TOXICOLOGICAL SCIENCES **68,** 361–371 (2002)

Placental Transfer of a Hydroxylated Polychlorinated Biphenyl and Effects on Fetal and Maternal Thyroid Hormone Homeostasis in the Rat

Ilonka A. T. M. Meerts,*,[1] Yvonne Assink,* Peter H. Cenijn,* Johannes H. J. van den Berg,* Bert M. Weijers,† Åke Bergman,‡ Jan H. Koeman,* and Abraham Brouwer*,§

**Toxicology Group, Wageningen University and Research Center, Tuinlaan 5, 6703 HE Wageningen, The Netherlands; †Laboratory Animal Center, Wageningen University and Research Center, Bomenweg 2, Wageningen, The Netherlands; ‡Department of Environmental Chemistry, Wallenberg Laboratory, Stockholm University, Stockholm, Sweden; and §Institute of Environmental Studies, Vrije Universiteit, Amsterdam, The Netherlands*

Received February 5, 2002; accepted April 8, 2002

Earlier studies at our laboratory indicated that several hydroxylated polychlorinated biphenyls (OH-PCBs) detected in human blood could specifically inhibit thyroxine (T_4) transport by competitive binding to the thyroid hormone transport protein transthyretin (TTR) *in vitro*. In the present study we investigated the effects of prenatal exposure to 5 mg/kg body weight of [^{14}C]-labeled or unlabeled 4-OH-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107), one of the major metabolites of PCBs detected in human blood, from gestation days (GD) 10 to 16 on thyroid hormone status and metabolism in pregnant rats and their fetuses at GD 17 and GD 20. 4-OH-CB107 is a metabolite of both 2,3,3′,4,4′-pentachlorobiphenyl (CB-105) and 2,3′,4,4′,5-pentachlorobiphenyl (CB-118). We were able to show the accumulation of 4-OH-CB107 in the fetal compartment. The fetal/maternal ratios at GD 20 in liver, cerebellum, and plasma were 11.0, 2.6, and 1.2, respectively. The ^{14}C-4-OH-CB107-derived radioactivity in plasma was bound to TTR in both dams and fetuses. Fetal plasma TT_4 and FT_4 levels were significantly decreased at GD 17 and GD 20 (89% and 41% respectively at GD 20). Fetal thyroid stimulating hormone levels were increased by 124% at GD 20. The T_4 concentrations in fetal forebrain homogenates at GD20 were reduced by 35%, but no effects could be detected on brain T_3 concentrations. The deiodination of T_4 to T_3 was significantly increased in fetal forebrain homogenates at GD 17, and unaltered at GD 20. In addition, no alterations were observed in maternal and fetal hepatic T_4-UDP-glucuronosyltransferase activity, type I deiodinase activity, and EROD activity. In conclusion, exposure of pregnant rats to 4-OH-CB107 results in the distribution of the compound in the maternal and fetal compartment, which is probably caused by the binding of the PCB metabolite to TTR. Consequently, TT_4 levels in fetal plasma and brain samples were reduced. Despite reductions in fetal brain T_4 levels, the active hormone (T_3) in fetal brains remained unaffected.

Key Words: **hydroxylated PCB; T_3; T_4; thyroid stimulating hormone; glucuronosyltransferase.**

Polychlorinated biphenyls (PCBs) are widespread, persistent environmental pollutants that have been reported to cause a variety of toxic effects, including neurotoxicity, developmental toxicity, reproductive toxicity, and carcinogenesis (reviewed in Peterson *et al.*, 1993; Safe, 1990, 1994; Schantz, 1996; Seegal, 1996). In recent years it has become evident that exposure to PCBs can also lead to thyroid hormone disturbances in laboratory animals, wildlife, and even humans as reviewed by Brouwer *et al.* (1998). Decreased levels of circulating plasma thyroxine (T_4) following PCB exposure have been shown in both adult (Barter and Klaassen, 1994; Byrne *et al.*, 1987; Van den Berg *et al.*, 1988) and developing organisms (Collins and Capen, 1980; Darnerud *et al.*, 1996; Morse *et al.*, 1993, 1996a; Ness *et al.*, 1993; Seo *et al.*, 1995). Plasma thyroid hormone levels can be decreased by xenobiotic compounds by at least 3 known mechanisms. Firstly, a direct effect of compounds on the thyroid gland can lead to a decreased synthesis of thyroid hormones, which has been reported in rats after exposure to the commercial PCB mixture Aroclor 1254 (Collins and Capen, 1980). Secondly, the reduction in thyroid hormone levels can be caused by enhanced biliary excretion of T_4 due to the induction of UDP-glucuronosyltransferases (UDP-GT; Barter and Klaassen, 1992; Bastomsky, 1974; Van Birgelen *et al.*, 1995). The third known mechanism involved in reduced plasma T_4 levels is the observed binding of PCB metabolites to the plasma thyroid hormone transport protein, transthyretin (TTR), thereby displacing the natural ligand T_4 (Brouwer and van den Berg, 1986; Darnerud *et al.*, 1996; Morse *et al.*, 1996a; Rickenbacher *et al.*, 1986).

TTR is the only thyroid hormone binding plasma protein that is synthesized both in liver and brain. It is suggested to serve a role in mediating the delivery of T_4 across the blood-brain barrier and the maternal to fetal transport through the placenta (Schreiber *et al.*, 1995; Southwell *et al.*, 1993). In addition, TTR plays an essential role in the determination of free T_4 levels in the extracellular compartment of the brain, which is independent of the homeostasis of T_4 in the body (Schreiber *et*

[1] To whom correspondence should be addressed at present address: NOTOX Safety and Environmental Research B.V., Hambakenwetering 7, 5231 DD's-Hertogenbosch, The Netherlands. Fax: +31-73-6406799. E-mail: ilonka.meerts@notox.nl.

al., 1995). T_4 in the brain is then converted to the active thyroid hormone, triiodothyronine (T_3) by specific deiodinases (type II deiodinase). An increasing number of chemicals have been reported to bind to human TTR *in vitro.* Parent PCB congeners (Chauhan *et al.,* 2000; Cheek *et al.,* 1999; Rickenbacher *et al.,* 1986) but especially hydroxylated metabolites of PCBs, dibenzo-*p*-dioxins and dibenzo-*p*-furans (Lans *et al.,* 1993) showed competitive binding to human TTR. Recently we were able to detect a new class of compounds, the brominated flame retardants (e.g., polybrominated diphenyl ethers, brominated bisphenols), with high *in vitro* T_4-TTR competition binding potency (Meerts *et al.,* 2000).

The *in vivo* effects of the high binding affinity of xenobiotics such as hydroxylated polychlorinated biphenyls (OH-PCBs) to TTR is hypothesized to result in (1) a selective retention of these compounds in plasma, (2) facilitated transport of the metabolites over the placenta to the fetal compartment, and (3) decreased maternal and fetal plasma T_4 levels by competition with the natural ligand T_4 (reviewed by Brouwer *et al.,* 1998). Several studies support this hypothesis. Bergman *et al.* (1994) detected several OH-PCBs, with high *in vitro* T_4-TTR binding potency, in human serum and wildlife samples environmentally exposed to PCBs. Exposure of rats to 3,3′,4,4′-tetrachlorobiphenyl resulted in selective retention of hydroxylated metabolites in plasma and caused marked reductions in plasma thyroxine levels (Brouwer *et al.,* 1990) and vitamin A transport (Brouwer and Van den Berg, 1986) via their binding to TTR. In addition, maternal exposure of rats to Aroclor 1254 from gestation days 10 to 16 resulted in selective accumulation of the metabolite 4-OH-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107) in fetal plasma and brain (Morse *et al.,* 1996a) and was accompanied by very low concentrations of T_4 in both tissues. In a comparable study conducted in mice, Darnerud *et al.* (1996) showed a high and selective accumulation of 4-OH-3,3′,4′,5-tetrachlorobiphenyl in fetal mouse plasma and reductions in thyroid hormone levels after maternal exposure to 3,3′,4,4′-tetrachlorobiphenyl. They were able to identify the metabolite in fetal plasma bound to TTR.

In vivo toxicity data on the effects of hydroxylated PCB-congeners on thyroid hormone homeostasis are scarce, since most *in vivo* studies are conducted with parent compounds that can exert effects of their own (e.g., induction of UDP-GT) and undergo metabolism in the exposed animal to different metabolites. Therefore, in the present study we investigated the effects of maternal exposure to the synthesized PCB metabolite 4-OH-CB107 on maternal and fetal rat thyroid hormone homeostasis. To determine maternal to fetal transfer, we also studied the uptake and distribution of ^{14}C radiolabeled 4-OH-CB107. We chose this metabolite, because it was one of the major metabolites identified in human blood samples (Bergman *et al.,* 1994), and was shown to accumulate in fetal plasma and brain after maternal exposure to Aroclor 1254 (Morse *et al.,* 1996a). Furthermore, 4-OH-CB107 was shown to be a metabolite, formed via a 1,2-shift of a chlorine atom, of 2,3,3′,4,4′-pentachlorobiphenyl (CB-105) and of 2,3′,4,4′,5-pentachlorobiphenyl (CB-118; Sjödin *et al.,* 1998). Both PCB congeners are present in adipose tissue of humans and wildlife and can thus slowly be biotransformed to the 4-OH-CB107 that is retained in the blood. We especially focused on testing the hypothesis that binding of a PCB metabolite to transthyretin *in vivo* would lead to facilitated transfer of the compound to the fetal compartment resulting in decreased thyroid hormone levels in fetal plasma and brain.

