Low Hepatic Ethoxyresorufin-O-Deethylase (EROD) Induction with no Detectable Cytosolic Ah-Receptor Levels in Flounder (*Platichthys flesus*) Exposed to the Polychlorinated Biphenyl (PCB) Mixture, Clophen A50.

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ABSTRACT

Flounder (*Platichthys flesus*) were i.p. dosed with corn oil or the technical PCB-mixture Clophen A50. After 2 and 10 days they were analyzed for hepatic microsomal cytochrome P450 content and ethoxyresorufin-O-deethylase (EROD) activity. No effect on cytochrome P450 content was observed. A slight but significant increase in EROD activity was found in flounder receiving 500 mg Clophen A50/kg b.w. only at day 10. The Aryl hydrocarbon (Ah) receptor could not be detected in the flounder liver cytosol.

INTRODUCTION

Populations of flatfish, especially flounder (*Platichthys flesus*) in the Dutch coastal waters have high incidences of skin infections and liver nodules[1]. It is assumed that polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) may be involved[2]. Field studies have shown that flounder from areas with high levels of these contaminants show higher incidences of skin diseases and liver tumours than in relatively unpolluted areas[3].

Some of the toxic effects of PCBs reported in mammals include hepatotoxicity, immunotoxicity, skin lesions and carcinogenicity. Most of the toxicological effects of PCBs are mediated by the Aryl hydrocarbon (Ah) receptor. There is a good structure dependent correlation between Ah-receptor binding and the potency to induce cytochrome P450 1A (cyp 1A) and associated ethoxyresorufin-O-deethylase (EROD) activity[4]. Induction of EROD activity by PCBs has been reported in several mammals as well as various fish species[5].

The objective of this study is to evaluate the cyp 1A induction potential in liver microsomes of flounder exposed to the PCB mixture, Clophen A50. Also the presence of the Ah-receptor levels in flounder liver cytosol was studied.

METHODS

Chemicals: Clophen A50 was a kind gift from Dr.J.P.Boon (Netherlands Institute for Sea Research, Den Burg, Texel, the Netherlands). A small amount of [^{3}H]-2,3,7,8-

tetrachlorodibenzo-*p*-dioxin ([^{3}H]-TCDD) was obtained from Givaudan, Switzerland. Unlabelled 2,3,7,8-tetrachlorodibenzofuran (TCDF) was purchased from C.N. Schmidt b.v., Amsterdam, the Netherlands.

Animal collection and treatment: Flounder were collected by beam-trawling between the 13th and 17th of June 1992 in the Western Wadden Sea. To avoid damage to the fish the ship towed a 5 m beam-trawl for only 10 minutes. After hauling the net was opened and gonadally immature fish in the size class between 18.0 and 22.0 cm were selected. The fish were kept in fresh water for 1 day, followed by 1 day in sea water and again 1 day in fresh water to free the animals from parasites. After this treatment the fish were acclimatized at 13°C for 4 weeks. During this period fish were kept in 450 L glass tanks with recirculated (biologically filtered) and aerated semi-synthetic seawater (HW Marinemix + Bio-Elements, Wimex, Wiegandt, Krefeld, Germany). Fish were divided into 4 groups of 20 animals each. Three groups received a single i.p. injection of Clophen A50 , 20 mg/kg, 100 mg/kg or 500 mg/kg, dissolved in corn oil. 20 fish were injected with corn oil only. After 2 and 10 days, 10 fish of each group were terminated by cervical transection. Livers were carefully dissected free from gall bladder, washed in ice-cold 0.9% NaCl, weighed and prepared for cytosol and microsomes. Cytosol and microsomes were stored at -80°C until analysis.

Total cytochrome P-450 content: Total cytochrome P-450 content in the microsomal fraction of flounder liver was determined using the method described by Omura and Sato[6]. Protein levels were analyzed by the Bio-Rad assay system.

Enzyme induction: Microsomal 7-ethoxyresorufin-O-deethylation (EROD) activity was determined using the method of Prough *et al.*[7]. The assay was optimized for flounder and executed at 25°C on a Hitachi fluorescence spectrophotometer F-2000.

