Polychlorinated Biphenyl Congeners that Increase the Glucuronidation and Biliary Excretion of Thyroxine Are Distinct from the Congeners that Enhance the Serum Disappearance of Thyroxine

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ABSTRACT:

Polychlorinated biphenyl (PCB) congeners differentially reduce serum thyroxine (T_4) in rats, but little is known about their ability to affect biliary excretion of T_4 . Thus, male Sprague-Dawley rats were orally administered Aroclor-1254, Aroclor-1242 (32 mg/kg per day), PCB-95, PCB-99, PCB-118 (16 mg/kg per day), PCB-126 (40 μ g/kg per day), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (3.9 μ g/kg per day), or corn oil for 7 days. Twenty-four hours after the last dose, [125 I] T_4 was administered intravenously, and blood, bile, and urine samples were collected for quantifying [125 I] T_4 and in bile [125 I] T_4 metabolites. Serum T_4 concentrations were reduced by all treatments, but dramatic reductions occurred in response to Aroclor-1254, PCB-99 [phenobarbital (PB)-type congener], and PCB-118 (mixed-type congener). None of the treatments increased urinary excretion of [125 I] T_4 . Aroclor-1254, PCB-118, TCDD, and

PCB-126 (TCDD-type congener) increased biliary excretion of T_4 -glucuronide by 850, 756, 710, and 573%, respectively, corresponding to marked induction of hepatic UDP-glucuronosyltransferase (UGT) activity toward T_4 . PCB-95 and PCB-99 did not induce UGT activity; therefore, the increased biliary excretion of T_4 -glucuronide was related to the affinity of congeners for the aryl hydrocarbon receptor. The disappearance of [125 I] T_4 from serum was rapid (within 15-min) and was increased by Aroclor-1254, PCB-99 and PCB-118. Thus, reductions in serum T_4 in response to PCBs did not always correspond with UGT activity toward T_4 or with increased biliary excretion of T_4 -glucuronide. The rapid disappearance of [125 I] T_4 from the serum of rats treated with PB-like PCBs suggests that increased tissue uptake of T_4 is an additional mechanism by which PCBs may reduce serum T_4 .

Introduction

Glucuronidation plays an essential role in thyroid hormone homeostasis not only by facilitating excretion, but also by providing the first step in enterohepatic recycling of triiodothyronine (T_3) and thyroxine (T_4) (Visser, 1990; Vansell and Klaassen, 2001, 2002). Reductions in serum T_4 and corresponding increases in T_4 glucuronidation occur in rats in response to the anticonvulsant phenobarbital (PB) (McClain et al., 1989), the selective antagonist of corticotropin-releasing factor receptor-1 DMP 904 (Wong et al., 2005; Lecureux et al., 2009), the

synthetic steroids spironolactone and pregnenolone-16 α -carbonitrile (PCN) (Semler et al., 1989; Liu et al., 1995), and the polycyclic aromatic hydrocarbons 3-methylcholanthrene (3-MC), tetrachlorodibenzo-*p*-dioxin (TCDD), and polychlorinated biphenyls (Hood and Klaassen, 2000; Craft et al., 2002).

Polychlorinated biphenyl (PCB) mixtures such as Aroclor-1254 are especially effective in reducing circulating levels of T_4 in rats (Collins and Capen, 1980; Liu et al., 1995). Although there are a number of theories regarding the mechanisms by which PCBs exert their effects on thyroid hormones, including direct effects on the gland itself and interference with serum transport proteins (Capen, 1994; Li and Hansen, 1997), the major focus has been on increased biotransformation. As early as the 1970s, increased glucuronidation of T_4 and increased biliary excretion of T_4 -glucuronide after exposure to Aroclor-1254 were demonstrated (Bastomsky, 1974). Subsequently, studies with thyroidectomized rats demonstrated that this effect produced by PCBs or TCDD was due to an extrathyroidal mechanism, most likely increased glucuronidation (Barter and Klaassen, 1992; Schuur et al., 1997).