MATERIALS AND METHODS

Chemicals. 4-Hydroxy-2,3,3′,4′,5-pentachloro-[^{14}C]biphenyl (specific activity: 15.6 mCi/mmol) was prepared from 3,4-dichloroiodo-[^{14}C]benzene, prepared from 3,4-dichloro-[^{14}C]aniline after this compound had been methylated with diazomethane and reacted with iodine, and 2,3,6-trichloro-4-iodoanisol via an Ullman reaction (Bergman *et al.,* 1990). 4-Hydroxy-2,3,3′,4′,5-pentachloro-[^{14}C]biphenyl was isolated in a chemical and radiochemical purity $>$ 98%. Unlabeled 4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107, $>$ 99%) was synthesized as described by Bergman *et al.* (1995). Isopropanol, bovine serum albumin, sucrose, Tris, hydrogen peroxide (H_2O_2), potassium hydroxide, Triton X-100, diisopropyl ether, dithiothreitol, and methanol (all solvents were analytical grade) were purchased from Merck Chemical Company (Darmstadt, Germany). Human prealbumin (TTR, 98% pure) was obtained from Sigma Chemical Company (St. Louis, MO). [^{125}I]-L-3′,5′-Thyroxine (spec. act. 46 μCi/μg) was from Orange Medical (Tilburg, The Netherlands).

Animals. All experimental procedures involving animals were approved by the Animal Welfare Committee of the Wageningen University. Wistar WU rats (60 females, 30 males; 14 weeks old) were purchased from Charles River (Sulzfeld, Germany) and allowed to acclimatize for 3 weeks. Throughout the experiment, animals were kept in an artificial 12:12-h light-dark cycle with lights on at 0600 h. Room temperature was maintained at 21 $\pm$ 2°C and humidity at 50 $\pm$ 10%. Animals were provided rat chow (Hope Farms, Woerden, The Netherlands) and tap water *ad libitum.*

After the acclimatization period 2 females were placed in a cage with 1 male overnight from 1700 to 800 h. Copulation was determined each morning by checking the presence of sperm in the vaginal smear. When spermatozoa were found, this day was designated as day 0 of gestation (GD 0) and females were housed individually. Body weight of the dams was measured throughout gestation. On day 10 of gestation the pregnant rats were divided randomly into the different treatment groups and transferred to a macrolon, stainless steel cage to facilitate the collection of feces.

Study on uptake and distribution of [^{14}C]-labeled 4-OH-CB107 in dams and fetuses. For investigating the uptake and distribution, 6 pregnant rats received a daily po dose of 2.3 μCi [^{14}C]-labeled 4-OH-CB107 per kg body weight diluted with unlabeled 4-OH-CB107 for a total exposure dose of 14.6 μmol (5 mg) 4-OH-CB107 per kg body weight per day from GD 10 to 16. The metabolite was dissolved in corn oil, 5 mg/2 ml. Feces and urine were collected daily. On GD 17 and GD 20, 3 dams per time point were sacrificed under ether anesthesia and maternal blood was collected via the *vena cava* in heparinized tubes. Maternal kidneys, liver, adrenals, pancreas, lungs, thymus, forebrain, cerebellum, brown adipose tissue, skeletal muscle, and abdominal fat were collected for radioactivity analyses. Individual placental/fetal units were carefully removed from the uterus. Fetuses were separated from the placenta, blotted dry with tissue paper, and weighed. Fetal trunk blood, obtained by decapitation, was collected in heparinized tubes, pooled per litter, and stored on ice until plasma was prepared for thyroid hormone analysis and radioactivity determinations. From 17-day-old fetuses, livers and brains (separated into forebrain and cerebellum) were collected and pooled per litter. From 20-day-old fetuses, lungs and kidneys were additionally collected and pooled per litter. Organs and placentas were rinsed with 0.9% sodium chloride, blotted

dry with tissue paper, weighed, and stored at −80°C. Carcasses were stored at –20°C. Cages were rinsed with 200 ml Triton X-100 at the end of the experiment to determine losses of radioactivity. Maternal and fetal plasma, liver, and brain samples were also used in biochemical assays described below ($n = 3$ per time point).

Study on biochemical effects of 4-OH-CB107 in dams and fetuses. In a parallel experiment, pregnant rats received a daily po dose of 0 or 5 mg 4-OH-CB107 per kg body weight dissolved in corn oil (2 ml/kg body weight) from GD 10 to GD 16. On GD 17 and GD 20, 4 dams per time point and exposure were sacrificed under ether anesthesia and maternal blood was collected via the *vena cava* in heparinized tubes. Fetuses were removed and weighed. Fetal trunk blood was collected in heparinized tubes and pooled per litter. Fetal liver and thymus were collected, weighed, frozen on dry ice, and pooled per litter. Fetal brains were removed, separated into forebrain and cerebellum, and frozen on dry ice. One fetal forebrain per litter was saved separately for thyroid hormone analysis, the remaining forebrains and cerebella were pooled per litter for analysis of thyroid hormone metabolism and stored at −80°C. From the dams, liver, brain, thymus, and plasma were isolated, frozen in liquid nitrogen and stored at −80°C until analysis.

Tissue radioactivity concentrations. Approximately 60 to 100 mg of tissues or tissue homogenates and 25–50 μl of the plasma samples were dissolved in 1 ml Soluene-350 (Packard, St. Louis, MO) in glass scintillation vials. Samples were bleached with 0.5 ml 30% H_2O_2, and total radioactivity was measured 2 days later with 20 ml Hionic Fluor scintillation fluid (Packard) in a Packard 1600 liquid scintillation counter (LSC). Fecal samples were homogenized with a mortar under liquid nitrogen. Aliquots (± 50 mg) of feces homogenates were exactly weighed and solubilized with 1 ml Soluene-350 at 50°C during 1–2 h in closed glass scintillation vials. After addition of 0.5 ml isopropanol samples were incubated at 50°C for another 2 h. Samples were bleached by the addition of 0.6 ml 30% H_2O_2, and total radioactivity was measured 2 days later with 20 ml Hionic Fluor by LSC. The carcasses of dams and fetuses were dissolved in 700 ml (dams) or 200 ml (fetuses) 1.5 M potassium hydroxide containing 20% ethanol (v/v). After homogenization using an Ultra Turrax 0.5 ml aliquots (in total $n = 10$) were bleached with 0.6 ml 30% H_2O_2 and total radioactivity was measured 2 days later with 20 ml Hionic Fluor. The efficiency of counting was determined by quenching correction curves for the various additions and scintillation fluids. In order to estimate total radioactivity concentrations in plasma and skeletal muscle, the total weight of plasma and skeletal muscle was set at 4 and 40% of the total body weight, respectively.

Sample processing for biochemical purposes. Livers were thawed on ice and homogenized in ice-cold 0.1 M Tris–HCl buffer, pH 7.5, containing 0.25 M sucrose (3 ml/g liver) using a Potter tube. The homogenate was centrifuged for 30 min at 9000 × *g* (4°C). The resulting supernatant was centrifuged for 90 min at 105,000 × *g* (4°C). The microsomal pellet was resuspended in 0.1 M phosphate buffer (pH 7.5). Microsomes were stored in aliquots of 1 ml at −80°C until further analysis.

Maternal and fetal (pooled per litter) forebrains were homogenized in a Potter tube in 8 volumes ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 1 mM dithiothreitol and stored at −80°C until further analysis. Protein levels in different tissue fractions were determined using the BioRad Protein reagent (Bradford, 1976).

Thyroid hormone analysis. Plasma total T_4 (TT_4), free T_4 (FT_4), and total T_3 (TT_3) were analyzed in duplicate using chemiluminescence kits. Plasma thyroid stimulating hormone (TSH) concentrations were analyzed with a specific rat TSH immunoassay. All kits were purchased from Amersham (Amersham, Buckinghamshire, UK).

Brain T_4 and T_3 concentrations were determined by specific RIAs in purified extracts, as described before (Morreale de Escobar *et al.*, 1985). Briefly, maternal and fetal forebrain and cerebellum samples were homogenized in methanol, extracted in chloroform-methanol and back-extracted into an aqueous phase. This aqueous phase was purified through Bio-Rad AG 1×2 resin columns (Bio-Rad Laboratories, Richmond, VA), and the iodothyronines were eluted with 70% acetic acid, which was evaporated to dryness. The iodothyronines were analyzed in highly sensitive RIAs in duplicate at 2 different dilutions. Recovery of the extraction procedure was determined in each homogenate by the addition of tracer amounts of [^{131}I]-T_4 and [^{125}I]-T_3.

Thyroid hormone metabolism. Hepatic microsomal T_4 uridine diphosphoglucuronosyl transferase activity (UDP-GT) was determined as described by Beetstra *et al.* (1991) and Visser *et al.* (1993). In short, microsomes (1 mg protein per ml) were incubated for 30 min at 37°C with 1 μM T_4 and 50,000 cpm [^{125}I]-T_4, 5 mM uridine 5′-diphosphoglucuronic acid, 3.75 mM $MgCl_2$ and 0.125% (w/v) BSA in 75 mM Tris–HCl buffer (pH 7.8). The final reaction volume was 0.2 ml. The reaction was stopped by addition of 0.2 ml ice-cold methanol, and after centrifugation 0.2 ml supernatant was mixed with 0.8 ml 0.1 N HCl. The amount of [^{125}I]-T_4 glucuronide was analyzed by Sephadex LH-20 chromatography (Rutgers *et al.*, 1989).

