IN VITRO METABOLISM OF 3,4,3',4'-TETRACHLOROBIPHENYL BY MICROSOMES OF WILDLIFE SPECIES COMPARED TO THE RAT

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ABSTRACT
Hepatic microsomes of common tern (*Sterna hirundo*), eider duck (*Somateria mollissima*), rainbow trout (*Salmo gairdneri*), flounder (*Platichthys flesus*) and rat were incubated with ^{14}C labelled 3,3',4,4'-tetrachlorobiphenyl ([^{14}C]TCB). The birds were able to metabolize TCB. The pattern of the resulting hydroxylated metabolites differed between birds and rat. Surprisingly the trout exhibited an induced EROD activity, but did not metabolize any TCB. The flounder exhibited neither EROD activity nor any metabolism of TCB.

INTRODUCTION
Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants that accumulate in top predators of the aquatic foodchain like fish eating birds and mammals. In lung breathing animals PCBs have to be catabolyzed by the cytochrome P450 mono-oxygenase system before elimination can take place. Metabolic capacities of species have mostly been investigated by comparing PCB-congener composition of internal burden with that of food[1]. This method is however indirect and untill recently 3,3',4,4'-tetrachlorobiphenyl (TCB) and some other congeners could not be separated from each other on the chromatogram without special sample preparation. There is little or no data on in vitro biotransformation of TCB in fish, fish eating birds, marine mammals. We therefore performed an in vitro metabolism experiment with [^{14}C]-labelled TCB in hepatic microsomes of some wildlife species, including fish, fish eating birds and marine mammals. The results of the marine mammals are reported elsewhere[2]. A comparison is made between level of induction of cytochrome P450 1A1 activity, measured as ethoxyresorufin deethylase (EROD) activity, and the amount of metabolites formed.

MATERIALS AND METHODS
[^{14}C]TCB incubation and HPLC analysis: Four standard metabolites: 2-hydroxy-3,3',4,4'-TCB (2-OH-TCB), 4-hydroxy-3,3',4',5-TCB (4-OH-tetraCB) 5-hydroxy-3,3',4,4'-TCB (5-

OH-TCB) and 6-hydroxy-3,3',4,4'-TCB (6-OH-TCB) were synthesized as described elsewhere[3]. Purified[4] [^{14}C]TCB (Sigma Chemical Co.) with a specific activity of 37.1 mCi/mmol was incubated in duplicate and analyzed on HPLC according to Morse et al.[4]. Aliquots op 1 mg hepatic microsomal suspension with 10μM [^{14}C]TCB and 50 nM Tris-HCl buffer (pH=7.5) were pre-incubated for 2 minutes at 37°C. The reaction was started by adding NADPH (final concentration 1 nM) and ended after 5 minutes by adding 1 volume ice-cold methanol to the reactionmix. [^{14}C]TCB and metabolites were extracted with 2 volumes diisopropylether. The ether extract was evaporated under nitrogen, resuspended in 50 μl of methanol. Samples of 20 μl were injected on the HPLC. Separation of metabolites and TCB was performed with an eluent of 78% methanol and 22% water for 15 minutes, then 100% methanol for 16 minutes on a Perkin Elmer 30x3 mm coupled to a 83x3 C-18 3 μm column. Flow rate was 0.8 ml/min, 0.4 minute fractions were collected and radioactivity counted. The pattern of radioactive peaks was compared with the position of the standard metabolites on the UV-absorbance chromatogram (265 nm).

Origin of the microsomes: **Eider duck** microsomes originated from a semi-field experiment with eider ducklings dosed i.p. with 200 mg Clophen A50/kg bw and with 50 mg TCB/kg bw, killed 10 days after exposure[5]. **Common tern** microsomes were from one day old chicks artificially bred from eggs collected natural colonies[6]. **Trout** microsomes were prepared from orally dosed animals with 54 or 215 mg CloA50/kg bw, and killed after 10 or 20 days. **Flounder** microsomes were from an animal dosed i.p. with 500 mg Clophen A50/kg, and killed after 10 days[7]. **Rat** microsomes were obtained from a 16 week old Wister rat, treated 3 times i.p. with 30 mg BNF/kg bw, sacrificed 24 hours after the last treatment.

Protein levels were measured according to Bradford[8], using the Bio-Rad protein assay dye.

EROD activity was determined described by Prough et al.[9], using a Hitachi F-2000 Fluorescence spectrophotometer.