Receptor assay: Ah-receptor levels were determined by the hydroxylapatite (HAP) adsorption assay as described by Gasiewicz and Neal[8]. Briefly, ice-cold fish cytosol and rat cytosol (positive control) were diluted to 2 mg/ml protein and incubated for 2 hr at 20°C with [^{3}H]-TCDD (2 nM final concentration) in presence or absence of a 100-fold molar excess of unlabelled TCDF. Labelled cytosols were adsorbed to hydroxylapatite, washed with HEDG-buffer (25 mM Hepes, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol) containing 0.5% Tween 80 (v/v), pelleted and counted on a Packard liquid scintillation analyzer 1600 TR. Specific binding was calculated as the difference between total binding (in absence of TCDF) and nonspecific binding (in presence of TCDF).

Statistics: Data were analyzed for difference to control using the Mann-Withney test.

RESULTS

No effect on total cytochrome P450 levels was observed after 2 days as well as 10 days following Clophen A50 treatment. Even flounder injected with 500 mg Clophen A50/kg bodyweight did not show altered total cytochrome P-450 levels. In contrast, a slight but significant increase in EROD activity was found in flounder receiving 500 mg Clophen A50/kg bodyweight 10 days after exposure (figure 1), which appeared to be dose-dependent. The results of Ah-receptor levels in control rat cytosol and control flounder cytosol are presented in Table 1. Although the presence of the Ah-

receptor in rat cytosol could be demonstrated, it was not possible to trace an Ah-receptor-like entity in flounder cytosol using the method described.

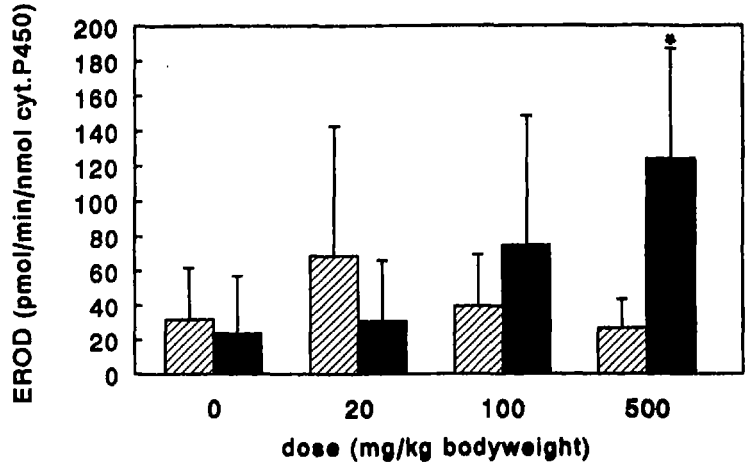

Figure 1. Effect of Clophen A50 on EROD activity in liver microsomes of flounder, 2 (▨) or 10 (■) days after i.p. dosing. Significant differences between treated and control flounder from the same day of termination are given ($P<0.05$).

Table 1. *Concentration of the TCDD receptor in rat and flounder hepatic cytosol.*

species	Ah-receptor (fmol/mg protein)
Wistar rat (3)	34.3 ± 2.5
flounder (6)	N.D.*

Note. Each value represents the mean ± SD of (x) determinations.
* Not detected.

DISCUSSION

Several authors have reported a cytochrome P450 and EROD induction in flounder exposed to environmental pollutants (for example polyhalogenated aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Most of these studies were performed using flounder originating from heavily polluted area[9]. Whether the induction seen in these animals is caused by PCBs, PAHs or other xenobiotics is unclear. Eggens[10] showed

a significant increase in cytochrome P-450 induction and EROD activity in flounder orally dosed with B(a)P. In the present study, flounder were i.p. dosed with the PCB mixture Clophen A50. The results of this study suggest that flounder is not very sensitive to PCB exposure in terms of cytochrome P450 and EROD induction. No in vitro metabolism capacity of [^{3}H]-3,4,3',4'-TCB in flounder hepatic microsomes was found. In this assay microsomes with a relatively high EROD activity were used but compared to the EROD activity in trout hepatic microsomes the activity in flounder was very low (Murk *et al.*[11]). Also, we were not able to demonstrate the presence of the Ah-receptor in the liver cytosol of flounder.

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