Hepatic type I 5′-deiodinase activity (D-I) was measured in duplicate in microsomes as described by Mol and Visser (1985). Briefly, microsomes (25 μg protein/ml) were incubated for 30 min at 37°C with 1 μM rT_3 and 100,000 cpm [^{125}I-rT_3] in 0.1 M phosphate buffer (pH 7.4) containing 2 mM EDTA and 5 mM DTT. The final reaction volume was 0.2 ml. The reaction was stopped by addition of 0.75 ml 0.1 M HCl, and the resulting [^{125}I] was separated from the reaction mixture by Sephadex LH-20 chromatography according to Rutgers *et al.* (1989). Blanks contained microsomes, inactivated by heating.

Brain type II thyroxine 5′-deiodinase activity (D-II) was analyzed as described by Visser *et al.* (1982) with slight modifications. Briefly, brain homogenates (0.8 mg protein/ml) were incubated with 2 nM T_4 and ± 50,000 cpm [^{125}I]-T_4, 500 nM T_3 and 1 mM propyl-2-thiouracil in 0.1 M phosphate buffer pH 7.2 containing 1 mM EDTA and 25 mM DTT in a total volume of 0.2 ml. Incubations were carried out at 37°C for 60 min. The reaction was stopped on ice by the addition of 0.1 ml 7% (w/v) BSA, followed by 0.5 ml 10% (w/v) trichloroacetic acid. The tubes were centrifuged at 4000 rpm in an eppendorf centrifuge for 5 min and the amount of radioiodide released was determined in 0.5 ml of the supernatant using Sephadex LH-20 chromatography as described above. Blanks contained brain homogenates, which were inactivated by boiling for 10 min.

Ethoxy- and pentoxyresorufin-O-deethylase activity. Ethoxyresorufin-O-deethylase (EROD) activity was measured according to the method of Burke *et al.* (1977) adapted for the use in 96-well plates and a fluorospectrophotometric plate reader (Cytofluor 2350, Millipore, Etten-Leur, the Netherlands). The reaction was performed with 0.1 mg liver microsomal protein per ml in 0.1 M Tris–HCl (pH 7.8) containing 0.4 μM ethoxyresorufin (ER), 1 mg/ml BSA, and 0.1 mM NADPH in a total volume of 0.2 ml. The reaction mixtures were preincubated at 37°C for 2 min, and the reaction was started by the addition of NADPH. Reactions were stopped after 10 min by adding 50 μl 1 M NaOH. The formation of resorufin was detected fluorimetrically (excitation 530 nm, emission 590 nm) and compared with a calibration curve (0–150 nM resorufin). Incubations were carried out in duplicate and results were corrected for blank microsomal incubations without NADPH.

Pentoxyresorufin-O-deethylase (PROD) activity was measured following the same procedure as described above for EROD, with final concentrations of 2 μM pentoxyresorufin (PR) and 0.1 mg microsomal protein/ml.

Plasma protein separation and [^{125}I]-T_4 competition binding. To determine the binding of the PCB metabolite to plasma proteins *in vivo*, plasma samples from [^{14}C]-4-OH-CB107 treated animals (dams and fetuses) were separated by polyacrylamide gelelectrophoresis (PAGE) as described by Brouwer and Van den Berg (1986). In addition, the determination of [^{125}I]-T_4-competitive binding to specific plasma proteins was performed as described by Lans *et al.* (1993) and Darnerud *et al.* (1996). In short, plasma samples for gel slices (40 μl) were mixed 1:1 with a 50 mM Tris/38 mM glycine buffer (pH 8.3) containing 4.5% saccharose. Plasma samples for [^{125}I]-T_4 competition binding (25 μl) were incubated overnight with 100,000 cpm [^{125}I]-T_4 (in 5 μl 50 mM Tris–HCl buffer, pH 8.0) at 4°C. Aliquots of 20 μl of the different samples were run on a 10% native separating gel for 4 h at 4°C at a constant current of 50 mA. Each gel also contained plasma samples for protein staining (5 μl) and pure BSA and human TTR as a reference. After electrophoresis, the part of the gel containing the reference proteins was stained in 0.04% Coomassie Brilliant Blue in 3.5% per-

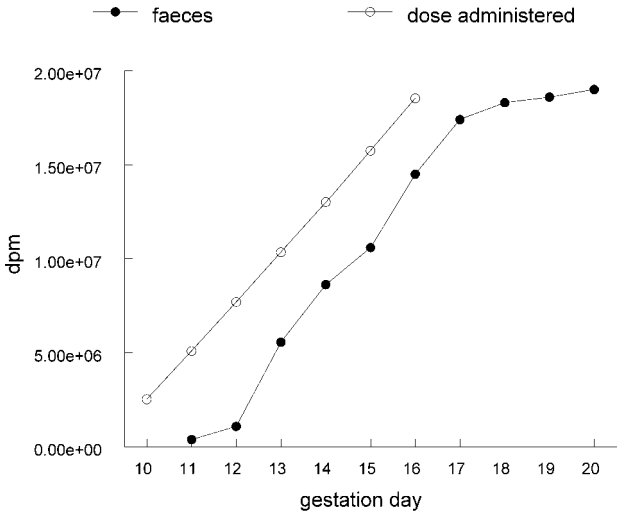

FIG. 1. Cumulative fecal excretion of [^{14}C]-derived radioactivity from pregnant rats after po exposure to 5 mg [^{14}C]-4-OH-CB107 per kg per day from GD 10–16. Data are expressed in dpm (result of 1 representative animal).

chloric acid for 60 min, and subsequently destained with 7% acetic acid for 24 h to determine the position of the proteins on the gel. The part of the gel for radioactivity measurements was frozen on the glass plate at –20°C overnight. The acrylamide gel was subsequently sliced into 1 mm pieces by a standardized procedure. Proteins in slices containing [^{14}C]-4-OH-CB107-derived radioactivity were first eluted by incubating the gel slices in tubes with 1 ml water overnight at 4°C. Four ml of scintillation fluid was added (Ultima Gold, Packard) the next day and the amount of radioactivity in each gel slice was quantified by LSC. Gel slices containing plasma samples incubated with [^{125}I]-T_4 were placed in RIA tubes and counted directly in a γ-counter (Cobra Auto Gamma Counter, Canberra Packard). The PAGE gel profile was made by plotting the [^{125}I]-T_4-radioactivity against the migration distance on the gel.

In vitro *T_4-TTR competition binding study with 4-OH-CB107.* The *in vitro* potency of 4-OH-CB107 to compete with T_4 for binding to human transthyretin was performed as described by Lans *et al.* (1993) with modifications (Meerts *et al.*, 2000). Briefly, 30 nM human TTR, a mixture of [^{125}I]-labeled and unlabeled T_4 (70,000 cpm, 55 nM), and 4-OH-CB107 (in concentrations ranging from 10^{-9} to 10^{-4} M) were dissolved in 0.1 M Tris–HCl-buffer (pH 8.0, containing 0.1 M NaCl and 0.1 mM EDTA). The incubation mixture was allowed to reach binding equilibrium overnight at 4°C. Protein-bound and free [^{125}I]-T_4 were separated on 1 ml Biogel P-6DG columns and spin-forced eluted with 0.2 ml Tris–HCl buffer (1 min at 100 × *g* in a precooled centrifuge, Difuge, Hereaus). Radioactivity in the eluate containing the protein-bound [^{125}I]-T_4 was determined by gamma counting and compared to control incubations. The competition binding curves for T_4 and 4-OH-CB107 were made by plotting relative [^{125}I]-T_4-protein binding (% of control) against concentration competitor.

Statistical analysis. Data are presented as mean values (± SEM). Comparisons between 2 groups of animals were performed using Student's *t* test.

RESULTS

Fecal and Urinary [^{14}C]-4-OH-CB107 Excretion

Fecal elimination of [^{14}C]-4-OH-CB107-derived radioactivity was high. After 1 day exposure (GD 11), 15.1 ± 1.8% of the administered dose could be detected in the feces (Fig. 1), but at GD 13 this level was raised to 60.6 ± 6.5% of the total dose administered. At GD 17 and GD 20, 78.4 ± 6.1% and 93.8 ± 6.9% of the total dose was excreted in the feces, respectively. Urinary excretion was lower than 1% of the total given dose (data not shown). The average recovery of radioactivity per rat for animals dissected at GD 17 or GD 20 was 91.2 ± 6.3% and 97.2 ± 5.3%, respectively.