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ABBREVIATIONS: T_3 , triiodothyronine; T_4 , thyroxine; PCN, pregnenolone-16 α -carbonitrile; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCB, polychlorinated biphenyl; AhR, aryl hydrocarbon receptor; PB, phenobarbital; PCB-126, 3,3',4,4',5-pentachlorobiphenyl; PCB-95, 2,2',3,5',6-pentachlorobiphenyl; PCB-99, 2,2',4,4',5-pentachlorobiphenyl; PCB-118, 2,3',4,4',5-pentachlorobiphenyl; HPLC, high-performance liquid chromatography; UGT, UDP-glucuronosyltransferase; EROD, ethoxyresorufin-*O*-deethylase; PROD, pentoxyresorufin-*O*-deethylase; 3-MC, 3-methylcholanthrene; Mrp2, multidrug resistance protein-2.

PCB mixtures are produced by random chlorination of the biphenyl molecule, which can theoretically produce 209 different congeners with varying degrees of chlorination and patterns of toxicity (De Voogt and Brinkman, 1989; Li and Hansen, 1997). The congeners are commonly divided into three types. The coplanar PCB congeners, which have no chlorine substitutions in the ortho positions, have affinity for the aryl hydrocarbon receptor (AhR), induce CYP1A, and are referred to as TCDD-type inducers. The noncoplanar PCB congeners, which have at least two chlorine substitutions in the ortho positions, have low affinity for the AhR (Murt et al., 1996), induce CYP2B, and are referred to as PB-type inducers. The mono-ortho coplanar PCB congeners have one chlorine substitution in an ortho position, which reduces but does not abolish affinity for the AhR. These congeners induce both CYP1A and CYP2B in rats and are referred to as mixed-type inducers (Safe et al., 1985).

We have previously shown that individual PCB congeners differentially reduce serum T_4 levels, with the PB-type and mixed-type PCBs being most effective (Martin and Klaassen, 2010). However, little is known about their ability to affect biliary excretion of T_4 -glucuronide. Thus, the present study was designed to test the hypothesis that the reduction of serum T_4 by these PCBs corresponds with their ability to induce the glucuronidation of T_4 and increase the biliary excretion of T_4 -glucuronide. PCB-126 (3,3',4,4',5-pentachlorobiphenyl, a coplanar TCDD-type congener), PCB-95 (2,2',3,5',6-pentachlorobiphenyl, noncoplanar PB-type congener), PCB-99 (2,2',4,4',5-pentachlorobiphenyl, noncoplanar PB-type congener), and PCB-118 (2,3',4,4',5-pentachlorobiphenyl, a mono-ortho coplanar mixed-type congener) were examined. Aroclor-1254 was used to compare the effects of the mixture with those of the congeners. Aroclor-1242 was used to compare the effects of a PCB mixture with a lower (42% by weight) degree of chlorination. TCDD was included as the prototypical AhR ligand for comparison with PCB-126. After a 7-day administration period, radiolabeled T_4 was administered intravenously to the rats, the disappearance of [125 I] T_4 from the plasma and excretion into bile was quantified, the radioactive metabolites in bile were separated by high-performance liquid chromatography (HPLC), and the induction of hepatic UDP-glucuronosyltransferase (UGT) activity toward T_4 was determined.

Materials and Methods

Chemicals and Reagents. Aroclor-1254 and Aroclor-1242 were donated by Dr. Lawrence Hansen (University of Illinois at Urbana). TCDD was a gift from Dr. Karl Rozman (University of Kansas Medical Center, Kansas City, KS). PCB-95, PCB-99, PCB-118, and PCB-126 were obtained from AccuStandard (New Haven, CT). Radioimmunoassay kits for assay of serum thyroid hormones were obtained from Diagnostics Products (Los Angeles, CA). [125 I] T_4 , radiolabeled on both outer-ring positions, was obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA). Heparin was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ). Ultima Flo-M Scintillant was purchased from PerkinElmer Life and Analytical Sciences. All other reagents were obtained from Thermo Fisher Scientific (Waltham, MA).

