DISTRIBUTION OF HYDROXYLATED PCB METABOLITES IN PREGNANT MICE

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Introduction

Polychlorinated biphenyls (PCBs) are highly lipophilic compounds that are quite resistent to degradation and therefore accumulate in biota. PCBs are mainly retained in adipose tissue and liver, but could also be metabolized by hepatic microsomal mixed function-oxidases to hydroxylated metabolites and eliminated chiefly *via* the bile as conjugates. In lactating animals, the major route of elimination is *via* milk[1)].

Earlier studies at our laboratory have shown that administration of 3,3´,4,4´-tetrachlorobiphenyl (CB-77) to pregnant mice gives rise to a high accumulation of hydroxylated metabolite(s) in the fetal compartment[2)]. One of the hydroxylated metabolites of CB-77 - 4-hydroxy-3,3´,4´,5-tetrachlorobiphenyl - has been suggested to affect fetal thyroxin levels, probably by transthyretin (TTR) binding and interference with plasma transport of the hormone[3,4)]. In addition, several hydroxylated PCB metabolites are selectively retained in blood from mammals, of which 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl, a metabolite of 2,3,3´,4,4´-pentachlorobiphenyl (CB-105) is one of the most abundant compounds[5)].

It is therefore of interest to study some toxicokinetic aspects of the *para*-substituted hydroxylated metabolites of CB-77 and CB-105 in the pregnant mouse.

Materials and methods

Chemical and animals. ^{14}C-labelled 4-hydroxy-3,3´,4´,5-tetrachlorobiphenyl (4-OH-TCB) and 4-hydroxy-2´,3,3´,4´,5-pentachlorobiphenyl (4-OH-PeCB) (spec. act. 4.9 and 7.4 µCi/µmol, respectively; purity ≥ 98%) were synthesized as follows: 4-Methoxy-3,3´,4´,5-tetrachloro-[^{14}C]biphenyl was synthesized from ^{14}C-labelled 3,4-dichloroaniline (Sigma chemicals) and 2,6-dichloroanisole *via* the Cadogan diaryl coupling reaction[6)]. 4-Methoxy-2´,3,3´,4´,5-pentachloro-[^{14}C]biphenyl was prepared in a multi-step synthesis from ^{14}C-labelled 4-hydroxy-acetanilide (Sigma chemicals). 4-OH-TCB and 4-OH-PeCB were

obtained after demethylation of the two methoxy-biphenyls by boron tribromide (1M) over night at ambient temperature.

NMRI and C57BL mice were purchased from Bomholtgård, Denmark. The animals were given free access to commercial pelleted food (R3, Ewos AB, Södertälje) and tap water, and were kept at 22 °C and at a 12 hr light-dark cycle. The mice were mated over night and the presence of a vaginal plug on the following day was defined as day 0 of the pregnancy.

Dosage. The labelled compounds were dissolved in DMSO and injected i.v. in a tail vein in near equimolar doses (ca. 2 µmol/kg body wt), except in the dose-dependent accumulation study (0.5-5 µmol/kg body wt.), in C57BL and NMRI (only 4-OH-TCB, day 16-17) pregnant mice as a single dose at day 16 of gestation. The animals were sacrificed at 1, 8, 24, 48 hours after administration. Whole blood (collected by cardiac puncture), liver and adipose tissue were obtained from dams, and whole blood (collected in heparinized capillary tubes), and liver were collected from the fetuses. Blood was centrifuged to obtain plasma. About 60-100 mg of tissues and 25-50 µl of the plasma were taken for quantitative measurements. The samples (tissue specimen after Soluene 350 digestion) were mixed with 10 ml scintillation fluid (Hionic Fluor, Packard) and the radioactivity was measured by liquid scintillation counting.

Results and discussion

Time dependent distribution. The two metabolites, 4-OH-TCB and 4-OH-PeCB, gave rise to a similar type of distribution; the maternal liver contained the highest absolute ^{14}C-concentrations, but in the fetus the relative uptake in the plasma exceeded that of the liver (Fig. 1). The concentration in fetal plasma surpassed that of maternal plasma between 8 and 24 hr after injection. Differences between the two substances were particularly seen in maternal liver and fetal plasma, where 4-OH-TCB showed a stronger retention at longer post-injection times; in maternal liver 4-OH-TCB had much longer calculated elimination half-life ($t_{1/2}$) (69.0 hr) than 4-OH-PeCB (17.0 hr). However, in maternal plasma the $t_{1/2}$ for both substances were equal (13.0 hr). The occurrence of multi-phase kinetics could perhaps lead to different values after a longer observation period than 48 hr.

Dose dependent tissue accumulation. The concentration of 4-OH-TCB in the investigated fetal and maternal C57BL mice tissues increased more or less linear with dose at 24 hours after the injection (Fig. 2). The fetal/maternal plasma ratio was 2.2-2.4 at the doses 0.5, 2.2 and 5.0 µmol/kg body wt. and no differences in tissue distribution pattern were observed.

Strain dependent accumulation. Results from the comparative tissue distribution of 4-OH-TCB in NMRI and C57BL mice showed that the fetal as well as maternal tissue concentration in the both mice strains were roughly the same (data not shown).

