2,3,7,8-TCDD Equivalent Concentrations in Livers from Swedish Otters determined with a Bioassay

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Introduction

During the past decades, otter (*Lutra lutra*) populations have declined in many parts of Western Europe. Exposure to PCB resulting in impaired reproduction is considered to be one of the major causes of this decline (1-4). The sensitivity of the otter to PCB is not known but the mink (*Mustela vison*), a mustelid that may be used in toxicological studies, is very sensitive to PCB, especially with regard to reproductive toxicity. Technical PCB preparations consist of numerous PCB congeners with different chlorination patterns. Certain of these congeners are agonists to the Ah receptor and exert dioxin-like biological responses. The dioxin-like PCB congeners seem to be responsible for the major part of the reproductive toxicity of technical PCB in mink (5).

Induction of ethoxyresorufin *O*-deethylase (EROD) is a biochemical response to Ah receptor agoniststs that can be measured in *in vitro* systems. Chicken embryos are very sensitive to dioxins and dioxin-like PCB congeners and these compounds strongly induce EROD in chicken embryo livers. The purpose of the present study was to use an *in vitro* assay based on EROD induction in chicken embryo livers (6) to determine the levels of Ah receptor agonists, expressed as bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (Bio-TEQs), in organic extracts from otter livers. The liver samples were obtained from Swedish otters killed by the traffic between 1981 and 1994. Based on analysis of residue levels of PCB, five samples from animals with a relatively low body burden and five samples from animals with a relatively high body burden of PCB were chosen. We also wanted to compare the Bio-TEQ levels in the otter livers with the levels in livers from mink experimentally treated with PCB at doses high enough to cause reproductive impairment. Therefore, liver extracts from mink exposed to the technical PCB mixture Clophen A50 (0.1 or 0.3 mg per animal and day) for 18 months (5) were also analysed in the bioassay.

Materials and Methods

PCB analysis: Muscle tissue samples from otters were analysed for residue levels of PCB (7). In order to facilitate comparison of the measured concentrations with previously published data, packed column gas chromatography was used. Extraction, fat determination, and determination of PCB concentrations were performed according to Jensen et al. (8).

Sample extraction: Following the procedure described by Jensen *et al.* (8,9), pieces of liver (5-13 g) from mink or otters were homogenized in hexane:acetone (1:2.5, v:v) using an Ultra Turrax homogenizer (IKA, Staufen, Germany) and the homogenates were then extracted with hexane/diethyl ether (9:1, v:v). The extracts were treated with sulphuric acid to rid them from lipids. The solvent was evaporated and the residues were then dissolved in DMSO.

Tissue culture: The method used was that described by Brunström *et al.* (6). Whole livers from 8-day-old chicken embryos were cultured in glass vials containing 3 ml medium to which test extracts had been added. Six livers were placed in each vial and exposed to one concentration of a test extract. The livers were cultured at 37 °C for 48 h, after which they were weighed and homogenized. The crude liver homogenates were used in the EROD assay.

EROD assay: EROD activities were determined using a slightly modified version of the end-point assay developed by Pohl and Fouts (10) as described by Brunström and Andersson (11). Six or seven concentrations of each extract were tested and concentration-response curves were drawn using computer-aided non-linear regression. EC50 values were determined and compared with the EC50 for TCDD to enable calculation of Bio-TEQ concentrations (6). Clophen A50 was used as a positive reference at a concentration of 0.33 µg/ml medium.

Results and Discussion

Large differences in EROD-inducing potency were found for the different otter liver extracts (Table 1). This variation reflects that there were large differences in PCB concentration in the otter livers analysed, because the samples were chosen to include animals with relatively high and low PCB levels. There was a good correlation between PCB concentrations and Bio-TEQ concentrations in the otter liver samples (Fig. 1).

In Fig. 2, the EROD-inducing potencies of the extracts are shown expressed as inverted EC50 values for the corresponding concentration-response curves. A dose-dependent increase in potency was observed for the mink liver extracts. In both PCB-treated groups of mink the reproduction was impaired, and in the high-dose group no viable kits were produced (5). The major part of the reproductive toxicity of Clophen A50 noted in this mink experiment was caused by the Ah receptor agonists in the mixture (5). The five otter liver samples from animals with muscle PCB concentrations above 50 mg/kg (lipid weight basis) were more potent than the samples from the mink exposed to the high PCB dose. If anticipating that the otter is as sensitive as the mink to Ah receptor agonists, then residue levels of dioxin-like compounds in many otter specimens in Sweden seem to be high enough to cause reproductive impairment.

Recent studies on PCB concentrations in 64 otters from northern Sweden that were traumatically killed show median concentrations of PCB (lipid weight) for the 1970s, 1980s, and 1990s of 27 µg/g, 22 µg/g, and 7.7 µg/g, respectively, and available data show an obvious increase of the otter population in this area in the 1990s (7). These observations together with the present results support the suspicion that PCB has caused detrimental effects on the European otter population.

Table 1. Muscle PCB concentrations and bioassay-derived TCDD equivalent concentrations (Bio-TEQs) in liver samples from ten otters collected in Sweden in 1981-1994.

Year/sex	total PCB (µg/g lipid)	Bio-TEQs (ng/g lipid)	lipid content (%)
1993/male	6	2.0	3.3
1993/female	8	2.6	2.5
1994/female	8	5.1	3.0
1994/male	3	1.4	5.7
1994/male	3	2.7	3.6
1981/male	300	70.6	2.8
1985/female	124	32.6	6.8
1990/female	237	45.7	3.9
1994/male	57	27.7	3.6
1994/male	63	26.9	2.7

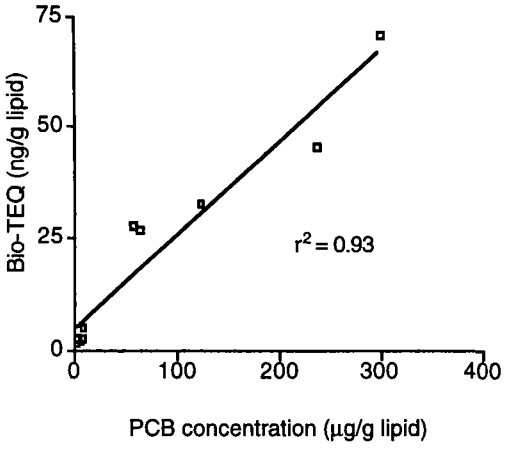

Figure 1. Correlation between PCB concentration and bioassay-derived TCDD equivalents (Bio-TEQs) in extracts from otter livers.

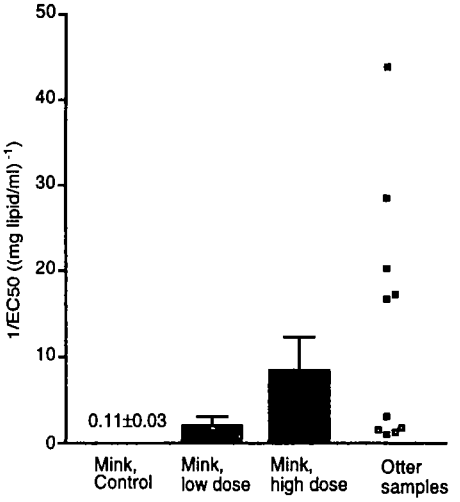

Figure 2. EROD-inducing potencies of extracts from mink and otter livers expressed as the inverted EC50 values for the concentration-response curves. Five mink were used for each dose and values shown are mean and SD.

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