Animals and Treatments. The Institutional Animal Care and Use Committee approved all protocols before initiation. Male Sprague-Dawley rats (Sasco, Omaha, NE), weighing 225 to 250 g, were individually housed in hanging wire-bottomed cages and were maintained at approximately 70°F on a 12-h light/dark cycle. All compounds were dissolved in corn oil (Mazola, Best Foods, Englewood Cliffs, NJ). Rats (six per group) were administered the corn oil solutions of Aroclor-1254, Aroclor-1242 (32 mg/kg per day), PCB-95, PCB-99, PCB-118 (16 mg/kg per day), PCB-126 (40 μ g/kg per day), or TCDD (3.9 μ g/kg per day) via gavage for 7 consecutive days at a dose volume of 5 ml/kg. The dose selection was based on our dose-response studies of these PCBs (Martin and Klaassen, 2010). Control rats were administered corn oil (5 ml/kg). All rats had ad libitum access to feed (Purina Rodent Chow, 5001; Purina, St. Louis, MO) and water. Body weights were recorded daily. Twenty-

four hours after the last dose, animals were anesthetized with sodium pentobarbital (50 mg/ml), combined with 1:1 potassium iodide (1 mg/ml) at 2 ml/kg to prevent uptake of [125 I] into the thyroid. The femoral vein and artery were cannulated (PE 50) and primed with saline or heparinized saline (33 units/ml), respectively. Approximately 1 ml of blood was sampled from the artery from which serum was prepared and stored at -70°C for further assay. The bile duct was cannulated (PE 10), and the body temperature of each animal was maintained at 37°C using a heat lamp with a rectal thermometer. Ten minutes later, each rat was given 1 ml of [125 I] T_4 i.v. at 15 μ Ci/ml in 10 mM NaOH saline + 1% normal rat serum, and bile was collected on ice at 30-min intervals for 2 h. Fifteen minutes after the start of bile collection and three more times at 30-min intervals, approximately 300- μ l aliquots of blood were collected from the femoral artery. At the end of the 2-h collection period, the urinary bladder was exposed, and urine was collected by puncture with needle and syringe. The liver was removed, weighed and snap frozen, and stored at -70°C . Bile and urine volumes were determined gravimetrically. Blood samples were centrifuged for 5 min to collect serum. Two aliquots (20 μ l each) were taken from the bile, serum, and urine samples for gamma spectroscopy. After the addition of methanol (1:2) and storage at -20°C for 1 h to precipitate protein, bile was centrifuged at 12,000g (4°C) for 10 min, and the supernatant was collected for analysis by HPLC.

HPLC Analysis. Beckman System Gold equipment and software (version 8.1) consisting of an Autosampler 507, Programmable Solvent Module 126, Radioisotope Detector 171 and 110B Solvent Delivery Module for pumping scintillation cocktail were used for HPLC (Beckman Coulter, Fullerton, CA). Reverse-phase HPLC was performed on a 10×0.3 cm ChromSpher C18 column in combination with both a ChromSep 10×2 mm reverse-phase guard column (Chrompack, Inc., Raritan, NJ) and a 7.5×4.6 mm Adsorbosphere C18 reverse-phase guard column (Alltech Associates, Inc., Deerfield, IL) with a 16 to 45% stepwise linear gradient of acetonitrile run against 0.02 M ammonium acetate, pH 4 for 1 h (Visser et al., 1993b). The sample volume injected was 20 μ l. To determine the amount of T_4 -glucuronide present, the percent total area of the T_4 -glucuronide peak was multiplied by the total biliary radioactivity.

Determination of Serum T_4 and T_3 . The concentrations of total (representing free and protein-bound) T_4 and T_3 in serum were determined by radioimmunoassay. The limits of detection of these kits were 0.25 μ g/dl and 7 ng/dl, respectively.

Microsome Preparation. UGT activity toward T_4 was determined in liver microsomes. Liver microsomes were prepared as described previously (Hood and Klaassen, 2000). In brief, liver tissue was homogenized in 2 volumes of ice-cold buffer containing 50 mM Tris and 150 mM potassium chloride (pH 7.5). Homogenates were centrifuged at 860g for 15 min, and the pellet was discarded. The supernatant was centrifuged at 23,300g for 25 min at 4°C , and the pellet was discarded. This second supernatant was decanted into ultracentrifuge tubes and was centrifuged at 105,000g for 1 h at 4°C . The resulting pellet was removed and homogenized in 1.15% potassium chloride containing 10 mM neutralized EDTA. The homogenate was centrifuged at 105,000g for 1 h at 4°C . The supernatant was discarded, and the microsomal pellet was homogenized in 0.4 ml/g tissue of 0.25 M sucrose. The homogenate was stored at -70°C until analysis. Protein concentrations in microsomes were determined according to the bicinchoninic acid method using a commercial assay kit with bovine serum albumin as the standard.