TABLE 1
Distribution of [^{14}C]-4-OH-CB 107-Derived Radioactivity in Maternal Tissues at Day 17 and Day 20 of Gestation

	Distribution by weight		Distribution by organ	
Tissue/organ	GD 17	GD 20	GD 17	GD 20
Plasma	39.02 ± 3.51	32.3 ± 5.09	415.5 ± 7.27	374.7 ± 6.90
Liver	4.85 ± 0.48	3.17 ± 0.07	49.08 ± 4.31	32.84 ± 1.44
Kidney	4.51 ± 0.4	2.86 ± 0.02*	7.70 ± 0.12	4.10 ± 0.17
Lung	4.27 ± 1.20	3.07 ± 0.62	4.96 ± 1.32	3.32 ± 0.87
Thyroid	3.79 ± 0.19	1.54 ± 0.18**	0.074 ± 0.02	0.04 ± 0.01
Thymus	3.22 ± 0.66	3.16 ± 0.89	1.05 ± 0.15	0.92 ± 0.29
Adrenals	3.12 ± 0.34	2.52 ± 0.06	0.23 ± 0.02	0.15 ± 0.003*
Pancreas	2.95 ± 0.35	2.16 ± 0.10	1.49 ± 0.20	0.73 ± 0.09
Forebrain	1.47 ± 0.08	0.96 ± 0.12*	1.84 ± 0.07	1.20 ± 0.14*
Cerebellum	1.52 ± 0.09	1.16 ± 0.34	0.68 ± 0.01	0.55 ± 0.18
Skeletal muscle	1.17 ± 0.25	0.28 ± 0.09	97.4 ± 12.1	30.8 ± 8.5
Abdominal fat	1.96 ± 0.04	1.18 ± 0.63	—	—
Brown adipose tissue	2.96 ± 0.19	2.19 ± 0.58	—	—

Note. After oral exposure to 5 mg/kg body weight from GD 10–16. Data are expressed as nmol/g tissue (first columns) or nmol/total organ (last columns), and presented as mean ± SE; $n = 3$ per time point.

*Significantly different from GD 17, $p < 0.05$.

**Significantly different from GD 17, $p < 0.01$.

TABLE 2
Distribution of [^{14}C]-4-OH-CB107-derived Radioactivity in Fetal Tissues at Day 17 and 20 of Gestation

	Distribution by weight		Distribution by organ	
Tissue/organ	GD 17	GD 20	GD 17	GD 20
Plasma	n.a.	37.2 ± 5.14	—	66.6 ± 13.8*
Liver	89.41 ± 8.17*	35.12 ± 8.91*	58.7 ± 9.18	89.08 ± 10.1
Kidney	n.a.	5.28 ± 0.88	—	1.76 ± 0.05*
Lung	n.a.	4.08 ± 0.24	—	4.69 ± 0.56
Forebrain	3.11 ± 0.03***	1.54 ± 0.49	2.02 ± 0.11	1.69 ± 0.40
Cerebellum	2.87 ± 0.03**	2.63 ± 0.34	0.99 ± 0.02***	1.42 ± 0.24*
Placenta	6.22 ± 0.46	5.02 ± 1.01	35.8 ± 3.66	28.6 ± 5.41

Note. After exposure of the dams to 5 mg/kg body weight from GD 10–16. Fetal samples were pooled by litter. Data are expressed as nmol per g tissue (first columns) or nmol per total organ (last columns), and presented as mean ± SE; $n = 3$ per time point; n.a., not analyzed.

*Significantly different from maternal levels at the same time point, $p < 0.05$.

**Significantly different from maternal levels at the same time point, $p < 0.005$.

***Significantly different from maternal levels at the same time point, $p < 0.0005$.

Tissue Distribution of [^{14}C]-4-OH-CB107

In the pregnant rat, high levels of [^{14}C]-4-OH-CB107-derived radioactivity could be detected in plasma, liver, and skeletal muscle on whole organ basis (Table 1). Organ levels in pregnant rats were higher at GD 17, i.e., 1 day after the last treatment, compared to GD 20. Significant decreases in radioactivity concentrations at GD 20 could be detected in kidneys, thyroid, and forebrain when levels were expressed in nmol per gram fresh weight, and in adrenals and forebrain when expressed in nmol per total organ.

The distribution in the fetal compartment was different from that in dams (Table 2). There is a substantial accumulation of [^{14}C]-4-OH-CB107-derived radioactivity in the fetal compartment. The total radioactivity concentrations in the fetal compartment were 51.7 ± 3.2% of the total maternal concentrations. Significantly higher amounts of radioactivity could be detected in fetal liver, forebrain, and cerebellum, whereas fetal plasma levels were comparable to maternal levels (Table 2). Fetal/maternal liver ratios were as high as 15.9 ± 0.6 at GD 17 and 11.0 ± 1.2 at GD 20 (Table 3). In addition, although levels in maternal organs tend to decrease from GD 17 to 20, amounts of [^{14}C]-4-OH-CB107 in fetal liver and cerebellum increased when corrected for total tissue weight, though not significantly.

Body and Organ Weights

No effects were observed on maternal body weight gain, mean and total fetal body weight, number of implantation sites, resorptions, number of fetuses, or sex ratio (data not shown). In addition, absolute and relative organ weights from dams (liver, brain, kidneys, adrenals, thyroid, thymus, spleen, pancreas) and fetuses (liver, brain, kidneys, lungs) were not affected by maternal exposure to 5 mg 4-OH-CB107 per kg body weight from GD 10 to 16.

Plasma Thyroid Hormone Levels

Thyroid hormone analysis revealed a significant decrease in maternal total thyroxine (TT_4) levels of 49% on GD 17 and 38% on GD 20 (Fig. 2A) following exposure to 5 mg 4-OH-CB107 per kg body weight from GD 10 to GD 16. Maternal free thyroxine (FT_4, Fig. 2B) and total triiodothyronine (TT_3, Fig. 2C) levels were not significantly reduced. At GD 20, fetal total thyroxine (TT_4) levels were drastically decreased by 89% and FT_4-levels were also significantly reduced by 41% after *in utero* exposure to the PCB metabolite (Figs. 3A and 3B). Fetal TT_4 levels on GD 17 could only be detected in the control group (0.3 ± 0.1 nM). The level in 4-OH-CB107-exposed fetuses was below 0.09 nM, suggesting a decrease of at least 70% compared to the control group. Due to the small sample size these measurements could not be repeated. Fetal plasma levels of TSH were significantly increased by 124% after

TABLE 3
Fetal/Maternal Ratios of [^{14}C]-4-OH-CB107-derived Radioactivity at Day 17 and 20 of Gestation

	Fetal/maternal ratios	
Tissue/organ	GD 17	GD 20
Plasma	—	1.16 ± 0.03
Liver	15.89 ± 0.63	11.02 ± 1.20
Kidney	—	1.84 ± 0.10
Lung	—	0.89 ± 0.09
Forebrain	2.12 ± 0.09	1.10 ± 0.10
Cerebellum	1.89 ± 0.14	2.58 ± 0.24

Note. Dams were exposed to 5 mg/kg body weight from GD 10 to 16. Fetal/maternal ratios were calculated with levels expressed as nmol/g (see Table 2). Fetal samples were pooled per litter; $n = 3$ per time point and exposure group.

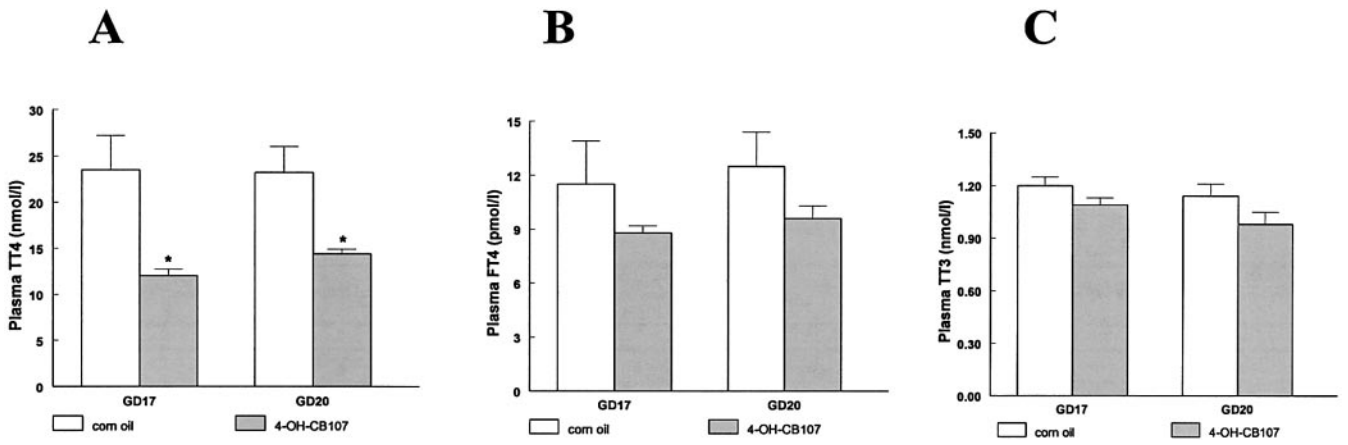

FIG. 2. Plasma levels of (A) maternal total thyroxine (TT_4); (B) free thyroxine (FT_4), and (C) total triiodothyronine (TT_3) after po exposure to 5 mg 4-OH-CB107/kg body weight from GD 10–16. Results are presented as mean ± SEM ($n = 7$). *Statistically significant differences with controls in Student's *t*-test ($p < 0.05$).

4-OH-CB107 treatment (Fig. 4). Maternal TSH-levels were unchanged.

Brain Thyroid Hormone Levels

At GD 17, fetal cerebellum T_4 and T_3 levels were not significantly changed (Table 4). Forebrain T_4 levels at GD 20 were significantly reduced by 35% in 4-OH-CB107 treated animals. Cerebellum T_4 levels at GD 20 were also reduced, though not significantly ($p = 0.051$). No reductions in fetal T_3 levels could be detected in forebrain or cerebellum at GD 20.

