EFFECTS OF VARIOUS FRACTIONS OF CLOPHEN A50 ON HEPATIC EROD ACTIVITIES IN ADULT FEMALE MINK AND THEIR KIDS

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ABSTRACT

Clophen A50, a technical preparation of polychlorinated biphenyls (PCBs), was separated into three fractions containing chlorobiphenyls with 0, 1 or 2-4 *ortho* chlorines and one fraction containing di- and tricyclic compounds such as naphthalenes and dibenzofurans. Unfractionated Clophen A50 and each of the fractions, as well as a synthetic mixture of non-*ortho*-chlorinated congeners, were mixed in the feed and given to female mink during the reproductive season. Hepatic EROD activities in the adult females were increased 2-3 times by Clophen A50 and by the fractions containing non- or mono-*ortho*-chlorinated congeners. No live kits were delivered by the females treated with unfractionated Clophen A50. EROD was induced in the kits by all other treatments, and the non- and mono-*ortho*-chlorinated congeners strongly enhanced enzyme activity to about 30 times the control value.

INTRODUCTION

The mink (*Mustela vison*) is a predatory species with high position in the food chain and is exposed to high concentrations of environmental pollutants. It is also highly sensitive to polychlorinated biphenyls (PCBs), especially with regard to reproduction (Platonow and Karstad, 1973; Jensen *et al.*, 1977; Bleavins *et al.*, 1980; Aulerich *et al.*, 1985).

Technical PCB preparations are mixtures of chlorobiphenyls having chloro-substituents in various positions. Theoretically, 209 various chlorobiphenyls are possible, about 100 of which are found in commercial PCB mixtures.

In the present communication, I describe the effects of various fractions of the technical PCB mixture Clophen A50 on hepatic ethoxyresorufin *O*-deethylase (EROD) activities in female mink and their newborn kits. The results presented here are part of a study on the effects of PCBs on mink during the reproductive season.

MATERIALS AND METHODS

The technical PCB preparation Clophen A50 was separated into three fractions containing chlorobiphenyls with 0, 1 or 2-4 *ortho* chlorines and one fraction containing di- and tricyclic compounds such as naphthalenes and dibenzofurans (Jensen and Athanasiadou, 1990). Each day for 13-14 weeks, groups of ten 2-year-old female mink were given 2 mg of Clophen A50 or one of the fractions in an amount equivalent to that present in 2 mg Clophen A50. The substances were mixed in a small portion of feed that was given before the daily main portion. The treatment was started 5 weeks before mating and continued until 5 days after whelping, when the animals were killed. One group of mink was treated with a synthetic mixture of 3,3',4,4'-tetra, 3,3',4,4',5-penta and 3,3',4,4',5,5'-hexachlorobiphenyl. The concentration of these chlorobiphenyls in the feed was the same as that in the feed mixed with the fraction containing non-*ortho*-chlorinated congeners. The coplanar PCBs were synthesized by Bergman *et al.*, Wallenberg Laboratory, University of Stockholm, Sweden.

Microsomes were prepared by homogenization of the livers in a Potter-Elvehjem homogenizer followed by centrifugation at 10 000 g for 15 min and further centrifugation of the supernatants at 105 000 g for 60 min. EROD activities of the fresh microsomal suspensions were determined according to Pohl and Fouts (1980).

RESULTS AND DISCUSSION

EROD activities in the livers of adult females and their kits treated with Clophen A50, each of the fractions of Clophen A50 and the mixture of synthetic non-*ortho*-substituted PCBs are shown in Table 1. Treatment with the complete mixture, the fraction containing the mono-*ortho*-chlorinated PCBs, the fraction containing the non-*ortho*-chlorinated congeners and the synthetic mixture of non-*ortho*-chlorinated PCBs significantly enhanced EROD activity (2-3 times the control value) in the adults. The enzyme activities observed after these treatments were similar, possibly reflecting the maximal EROD activity in adult female mink. In several studies, cytochrome P-450-dependent enzyme activities in adult mink were only weakly induced by PCBs (Shull *et al.*, 1982; Aulerich *et al.*, 1985; Gillette *et al.*, 1987). All four fractions of Clophen A50, as well as the mixture of synthetic non-*ortho*-chlorinated congeners, significantly induced EROD in the kits. No live kits were delivered by the females treated with the complete mixture. The mono-*ortho*-chlorinated congeners and the fraction containing non-*ortho*-chlorinated ones, as well as the synthetic mixture, were very potent inducers in the kits, causing an increase in enzyme activities to levels about 30 times higher than those measured in control kits. The fraction containing congeners substituted with 2-4 *ortho* chlorines and the one with di- and tricyclic compounds were considerably less potent inducers, causing about a threefold increase in enzyme activities compared with the controls. The fact that EROD was induced in the kits suggests that the *Ah* receptor is present in neonatal mink, and all fractions prepared seemed to contain substances which bind to the receptor. The fractions containing non- and mono-*ortho*-chlorinated congeners were very potent inducers in the kits. The PCBs being chlorinated in one *ortho*-position bind less avidly to the *Ah* receptor than the non-*ortho*-chlorinated chlorobiphenyls (Poland and Glover, 1977; Bandiera *et al.*, 1982). However, the concentrations of mono-*ortho*-chlorinated congeners in technical PCBs are high (up to 20%), and these congeners probably contribute significantly to the toxic effects of technical PCB mixtures.

Table 1. 7-Ethoxyresorufin *O*-deethylase (EROD) activities in livers of adult females and 5-day-old kits treated with polychlorinated biphenyls (PCBs). The mink were given a feed mixed with either Clophen A50 or one of four fractions prepared from Clophen A50. One group of mink was treated with a synthetic mixture of non-*ortho*-chlorinated congeners. For preparation of microsomes, pieces from each adult liver (≈3g) were used, whereas the livers from 2-3 kits in each litter were pooled.

Treatment	EROD activity[a] (mean ± S.D.)			
	Adults	n	Kits	n
Control	0.69 ± 0.50	10	0.035 ± 0.012	9
Clophen A50	1.5 ± 0.5**	7	-	-
0 *ortho* Cl	2.2 ± 0.7***	10	1.2 ± 0.4***	8
1 *ortho* Cl	2.0 ± 0.5***	9	1.1 ± 0.3***	8
2-4 *ortho* Cl	0.93 ± 0.29	10	0.089 ± 0.055*	10
di- and tricyclic	1.1 ± 0.5	9	0.086 ± 0.039**	9
0 *ortho* Cl (synthetic)	1.5 ± 0.4***	10	1.1 ± 0.2***	9

[a] Activity expressed as nmol resorufin x (mg protein)$^{-1}$ x (min)$^{-1}$

*,**,*** Significantly different from the corresponding control value (t-test, $P < 0.05$, 0.01 and 0.001, respectively)

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