Purification of [125 I] T_4 . Free [125 I] label was removed from the stock [125 I] T_4 before use in the UGT activity assay (Vansell and Klaassen, 2001). Stock [125 I] T_4 was applied to a Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO) column (0.5-ml bed volume), equilibrated with 0.1 N HCl. Free [125 I] T_4 was eluted from the column with 4 ml of 0.1 N HCl. The column was then rinsed with 4 ml of deionized water. Purified [125 I] T_4 was eluted by rinsing the column with 1.5 ml of an ethanol/ammonium hydroxide (98:2%) solution. The volume of the [125 I] T_4 was reduced to approximately 200 μ l using a stream of nitrogen gas.

UGT Activity Toward T_4 . UGT activity toward T_4 in liver microsomes was evaluated by determining the amount of [125 I] T_4 -glucuronide produced (Beetstra et al., 1991). All reactions contained 150 μ l of reaction mixture with 75 mM Tris-HCl, 7.5 mM MgCl_2 , 30 mM UDP-glucuronic acid, 1 μ M nonradioactive T_4 , approximately 100,000 counts per minute of [125 I] T_4 , and 0.1 mM propylthiouracil to inhibit outer-ring deiodinases. Reactions were

FIG. 1. Effects of PCBs and TCDD on liver/body weight ratios. Values represent the mean $\pm$ S.E.M. of six rats. *, significantly different from the control group ($p < 0.05$).

initiated by adding 50 μ l of microsomal protein (final concentration, 250 μ g/ml) before incubation for 1 h in a 37°C water bath. The reaction was terminated by placing the samples on ice and adding 200 μ l of ice-cold methanol. The samples were centrifuged at 12,000g (4°C) for 45 min. The supernatant (200 μ l) was poured over a Sephadex LH-20 column (1-ml bed volume) that had been equilibrated with 6 ml of 0.1 N HCl. Free 125 I was eluted by rinsing the column with 2 ml of 0.1 N HCl. Columns were subsequently rinsed with 2 ml of deionized water. The [125 I]T₄-glucuronide was eluted by rinsing with 3 ml of deionized water, and to ensure complete elution of the glucuronide, the columns were rinsed with an additional 3 ml of deionized water. The [125 I]T₄ that remained unconjugated was eluted from the columns using 3 ml of ethanol: 0.1 N NaOH (1:1 v/v). The amount of [125 I]T₄ in the eluates was determined by gamma spectroscopy.

Statistics. Differences between control and treated animals were determined using analysis of variance followed by the Duncan's multiple range post hoc test. Significant differences between treated and control groups ($p < 0.05$) are indicated by asterisks in the figures. Statistical analyses were performed using STATISTICA 4.5 (StatSoft, Inc., Tulsa, OK).

Results

No significant changes in body weight were produced by administration of PCBs or TCDD (data not shown). With the exception of PCB-95, all of the treatments produced significant increases in liver/body weight ratios (Fig. 1). Aroclor-1254 produced the greatest increase of 78%. PCB-99, PCB-118, and TCDD caused roughly equal increases in liver/body weight ratios of 42, 47, and 44%, respectively. Aroclor-1242 and PCB-126 produced more moderate, but still significant, increases in liver/body weight ratios of 33 and 25%, respectively.