Thyroid Hormone Metabolism

Maternal and fetal hepatic microsomal type I deiodinase activities and T_4 uridine diphosphoglucuronosyl transferase activity (UDP-GT) were not altered by exposure to 4-OH-CB107 (data not shown).

The activity of brain type II 5′-thyroxine deiodinase (D-II) in forebrain homogenates from 17-day-old fetuses is very low compared to 20-day-old fetuses and maternal levels at GD 17 and GD 20. A significant increase of 67% compared to controls was observed at GD17 after exposure to 4-OH-CB107 (Fig. 5). However, in 20-day-old fetuses, brain D-II levels were unaffected. D-II levels in maternal forebrain homogenates were decreased following exposure to 4-OH-CB107, though not significantly.

Ethoxy- and Pentoxyresorufin-O-deethylase Activity

No effects were detected on maternal and fetal hepatic microsomal EROD and PROD activity (data not shown).

Plasma Protein Separation and [^{125}I]-T_4 Competition Binding

Plasma protein separation from animals treated with [^{14}C]-labeled 4-OH-CB107 revealed the binding of [^{14}C]-label to transthyretin in both maternal and fetal plasma (Fig. 6). The identification of the transthyretin peak was based on co-migration of the TTR reference. *In vitro* T_4-competition binding with maternal plasma and separation of the plasma proteins by gel electrophoresis showed 3 peaks with [^{125}I]-T_4 bound radioactivity (Fig. 7). The second peak represented transthyretin, and the last one represented free T_4. The third peak could not be identified. The binding of [^{125}I]-T_4 with maternal plasma

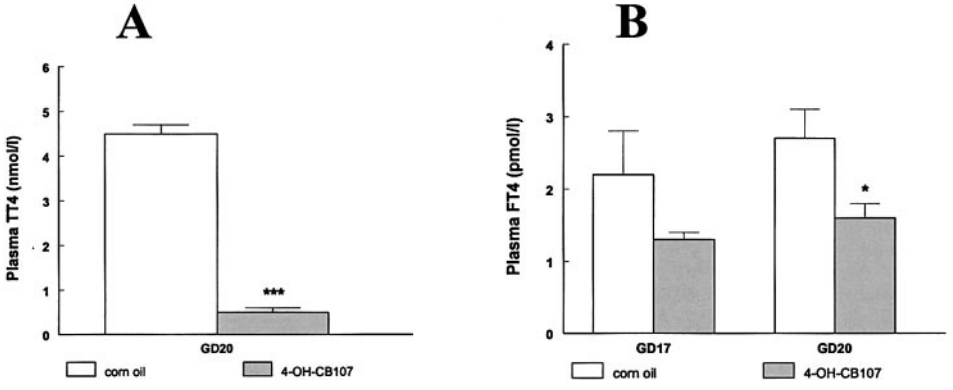

FIG. 3. Plasma levels of (A) fetal total thyroxine (TT_4) and (B) free thyroxine (FT_4) after po exposure to 5 mg 4-OH-CB107/kg body weight from GD 10–16. Results are presented as mean ± SEM ($n = 7$). Statistically significant differences with controls in Student's *t*-test, *($p < 0.05$) and ***($p < 0.0001$).

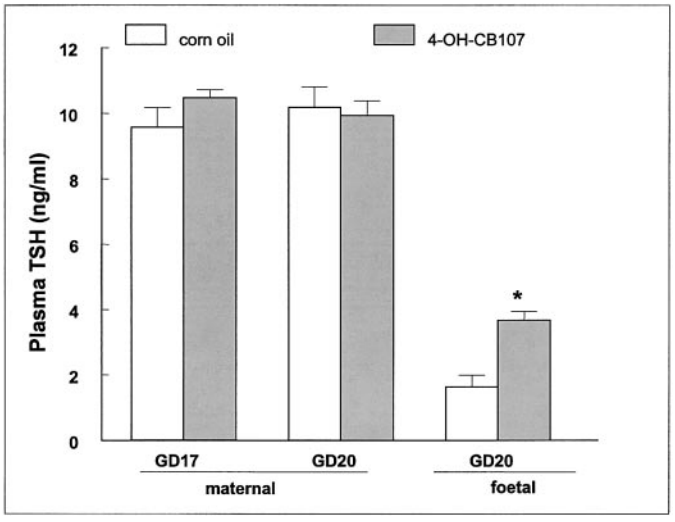

FIG. 4. Plasma levels of thyroid stimulating hormone (TSH) in dams and fetuses following prenatal exposure of rats to 5 mg 4-OH-CB107 per kg body weight from GD 10–16. Results are presented as mean ± SEM ($n = 7$ per exposure and time point). *Statistically significant differences with controls in Student's *t*-test ($p < 0.05$).

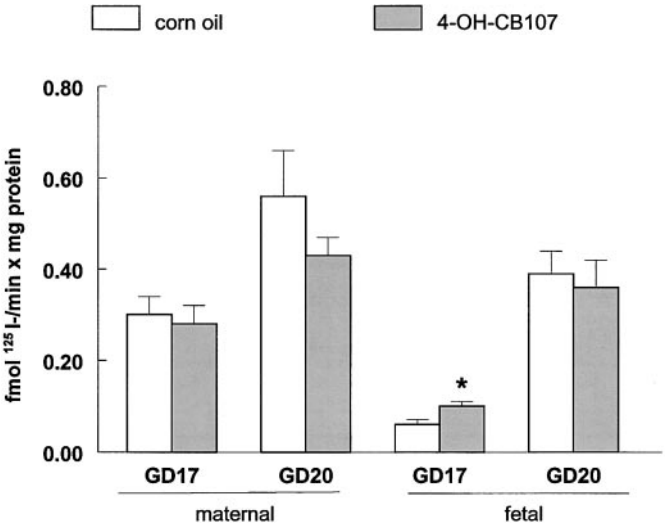

FIG. 5. Type II thyroxine 5′ deiodinase (D-II) activity in forebrain homogenates from dams and fetuses at GD 17 and 20, following prenatal exposure to 0 or 5 mg 4-OH-CB107 per kg body weight from GD 10–16. Results are presented as mean ± SEM ($n = 7$). *Statistically significant differences in Student's *t*-test ($p < 0.05$).

showed a significant decrease of 45% in the amount of [^{125}I]-T_4 bound to TTR in 4-OH-CB107 treated dams compared to controls (Fig. 7A). The unbound radioactivity can be detected at the front of the gel. In fetal plasma, this shift in the position of radioactivity is not very clear (Fig. 7B). The amount of [^{125}I]-T_4 bound to TTR in fetuses treated *in utero* with 4-OH-CB107 was slightly though not significantly decreased.

In Vitro T_4-TTR Competition Binding Study with 4-OH-CB107

The binding affinity (K_a) and IC_{50} value of 4-OH-CB107 as determined in the *in vitro* T_4-TTR competition binding assay were 1.19 (± 0.01) × 10^8 M^{-1} and 24.4 ± 2.2 nM, respectively (Fig. 8). The relative potency compared to the natural ligand T_4 (IC_{50} of 80.7 nM) was 3.3 ± 0.3.

TABLE 4
Fetal Brain Thyroid Hormone Levels

Exposure	Corn oil	4-OH-CB107
GD 17		
Forebrain T_4	n.a.	n.a.
Forebrain T_3	n.a.	n.a.
Cerebellum T_4	0.53 ± 0.08 (6)	0.49 ± 0.06 (6)
Cerebellum T_3	0.14 ± 0.02 (6)	0.12 ± 0.01 (6)
GD 20		
Forebrain T_4	1.79 ± 0.09 (4)	1.16 ± 0.07 (5)***
Forebrain T_3	0.91 ± 0.06 (4)	0.80 ± 0.05 (6)
Cerebellum T_4	1.38 ± 0.13 (4)	1.10 ± 0.04 (5)
Cerebellum T_3	0.18 ± 0.02 (4)	0.16 ± 0.01 (6)

Note. Data are presented as mean ± SEM. The number of animals is given in parentheses; n.a., not analyzed. Thyroid hormone levels are presented as ng T_4 or T_3 per g tissue.

***Significantly different from corn oil, $p < 0.001$.

DISCUSSION

The results of the present study show that maternal exposure to the PCB metabolite 4-OH-2,3,3′,4′,5-pentaCB from GD 10–16 results in considerable transfer of this metabolite from the mother to the fetus, thereby affecting both maternal and especially fetal thyroid hormone levels. Detection of 4-OH-CB107 bound to transthyretin in fetal and maternal plasma suggests that binding of a compound to TTR *in vivo* can lead to facilitated maternal to fetal transfer, decreased maternal and fetal plasma T_4 levels, and decreased fetal brain T_4 levels. Hepatic UDP-GT levels were not induced in dams or fetuses, indicating that this mechanism did not play a role in the observed decrease in plasma thyroid hormone levels as shown for e.g., TCDD and parent PCB-compounds (Darnerud *et al.*, 1986; Van Birgelen *et al.*, 1995).