The differences in tissue distribution of the two metabolites are very small in comparison to the differences in tissue distribution shown by the parent compounds, CB-77 and CB-105[7] but are probably explained by the differences in lipophilicity and rate of metabolism[8,9]. It is notable that the observed hepatic $t_{1/2}$ of 4-OH-TCB in C57BL mice is longer than the parent compound, CB-77[10], whereas the opposite is true for the plasma $t_{1/2}$.

The distribution of 4-OH-TCB in C57BL mice is similar to that of NMRI. In contrast, big differences have earlier been observed when the parent compound was compared in these two strains (fetal uptake ca five times higher in C57BL)[7]. Our hypothesis that strain differences in metabolic activity[11] could explain the observed dissimilarities in fetal uptake is therefore strengthened by the present results, where the metabolite itself was administered and the initial metabolic step consequently omitted.

Fig. 1. Tissue concentration of ^{14}C-labelled 4-OH-TCB versus time in dams (A) and fetuses (B) and of ^{14}C-labelled 4-OH-PeCB in dams (C) and in fetuses (D) after i.v. administration a single dose of 4-OH-TCB (2.16 μmol/kg body wt.) or of 4-OH-PeCB (1.87 μmol/kg body wt.) to C57BL pregnant mice at day 16. Semilogarithmic/linear regression graphs are used for the dams' tissue levels. Each point represents the mean ± SD for four animals. The fetal samples are pooled from 3-9 fetuses/animal (D, 48 hr: 3 fetuses from one animal).

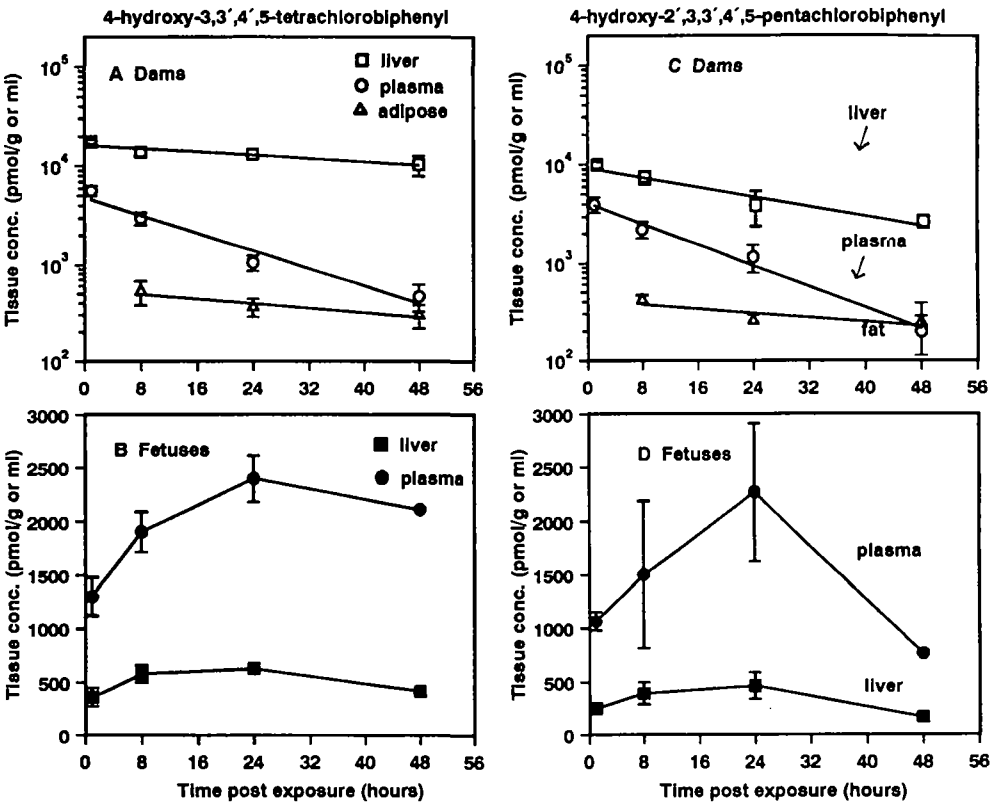
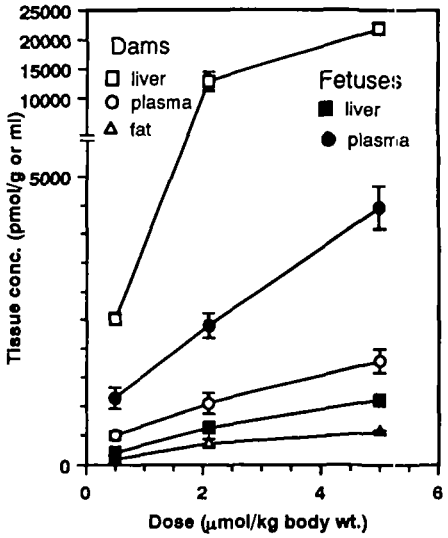


Fig. 2. Concentration of ^{14}C-4-OH-TCB (pmol/ml plasma or g tissue) in certain C57BL dam and fetus tissues, one day after i.v. administration of different doses in μmol/kg body wt.(day 17 of gestation). Each value represents the mean with S.D. from four animals. The fetal samples are pooled from 3-9 fetuses/animal. The curved appearance of the uptake in dams liver could chiefly be explained by a shift in scale.



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