The effects of the PCBs on serum thyroid hormone concentrations after the 7-day dosage period are shown in Fig. 2. Each of the seven treatments produced significant reductions in serum total T₄ (Fig. 2, top). The most dramatic reductions in serum T₄ occurred in response to treatment with Aroclor-1254, PCB-99, and PCB-118 (95, 87, and 93%, respectively). Aroclor-1242, PCB-95, PCB-126, and TCDD caused nearly equal decreases of 73, 64, 74, and 71%, respectively. Serum total T₃ levels were also significantly reduced by each of the compounds (Fig. 2, bottom). As with T₄, the largest decreases in T₃ occurred in response to treatment with Aroclor-1254, PCB-99, or PCB-118. These decreases were 53, 41, and 52%, respectively. Aro-

clor-1242, PCB-95, PCB-126, and TCDD caused decreases of 37, 36, 32, and 17%, respectively.

After the intravenous administration of radiolabeled T₄ ([125 I]T₄), the concentration of [125 I]T₄ in serum was determined at 30-min intervals, beginning at 15 min after injection (Fig. 3). Each of the treatments significantly enhanced the disappearance of [125 I]T₄ from serum. Aroclor-1254, PCB-99, and PCB-118 decreased the serum concentration of [125 I]T₄ by more than 70% within 15 min of administration. Serum concentrations of [125 I]T₄ in these PCB-treated rats continued to be significantly lower, compared with the control group values, over the entire 2-h collection period by an average of 77%. Aroclor-1242, PCB-95, and PCB-126 also significantly enhanced the disappearance of [125 I]T₄ from serum within 15 min and continuing over the entire collection period; the reductions in serum concentrations of [125 I]T₄ caused by these three treatments were less dramatic than those caused by Aroclor-1254, PCB-99, and PCB-118, averaging approximately 45%. In contrast, TCDD did not significantly reduce serum [125 I]T₄ until the 45-min time point. The average reduction caused by TCDD was only 25% (9, 23, 32, and 39% at the 15, 45, 75, and 105-min time points, respectively).

The effect of PCBs and TCDD on bile flow was not appreciable (data not shown). Only Aroclor-1242 significantly increased bile flow and only at the 30- and 60-min time points. These increases were 36 and 37%, respectively.

The biliary excretion rate for total [125 I]T₄ (T₄ and all metabolites) was significantly increased by Aroclor-1254, PCB-118, PCB-126, and TCDD (Fig. 4). Aroclor-1254 had the greatest effect, increasing the

FIG. 2. Effects of PCBs and TCDD on serum total T₄ (top) and total T₃ (bottom). Values represent the mean $\pm$ S.E.M. of six rats. *, significantly different from the control group ($p < 0.05$).

FIG. 3. Effects of PCBs and TCDD on the disappearance of [125 I] T_4 from serum. Values represent the mean $\pm$ S.E.M. of six rats. *, significantly different from the control group ($p < 0.05$).

biliary excretion rate above the control rate by 558, 679, 444, and 334% at the 30, 60, 90, and 120-min time points, respectively. PCB-118 was nearly as effective as Aroclor-1254 in increasing the rate of biliary excretion of [125 I] T_4 , causing 421, 452, 430, and 386% increases, compared with the control rate, at the 30, 60, 90, and 120-min time points, respectively. PCB-126 and TCDD were somewhat less effective, causing average increases in the rate of biliary excretion of 280 and 373%, respectively, over the 2-h collection period. Aroclor-1242, PCB-95, and PCB-99 did not significantly increase the rate of biliary excretion of [125 I] T_4 .

FIG. 4. Effects of PCBs and TCDD on the rate (top) and amount (bottom) of biliary excretion of [125 I] T_4 . Values represent the mean $\pm$ S.E.M. of six rats. *, significantly different from the control group ($p < 0.05$).

The cumulative biliary excretion of [125 I] T_4 is shown in the bottom of Fig. 4. Consistent with the increases in the rate of excretion, Aroclor-1254, PCB-118, PCB-126, and TCDD produced significant increases in the amount of [125 I] T_4 excreted into bile over the 2-h collection period. As with the excretion rate, Aroclor-1254 was most effective in significantly increasing the cumulative amount of [125 I] T_4 present in bile at each of the time points, compared with the control group values. These increases were 561, 616, 565, and 508% at the 30-, 60-, 90-, and 120-min time points, respectively (average increase of 563%). PCB-118 caused similar increases, with an average increase of 456% over the 2-h collection period. PCB-126 and TCDD also significantly increased the cumulative amount of [125 I] T_4 present in bile, compared with the control, with average increases of 277 and 372%, respectively. Aroclor-1242, PCB-95, and PCB-99 did not significantly increase the amount of biliary excretion of [125 I] T_4 .

