies it is obvious that biochemical effects, like changes in plasma thyroxin levels and cytochrome P 450 activities, are more sensitive parameters than thymic atrophy and reduction in bodyweight gain. Cytochrome P 450 induction was found to be more a sensitive parameter than reduction in plasma thyroxin levels. Applying a safety factor of 250 and the most sensitive parameters in our experiments the following TDI (tolerable daily intake) values might be suggested: PCB 153 <2 µg/kg/day (based on cytochrome P 450 2B activity) and PCB 156 <0.3 µg/kg/day (based on plasma thyroxin reduction and cytochrome P450 1A+2B activity).

In summary we can conclude that the TEFs based on NOELs and LOAELs for the PCBs 126, 156, and 153 (see table 3) correspond well to the TEF values proposed by Safe, i.e. 0.1, 0.001, and 0.00002, respectively[1]. These results indicate that NOELs for PCB congeners might be derived from the NOEL for TCDD using TEF values.

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