The internal dose of [^{14}C]-4-OH-CB107 in pregnant dams was low, since most of the radioactivity was excreted in the feces. However, relatively high levels of [^{14}C]-4-OH-CB107-derived radioactivity could be detected in the fetal compartment (i.e., 52% of the total maternal concentrations), indicating a high placental transfer of the compound. At GD 17 and 20, fetal liver, forebrain, and cerebellum levels were all higher than maternal levels, whereas fetal plasma levels were almost equal to maternal plasma levels (fetal/maternal ratio of 1.16 ± 0.03 at GD 20). The approximate 16- and 11-fold higher levels in fetal livers at GD 17 and 20, respectively, compared to mater-

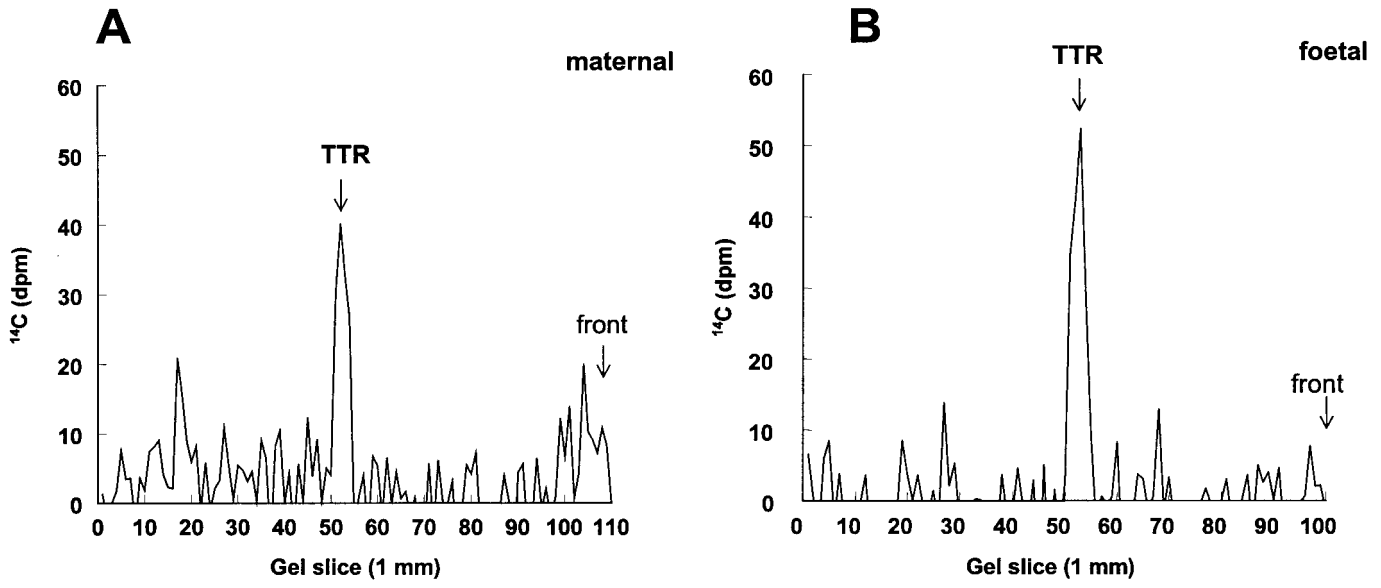

FIG. 6. Distribution of [^{14}C]-derived radioactivity in maternal (A) and pooled fetal (B) plasma at GD 20 after native polyacrylamide gelelectrophoresis. Pregnant rats were exposed to 5 mg [^{14}C]-labeled 4-OH-CB107 per kg body weight from GD 10–16.

nal livers are striking. This may be due to the fact that the liver is one of the major sites of TTR synthesis in the body (Dickson *et al.,* 1985). In addition, the 3.3-fold higher affinity of 4-OH-CB107 for TTR *in vitro* compared to the natural ligand T_4 (this study) and the observed *in vivo* binding of [^{14}C]-4-OH-CB107-derived radioactivity (this study) are in line with this explanation.

Significant reductions (approximately 90%) in fetal plasma TT_4 levels at GD 20 could be detected after maternal exposure to 4-OH-CB107, with fetal plasma metabolite levels of 12.7 μg/g on wet weight basis. As a comparison, rats exposed to 5 or 25 mg/kg Aroclor 1254 in the same experimental setup resulted in a 52 or 74% decrease in fetal plasma TT_4, respectively, with fetal plasma 4-OH-CB107 concentrations of approximately 0.6 and 1.6 μg/g (Morse *et al.,* 1996a). The higher decreases in fetal free T_4 levels and maternal total and free T_4 levels after Aroclor 1254 exposure can be explained by the additional induction of hepatic UDP-GT by Aroclor 1254 and consequently induced biliary excretion of T_4 after glucuronidation. Surprisingly, exposure of pregnant mice to one single iv

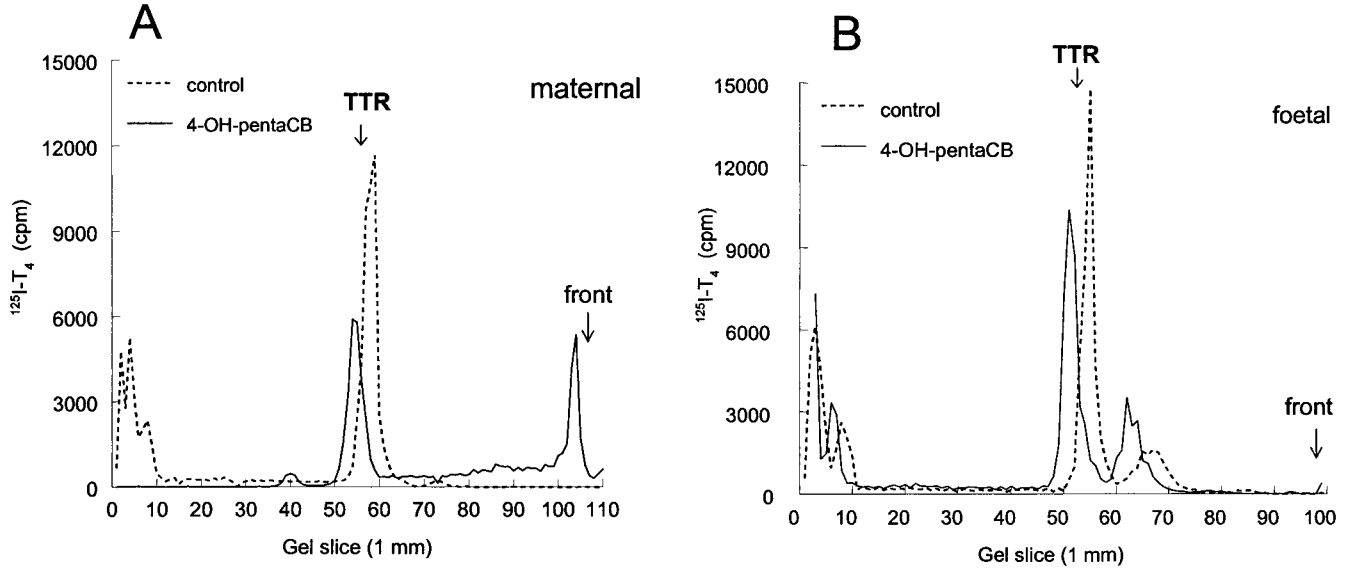

FIG. 7. Distribution of [^{125}I]-T_4-derived radioactivity in maternal (A) and pooled fetal (B) plasma after *in vitro* incubation with [^{125}I]-T_4 and native PAGE. Pregnant rats were treated with corn oil (dotted lines) or 5 mg 4-OH-CB107 per kg body weight from GD 10–16.

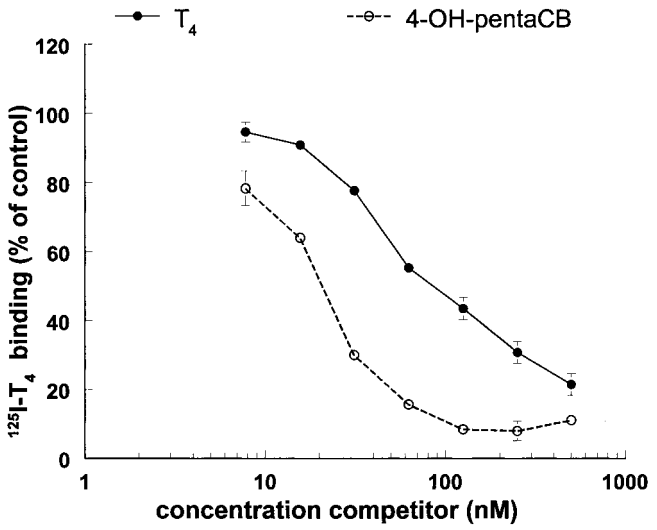

FIG. 8. Displacement of T_4 from TTR by 4-OH-CB107. Data points are mean values ± SD of 1 representative measurement in duplicate. If no error bar is visible, it is smaller than the marker. Relative [^{125}I]-T_4-TTR binding is presented as % of control value.

dose of 50 μmol (= 17.1 mg) per kg body weight of another PCB metabolite, 4-OH-2′,3,3′,4′,5-pentaCB (5.9-fold higher affinity for TTR *in vitro* compared to T_4, Lans *et al.*, 1993) resulted in an only 14% reduction of fetal plasma T_4 levels compared to control levels (Sinjari and Darnerud, 1998). However, comparison with this latter study is difficult, since the route of exposure, species, and time point of analysis were all different.