HPLC analysis of bile samples revealed the predominant T_4 metabolite to be T_4 -glucuronide (a representative chromatogram is depicted in Fig. 5). In control animals, the T_4 -glucuronide represented, on average, 86% of the radiolabeled iodothyronines present in bile. Figure 6 shows both the rate (top) and the cumulative amount (bottom) of biliary excretion of T_4 -glucuronide after pretreatment of the rats with the various polychlorinated biphenyls. The effects on the rate of excretion of T_4 -glucuronide were consistent with the effects on the overall rate of excretion of [125 I] T_4 (Fig. 4, top). The rate of biliary excretion of T_4 -glucuronide was significantly increased by Aroclor-1254, PCB-118, PCB-126, and TCDD. Aroclor-1254 was the most effective, causing an average increase of 850% over the 2-h collection period. PCB-118, PCB-126, and TCDD also markedly increased the

FIG. 5. A representative chromatogram of HPLC analysis of bile samples. T₄-glucuronide was eluted at 19.14 min as a predominant T₄ metabolite.

rate of biliary excretion of T₄-glucuronide, with increases over the 2-h collection period averaging 756, 573, and 710%, respectively. These four compounds consistently increased the amount of T₄-glucuronide excreted into bile at 30, 60, 90, and 120 min: Aroclor-1254 by 992, 1100, 1000, and 938%; PCB-118 by 734, 848, 820, and 784%; PCB-126 by 525, 652, 664, and 614%; and TCDD by 602, 758, 769, and 705%, respectively. Aroclor-1242, PCB-95, and PCB-99 did not significantly increase the rate or amount of biliary excretion of T₄-glucuronide. No other T₄ metabolites were present in sufficient quantities to analyze.

The amount of [¹²⁵I]T₄ and its metabolites excreted in urine during the 2-h collection period was only a small percentage (0.8 to 27%) of that present in bile (data not shown). Administration of PCBs and TCDD did not result in significant differences in urinary excretion of [¹²⁵I]T₄.

The effect of PCBs and TCDD on the activity of UGTs toward T₄ was evaluated in liver microsomes and is shown in Fig. 7. Aroclor-1254, PCB-126, and TCDD were the most effective inducers of UGT activity toward T₄, causing 789, 709, and 824% increases, respectively, in UGT activity compared with the control group value. Aroclor-1242 and PCB-118 were slightly less effective, producing increases in activity of 307 and 514%, respectively. PCB-95 and PCB-99 did not significantly increase the activity of UGTs toward T₄.

Discussion

It is generally thought that the reduction in serum T₄ produced by PCBs is due to increased glucuronidation of T₄ followed by increased biliary excretion of T₄-glucuronide (Bastomsky, 1974; Hood and Klaassen, 2000; Vansell and Klaassen, 2001; Craft et al., 2002). However, the present study demonstrates that at least one additional mechanism might be responsible for the reduction in serum T₄ on the basis of the observations that PCB mixtures and congeners used in this study significantly reduced serum levels of T₄ (Fig. 2), but not all of them significantly increased the *in vivo* excretion of T₄-glucuronide into bile (Fig. 4) or increased the UGT activity toward T₄ *in vitro* (Fig. 7).

We were not surprised that Aroclor-1254 and TCDD significantly increased the biliary excretion of T₄-glucuronide *in vivo*, because it has been reported previously (Bastomsky, 1974; Vansell and Klaassen, 2001). However, we were surprised that only the PCB congeners with TCDD-like activity, namely PCB-118 and PCB-126, significantly increased the biliary excretion of T₄-glucuronide *in vivo*, whereas the PB-type congeners, PCB-95 and PCB-99, did not. We have shown that PCB-118 and PCB-126 significantly increased CYP1A2 activity [as quantified by ethoxyresorufin-*O*-deethylase (EROD) activity], whereas PCB-95 and PCB-99 increased CYP1B2 activity [as quantified by pentoxyresorufin-*O*-deethylase (PROD) activity] (Martin and Klaassen, 2010).