RESULTS AND DISCUSSION

A wide range in EROD activities was observed in the samples used for in vitro metabolism of TCB (see table). Trout killed after the same period (10 or 20 days) showed the same EROD activity although doses differed by a factor of 4. The TCB dosed eider showed a high capacity to synthesize metabolites. The amount of metabolites formed by the Clophen A50 dosed eider is slightly lower when related to the EROD activity. The mean ratio for both eiders was 4.0, which is exactly the same as for the rat (see table). The total amount of TCB metabolized in the tern is low, also compared to the EROD activity (ratio is 1.1). The observed metabolism of TCB by the eider is in contrast to the conclusion based on comparison of PCB-congener patterns in Aroclor and in wild fish eating sea birds, that PCB-congeners with chlorine substituted at adjacent meta and para positions strongly bioaccumulate in birds[1]. However TCB itself was not determined in these studies, nor in other studies reviewed by Walker[10].

The most striking result of this experiment was the complete lack of TCB metabolite formation by all (highly induced) trout liver microsomes (see table). This suggests that the

EROD activity, metabolite formation and their ratio (%) of wildlife species compared to rat.

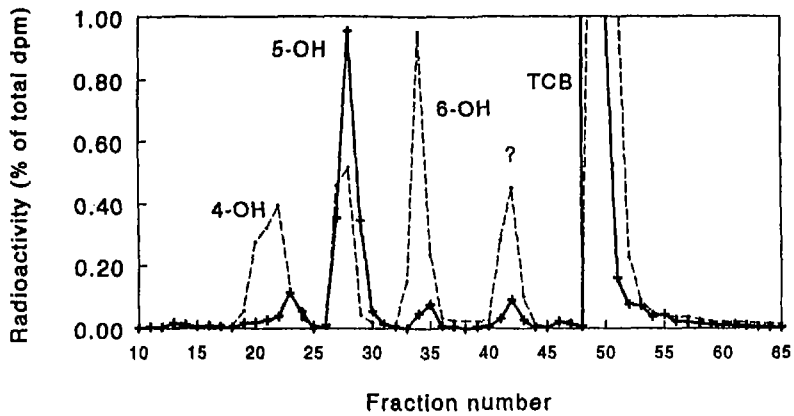
Species	Exposure	EROD act. pmol/mg.min	Metabolites pmol/mg.min	Ratio (x100) metabs./EROD
Eider duck	TCB (50 mg/kg)	1050	47	4.5
Eider duck	CloA50[a] (200mg/kg)	616	21	3.4
Common tern	Environment	130	1.4	1.1
Trout[b]	CloA50 (both doses)	330	0	0
Trout[c]	CloA50 (both doses)	256	0	0
Flounder	CloA50 (500 mg/kg)	2	0	0
Rat	BNF	2290	91.4	4.0

[a]CloA50 = Clophen A50 [b]killed after 10 days [c]killed after 20 days

substrate specificity of P4501A is different between the tested fish, birds and rat. The EROD activity of the flounder microsomes appeared to be very low despite high dose PCB-treatment. Consequently no TCB-metabolite formation was observed. Our findings of the inability of trout and flounder to metabolize TCB are in accordance with conclusions based on comparison of PCB-congener patterns, including TCB, in fish, that apart from the eel (Anquilla anquilla) none of the 14 tested (shell)fish species, can metabolize TCB[11].

The relative amount of OH-TCB metabolites formed differed between the rat (and marine mammals, data not shown) and the birds (see figure). The eiders and common tern formed mainly 5-OH-TCB (±75% of total amount of metabolites), and less 4-OH-tetraCB (±10%) and 6-OH-TCB (±8%). In the rat 24% of the metabolites is 5-OH-TCB, the major metabolite is 6-OH-TCB (31%) followed by 4-OH-tetraCB (28%). Both eiders and rat produced an unknown metabolite (see figure) which comprised respectively ±8% and ±19% of the total amount of metabolites.

From our experiments we conclude that although EROD activity is a valid measure of exposure to PCBs for most species, it is not neccesarily a measure of the ability of P450IA of a species to metabolize TCB. Within the same species however, biotransformation capacity of microsomes for TCB and EROD activity are related. The observed species differences in inducibility of P4501A1 and amount and composition of OH-metabolites formed may be a consequence of evolutionary differences, food specialization[12] and (in)ability to loose PCBs through the gills. This may pose serious problems for extrapolation of toxicity data, on the basis of EROD activity, from one type of species to another. Especially for those toxic effects that are related to OH-TCB metabolites, such as



The HPLC profile of TCB and its metabolites from hepatic microsomes of CB-77 induced eider (--) and BNF induced rat (+). The codes 4-OH, 5-OH and 6-OH indicate the position of the standard metabolites on the corresponding chromatogram.

interference in plasma transport of thyroid hormone and vitamin A[13], mitochondrial uncoupling of oxidative phosphorilation, inhibition of hepatic thyroxin 5'- deiodinase and interactions with estrogen receptors[14].

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