Fetal plasma thyroid stimulating hormone levels were significantly increased at GD 20, indicating that the hypothalamus-pituitary-thyroid (HPT) axis was stimulated in the fetuses. This was expected, since reductions in plasma T_4 levels are responsible for regulating fetal plasma TSH levels (Morreale de Escobar *et al.*, 1993). However, stimulation of this HPT axis occurs at a stage when the setpoint of homeostatic control is being developed, and it is possible that these disturbances might have a prolonged effect on the homeostatic control of thyroid hormones in these animals. Morse *et al.* (1996a) reported normal levels of plasma thyroid hormones in offspring exposed prenatally to 5 mg/kg Aroclor 1254 at day 21 postpartum, but a statistically significant elevation of plasma TT_4 levels in male offspring at 90 days postpartum.

Despite the very low T_4 levels in fetal plasma, fetal brain T_4 levels were reduced only in forebrain and cerebellum homogenates at GD 20, and not at GD 17. It should be stated however, that brain T_4 and T_3 levels at GD 17 were very difficult to measure because of small sample sizes, and we only used cerebellum samples at GD 17 for thyroid hormone analysis. No changes were observed in brain T_3 levels at GD 17 or GD 20. The induction of brain type II 5′-thyroxine deiodinase (D-II) is a well known response of the rat brain to maintain brain T_3 levels when circulating T_4 concentrations are decreased (Obregón *et al.*, 1984; Ruiz de Oña *et al.*, 1988; Silva and Matthews, 1984), and has been reported before in fetal and neonatal rats after maternal exposure to 3,3′,4,4′,5,5′-hexachlorobiphenyl (Morse *et al.*, 1993) and Aroclor 1254 (Morse *et al.*, 1996a). Maternal exposure to Aroclor 1254 caused a significant decrease in fetal forebrain T_4 levels, but not in T_3 levels.

The accumulation of the 4-OH-CB107 in fetal forebrain and cerebellum may have an effect on the neurodevelopment of the offspring. In a comparable study by Morse *et al.* (1996a), exposure of pregnant rats to Aroclor 1254 from GD 10 to 16 resulted in long-term alterations in glial and neuronal cell marker proteins in the offspring (Morse *et al.*, 1996b), and significant increases in 5-hydroxytryptamine (5-HT) metabolism (Morse *et al.*, 1996c). These adverse effects were likely caused by 4-OH-CB107, since this metabolite accumulated in fetal brains after maternal exposure to Aroclor 1254. Concentrations of 4-OH-CB107 in fetal brains (determined by GC/MS analysis) at GD 20 were approximately 0.16 ppm on fresh weight basis (Morse *et al.*, 1996a). In the current study, maternal exposure of rats to 5 mg/kg 4-OH-CB107 resulted in concentrations of 0.90 ppm 4-OH-CB107 in fetal cerebellum and 0.53 ppm in fetal forebrain. In total, the brain 4-OH-CB107 levels in this study were approximately 9 times higher compared to maternal exposure to 25 mg/kg Aroclor 1254. To investigate the effects of maternal 4-OH-CB107 exposure on the development and behavior of the offspring in more detail, a passive avoidance test (at PND 130) and catalepsy test (at PND 168–175) was performed on the offspring and neurotransmitter concentrations were measured at autopsy. This study revealed that developmental exposure to Aroclor 1254 affects both the dopaminergic and serotonergic system, whereas exposure to 4-OH-CB107 affects dopaminergic and noradrenergic systems (Meerts *et al.*, manuscript in preparation).

OH-PCBs can also exert several other effects on the endocrine system. OH-PCBs have been reported to interact with thyroid hormone metabolizing enzymes, such as iodothyronine 5′-deiodinase (Adams *et al.*, 1990; Lans, 1995; Rickenbacher *et al.*, 1989) and iodothyronine sulfotransferase (Schuur *et al.*, 1998) *in vitro.* In addition, some OH-PCBs competitively bind to the estrogen receptor and exhibit estrogenic activity in the mouse uterus (Korach *et al.*, 1988). Recently, Kester *et al.* (2000) demonstrated that various environmentally relevant OH-PCBs were extremely potent inhibitors of human estrogen sulfotransferases.

In conclusion, exposure of pregnant rats to the PCB metabolite 4-OH-CB107 results in drastic reductions in fetal plasma thyroid hormone concentrations, and to an accumulation of the compound in fetal liver, brain, and plasma. It is suggested that the observed binding of 4-OH-CB107 to TTR may play a role in the retention of the metabolite in plasma, in the maternal to fetal transport and in the distribution of 4-OH-CB107 in the fetal compartment. The question remaining is whether this

possible mechanism is also operating in humans. Even though in humans thyroxine binding globulin is the main thyroid hormone transport protein in the blood, TTR still plays a role in mediating the delivery of T_4 across the blood-brain barrier, transporting T_4 into the cerebrospinal fluid and transferring maternal-to-fetal T_4 over the placenta (Calvo *et al.*, 1990; Southwell *et al.*, 1993). In fact, current determinations of 4-OH-CB107 levels in human maternal plasma and cord blood show approximately 3-fold higher levels in cord blood (Bergman *et al.*, 1999), suggesting that indeed transport of OH-PCBs to the human fetus is possible. If this facilitated transport is also operating for other organohalogen compounds, further investigation is needed into the possible consequences of exposure to these compounds on neuronal development of the offspring.

ACKNOWLEDGMENTS

We thank the colleagues from the Division of Toxicology of the Wageningen University for their assistance at the animal autopsy. We are very grateful to Tatiana Cantillana from the Stockholm University (Sweden) for synthesis of the PCB metabolite and to Gabriella Morreale de Escobar for the analysis of thyroid hormone levels in brain. This research was financially supported by the European Commission, Environment and Climate Program (ENV4-CT96-0170).

REFERENCES

Adams, C., Lans, M. C., Klasson-Wehler, E., Van Engelen, J. G. M., Visser, J., and Brouwer, A. (1990). Hepatic thyroid hormone 5′-deiodinase, another target-protein for monohydroxy metabolites of 3,3′,4,4′-tetrachlorobiphenyl. *Organohalogen Comp.* **1,** 51–54.

Barter, R. A., and Klaassen, C. D. (1992). UDP glucuronyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Toxicol. Appl. Pharmacol.* **113,** 36–42.

Barter, R. A., and Klaassen, C. D. (1994). Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. *Toxicol. Appl. Pharmacol.* **128,** 9–17.

Bastomsky, C. H. (1974). Effects of a polychlorinated biphenyl mixture (Aroclor 1254) and DDT on biliary thyroxine excretion in rats. *Endocrinology* **95,** 1150–1155.

Beetstra, J. B., van Engelen, J. G. M., Karels, P., van der Hoek, H. J., de Jong, M., Docter, R., Krenning, E. P., Hennemann, G., Brouwer, A., and Visser, T. J. (1991). Thyroxine and 3,3′,5-triiodothyronine are glucuronidated in rat liver by different uridine diphosphate-glucuronyltransferases. *Endocrinology* **128,** 741–746.

Bergman, Å., Brouwer, A., Hagmar, L., Johnson, L., and Sauer, P. J. J. (1999). Final report from a European Commission Research and development program RENCO (ENV4-CR96–1070).

Bergman, Å., Klasson-Wehler, E., and Kuroki, H. (1994). Selective retention of hydroxylated PCB metabolites in blood. *Environ. Health Perspect.* **102,** 464–469.

Bergman, Å., Klasson-Wehler, E., Kuroki, H., and Nilsson, A. (1995). Synthesis and mass spectrometry of 50m methoxylated PCB. *Chemosphere* **30,** 1921–1938.

Bergman, Å., Nilsson, A., Riego, J., and Örn, U. (1990). Synthesis of ^{14}C-Labelled and unlabelled coplanar polychlorinated biphenyls (PCBs). *Acta Chem. Scand.* **44,** 1071–1076.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Brouwer, A., Blaner, W., Kukler, A., and Van den Berg, K. J. (1988). Study on the mechanism of interference of 3,4,3′,4′-tetrachlorobiphenyl with the plasma retinol-binding proteins in rodents. *Chem. Biol. Interact.* **68,** 203–217.

Brouwer, A., Klasson-Wehler, E., Bokdam, M., Morse, D. C., and Traag, W. A. (1990). Competitive inhibition of thyroxin binding to transthyretin by monohydroxy metabolites of 3,4,3′,4′-tetrachlorobiphenyl. *Chemosphere* **20,** 1257–1262.

Brouwer, A., Morse, D. C., Lans, M. C., Schuur, A. G., Murk, A. J., Klasson-Wehler, E., Bergman, A., and Visser, T. J. (1998). Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. *Toxicol. Ind. Health* **14,** 59–84.

Brouwer, A., and van den Berg, K. J. (1986). Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. *Toxicol. Appl. Pharmacol.* **85,** 301–312.

Burke, M. D., Prough, R. A., and Mayer, R. T. (1977). Characteristics of a microsomal cytochrome P-448-mediated reaction. Ethoxyresorufin O-deethylation. *Drug Metab. Dispos.* **5,** 1–8.

Byrne, J. J., Carbone, J. P., and Hanson, E. A. (1987). Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. *Endocrinology* **121,** 520–527.

Calvo, R., Obregon, M. J., Ruiz de Ona, C., Escobar del Rey, F., and Morreale de Escobar, G. (1990). Congenital hypothyroidism, as studied in rats. The crucial role of maternal thyroxine but not of 3,5,3′-triiodothyronine in the protection of the fetal brain. *J. Clin. Invest.* **86,** 889–899.

Chauhan, K. R., Kodavanti, P. R. S., and McKinney, J. D. (2000). Assessing the role of *ortho*-substitution on polychlorinated biphenyl binding to transthyretin (TTR), a thyroxine transport protein. *Toxicol. Appl. Pharmacol.* **162,** 10–21.