The effects of PCB congeners on UGT activity toward T₄ *in vitro* were consistent with the *in vivo* excretion results. Aroclor-1254, PCB-118, PCB-126, and TCDD were the most effective inducers of UGT activity toward T₄ *in vitro*. PCB-95 and PCB-99 (PB-type) had no effect on UGT activity toward T₄. These results are supported by the observation that PCB-110 (a PB-type congener) did not induce hepatic UGT activity unless it was contaminated with PCB-126 (Li et al., 1998). When a PCB-contaminated soil sample is filtered with charcoal to remove the coplanar (TCDD-like) congeners, induction of UGT activity is abolished (Li and Hansen, 1997). These data suggest that the ability of the PCBs to induce UGT activity toward T₄ is related to the level of TCDD-like activity of these compounds.

The mechanism by which TCDD induces gene transcription begins by interaction with a soluble cytoplasmic receptor known as the AhR. The ligand (TCDD) and the receptor are then translocated to the nucleus where the heterodimerization with the AhR nuclear translocator occurs. The receptor-ligand complex then interacts with xenobiotic response elements, which are *cis*-acting DNA sequences that induce transcription of genes (Whitlock, 1993). Transcriptional regulation of the genes encoding UGT1A6 in rat liver is mediated by xenobiotic response elements, and this gene is inducible by the AhR ligands 3-MC and TCDD (Emi et al., 1996; Masmoudi et al., 1997). It is currently thought that this UGT isoform is at least partially responsible for glucuronidation of T₄. Evidence for this includes impaired T₄ glucuronidation in Gunn rats, which have a genetic defect

FIG. 6. Effects of PCBs and TCDD on the rate (top) and amount (bottom) of biliary excretion of [125 I]T₄-glucuronide. Values represent the mean $\pm$ S.E.M. of six rats. *, significantly different from the control group ($p < 0.05$).

in all UGT1 gene products (Visser et al., 1993a). In addition, induction of UGT activity toward *p*-nitrophenol (a specific substrate of UGT1A6) by 3-MC, PCBs, and TCDD also increased the glucuronidation of T₄ but not T₃ (Beetstra et al., 1991; Visser et al., 1993b; van Raaij et al., 1993). In regard to PCBs, these data support the theory that induction of UGT activity toward T₄ is mediated by the TCDD-like activity of these compounds. This also explains the lack of

induction of UGT activity toward T₄ by the PB-type congeners, which do not bind to the AhR (Safe et al., 1985).

Whereas there appears to be a good correlation between the ability of these PCBs to increase UGT activity toward T₄ in vitro and to increase the excretion of T₄-glucuronide in vivo, there is not a good correlation with this ability to lower serum T₄ levels. The AhR ligands, Aroclor-1254, PCB-118, PCB-126, and TCDD, were the most effective inducers of UGT activity toward T₄ in vitro and produced the greatest increases in biliary excretion of T₄-glucuronide in vivo. The PB-type congeners (PCB-95 and PCB-99) did not induce UGT activity toward T₄ in vitro or increase the biliary excretion of T₄-glucuronide in vivo. According to present theory, one would anticipate that the TCDD-type congeners would decrease serum T₄ the most and the PB-type congeners would not be as effective. However, this hypothesis is not supported by the results of the current study. PCB-99, which was one of the most effective congeners at reducing serum levels of T₄, did not induce UGT activity toward T₄ or increase the biliary excretion of T₄-glucuronide. In addition, two of the compounds that increased UGT activity the most (TCDD and PCB-126) were less effective at reducing serum levels of T₄ than PCB-99. This suggests that the PB-like activity of the mixtures and congeners is responsible for perturbation of pathways that are not related to UGT induction.