Cheek, A. O., Kow, K., Chen, J., and McLachlan, J. A. (1999). Potential mechanisms of thyroid disruption in humans: Interaction of organohalogen compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environ. Health Perspect.* **107,** 273–278.

Collins, W. T., Jr., and Capen, C. C. (1980). Fine structural lesions and hormonal alterations in thyroid glands of perinatal rats exposed *in utero* and by the milk to polychlorinated biphenyls. *Am. J. Pathol.* **99,** 125–142.

Dickson, P. W., Howlett, G. J., and Schreiber, G. (1985). Rat transthyretin (prealbumin). Molecular cloning, nucleotide sequence, and gene expression in liver and brain. *J. Biol. Chem.* **260,** 8214–8219.

Darnerud, P. O., Brandt, I., Klasson-Wehler, E., Bergman, Å., D'Argy, R., Dencker, L., and Sperber, G. O. (1986). 3,3′,4,4′-tetrachloro[14C]biphenyl in pregnant mice: Enrichment of phenol and methyl sulphone metabolites in late gestational fetuses. *Xenobiotica* **16,** 295–306.

Darnerud, P. O., Morse, D., Klasson-Wehler, E., and Brouwer, A. (1996). Binding of a 3,3′,4,4′-tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. *Toxicology* **106,** 105–114.

Kester, M. H., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C. N., Coughtrie, M. W., Bergman, Å, Safe, S. H., Kuiper, G. G., Schuur, A. G., Brouwer, A. and Visser, T. J. (2000). Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: A novel pathway explaining the estrogenic activities of PCBs. *Endocrinology* **141,** 1897–1900.

Korach, K. S., Sarver, P., Chae, K., McLachlan, J. A., and McKinney, J. D. (1988). Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: Conformationally restricted structural probes. *Mol. Pharmacol.* **33,** 120–126.

Lans, M. C. (1995). Thyroid hormone binding proteins as novel targets for

hydroxylated polyhalogenated aromatic hydrocarbons (PHAHs): Possible implications for toxicity. Thesis, Wageningen Agricultural University, The Netherlands, ISBN 90-5485-430-8.

Lans M. C., Klasson-Wehler E., Willemsen M., Meussen E., Safe S, and Brouwer A. (1993). Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins and -dibenzofurans with human transthyretin. *Chem. Biol. Interact.* **88,** 7–21.

Meerts, I. A. T. M., van Zanden, J. J., Luijks, E. A. C., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, Å., and Brouwer, A. (2000). Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin *in vitro*. *Toxicol. Sci.* **56,** 95–104.

Mol, J. A., and Visser, T. J. (1985). Rapid and selective inner ring deiodination of thyroxine sulfate by rat liver deiodinase. *Endocrinology* **117,** 8–12.

Morreale de Escobar, G., Calvo, R., Escobar del Rey, F., and Obregón, M. J. (1993). Differential effects of thyroid hormones on growth and thyrotropic hormones in rat fetuses near term. *Endocrinology* **132,** 2056–2064.

Morreale de Escobar, G., Pastor, R., Obregon, M. J., and Escobar del Rey, F. (1985). Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues before and after the onset of fetal thyroid function. *Endocrinology* **117,** 1890–1900.

Morse, D. C., Groen, D., Veerman, M., van Amerongen, C. J., Koeter, H. B. W. M., Smits van Prooije, A. E., Visser, T. J., Koeman, J. H., and Brouwer, A. (1993). Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. *Toxicol. Appl. Pharmacol.* **122,** 27–33.

Morse, D. C., Klasson-Wehler, E., Wesseling, W., Koeman, J. H., and Brouwer, A. (1996a). Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol. Appl. Pharmacol.* **136,** 269–279.

Morse, D. C., Plug, A., Wesseling, W., van den Berg, K. J., and Brouwer, A. (1996b). Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. *Toxicol. Appl. Pharmacol.* **139,** 252–261.

Morse, D. C., Seegal, R. F., Borsch, K. O., and Brouwer, A. (1996c). Long-term alterations in regional brain serotonin metabolism following maternal polychlorinated biphenyl exposure in the rat. *Neurotoxicology* **17,** 631–638.

Ness, D. K., Schantz, S. L., Moshtaghian, J., and Hansen, L. G (1993). Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. *Toxicol. Lett.* **68,** 311–323.

Obregón, M. J., Mallol, J., Pastor, R., Morreale de Escobar, G., and Escobar del Rey, F. (1984). L-thyroxine and 3,5,3′-triiodo-L-thyroxine in rat embryos before onset of fetal thyroid function. *Endocrinol.* **114,** 305–307.

Peterson, R. E., Theobald, H. M., and Kimmel, G. L. (1993). Developmental and reproductive toxicity of dioxins and related compounds: Cross-species comparisons. *Crit. Rev. Toxicol.* **23,** 283–335.

Rickenbacher, U., Jordan, S., and McKinney, J. D. (1989). Structurally specific interaction of halogenated dioxin and biphenyl derivatives with iodothyronine-5′-deiodinase in rat liver. In *Probing Bioactive Mechanisms,* ACS Symposium Series **413**, Chapter 22, 354–365. American Chemical Society, Washington, D.C.

Rickenbacher, U., McKinney, J. D., Oatley, S. J., and Blake, C. C. F. (1986). Structurally specific binding of halogenated biphenyls to thyroxine transport protein. *J. Med. Chem.* **29,** 641–648.

Ruiz de Oña, C., Obregón, M. J., Escobar del Rey, F., and Morreale de Escobar, G. (1988). Developmental changes in rat brain 5′-deiodinase and thyroid hormones during the fetal period: The effects of fetal hypothyroidism and maternal thyroid hormones. *Pediatr. Res.* **24,** 588–594.

Rutgers, M., Pigmans, I. G. A. J., Bonthuis, F., Docter, R., and Visser, T. J. (1989). Effects of propylthiouracil on the biliary clearance of thyroxine (T4) in rats: Decreased excretion of 3,3,3′-triiodothyronine glucuronide and increased excretion of 3,3′,5′-triiodothyronine glucuronide and T_4 sulfate. *Endocrinology* **125,** 2175–2186.

Safe, S. H. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.* **21,** 51–88.

Safe, S. H. (1994). Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* **24,** 87–149.

Schantz, S. L. (1996). Developmental neurotoxicity of PCBs in humans: What do we know and where do we go from here? *Neurotoxicol. Teratol*. **18,** 217–227.

Schreiber, G., Southwell, B. R., and Richardson, S. J. (1995). Hormone delivery systems to the brain—transthyretin. *Exp. Clin. Endocrinol. Diabetes* **103,** 75–80.

Schuur, A. G., Legger, F. F., van Meeteren, M. E., Moonen, M. J. H., van Leeuwen-Bol, I., Bergman, Å, Visser, T. J., and Brouwer, A. (1998). *In vitro* inhibition of thyroid hormone sulfation by hydroxylated metabolites of polyhalogenated aromatic hydrocarbons. *Chem. Res. Toxicol.* **11,** 1075–1081.

Seegal, R. F. (1996). Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit. Rev. Toxicol.* **26,** 709–737.

Seo, B. W., Li, M. H., Hansen, L. G., Moore, R. W., Peterson, R. E., and Schantz, S. L. (1995). Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. *Toxicol. Lett.* **78,** 253–262.

Silva, J. E., and Matthews, P. S. (1984). Production rates and turnover of triiodothyronine in rat-developing cerebral cortex and cerebellum. Responses to hypothyroidism. *J. Clin. Invest.* **74,** 1035–1049.

Sinjari, T., and Darnerud, P. O. (1998). Hydroxylated polychlorinated biphenyls: Placental transfer and effects on thyroxine in the fetal mouse. *Xenobiotica* **28,** 21–30.

Sjödin, A., Tullsten, A.-K., and Klasson-Wehler, E. (1998) Identification of the parent compounds to selectively retained hydroxylated PCB metabolites in rat blood plasma. *Organohalogen Comp.* **37,** 365–368.

Southwell, B. R., Duan, W., Alcorn D., Brack, C., Richardson, S. J., Kohrle, J., and Schreiber, G. (1993). Thyroxine transport to the brain: Role of protein synthesis by the choroid plexus. *Endocrinology* **133,** 2116–2126.

Van Birgelen, A. P. J. M., Smit, E. A., Kampen, I. M., Groeneveld, C. N., Fase, K. M., Van der Kolk, J., Poiger, H., Van den Berg, M., Koeman, J. H., and Brouwer, A. (1995). Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: Use in risk assessment. *Eur. J. Pharmacol.* **293,** 77–85.

Van den Berg, K. J., Zurcher, C., and Brouwer, A. (1988). Effects of 3,4,3′,4′-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. *Toxicol. Lett.* **41,** 77–86

Visser, T. J., Kaptein, E. Gijzel, A. L., de Herder, W. W., Ebner, T., and Burchell, B. (1993). Glucuronidation of thyroid hormone by human bilirubin and phenol UDP-glucuronyltransferase isoenzymes. *FEBS Lett.* **324,** 358–360.

Visser, T. J., Leonard, J. L., Kaplan, M. M., and Larsen, P. R. (1982) Kinetic evidence suggesting two mechanisms for iodothyronine 5′-deiodination in rat cerebral cortex. *Proc. Nat. Acad. Sci. U.S.A.* **79,** 5080–5084.