The reductions in circulating levels of T₄ seem to correlate with the disappearance of [125 I]T₄ from the serum of these PCB-treated rats. Within 15 min of administration of [125 I]T₄, the serum level of [125 I]T₄ in the rats administered Aroclor-1254, PCB-99, or PCB-118

FIG. 7. Effects of PCBs and TCDD on the activity of hepatic UDP-UGTs toward T₄. Values represent the mean $\pm$ S.E.M. of six rats. *, significantly different from the control group ($p < 0.05$).

was less than 30% of that of controls (Fig. 3). PCB-95, Aroclor-1242, PCB-126, and TCDD also significantly increased the disappearance of [125 I]T₄ from serum, but much less dramatically. These results mirror those observed for circulating levels of T₄. The rapid disappearance of [125 I]T₄ from the plasma of rats treated with Aroclor-1254, PCB-99, or PCB-118 suggests that the PB-like activity of these compounds must be important for increased T₄ clearance. It is hypothesized that these compounds may increase tissue uptake of T₄ by increasing thyroid hormone transport across cell membranes.

Thyroid hormones most likely do not diffuse but are actively transported across cell membranes (Hennemann et al., 2001). Several transport proteins have been identified and cloned from rat liver that are thought to mediate the transport of thyroid hormones, including the organic anion-transporting polypeptides Oatp1a1, Oatp1a4, and Oatp1b2, as well as the sodium taurocholate cotransporting polypeptide Ntcp (Docter et al., 1997; Abe et al., 1998; Friesema et al., 1999; Fujiwara et al., 2001). Treatment of male Sprague-Dawley rats with PB increased Oatp1a4 protein levels in liver and enhanced the uptake of digoxin (substrate for Oatp1a4) (Hagenbuch et al., 2001). Another study reported the induction of Oatp1a4 protein by PB and PCN (Rausch-Derra et al., 2001). These reports reinforce findings in previous studies that pretreatment with PCN and PB increased the hepatic uptake and biliary excretion of cardiac glycosides (Klaassen, 1974) and suggest that the increased hepatic uptake of cardiac glycosides may be due, at least in part, to up-regulation of Oatp1a4.

It is conceivable that PCB-99, PCB-118, and Aroclor-1254, which have PB-like activity (induction of CYP2B) (Martin and Klaassen, 2010), may also induce Oatp1a4 and increase hepatic uptake of T₄. In fact, it has been shown that Oatp1a4 protein was increased 130% by PCB-99 but was decreased 72% by PCB-126 (Guo et al., 2002). If hepatic uptake of T₄ is increased by PCB-99 and decreased in response to PCB-126, this may, at least in part, explain the differential effects of PCB-99 and PCB-126 on serum T₄. PCB-99 effectively reduces serum T₄ without inducing UGT activity toward T₄ or increasing the biliary excretion of T₄-glucuronide, whereas PCB-126 is less effective at reducing serum levels of T₄ while significantly inducing UGT activity toward T₄. The other PB-type congener, PCB-95, also significantly increased the disappearance of [125 I]T₄ without inducing UGT activity or increasing the biliary excretion of T₄-glucuronide. However, PCB-95 was less effective than PCB-99 in decreasing plasma T₄ levels, which probably reflects the more labile, metabolizable structure of PCB-95 (Matthews and Dedrick, 1984).

The selective antagonist of corticotropin-releasing factor receptor-1, DMP 904, dramatically increased the biliary clearance of unconjugated [125 I]T₄ in rats, at least in part by enhanced hepatic uptake of [125 I]T₄ through induction of Oatp1a1 and Oatp1a4 (Wong et al., 2005). DMP 904 also induced a canalicular transporter, namely the multidrug resistance protein-2 (Mrp2) (Wong et al., 2005). Further study demonstrated that DMP 904 increased plasma clearance and hepatic uptake of [125 I]T₄ in Wistar but not in Mrp2-deficient TR(−) rats, suggesting that Mrp2 is responsible for biliary excretion of T₄-glucuronide and contributes in part to the excretion of T₄ (Lecœur et al., 2009). Sulfation is another pathway for thyroid hormone metabolism (Wu et al., 2005) and contributes a small part to the biliary excretion of T₃, which was increased by PCN but not by PCB (Vansell and Klaassen, 2002).

In conclusion, the present study provides evidence that the effects of PCB mixtures and congeners on serum levels of T₄ cannot be explained solely by induction of UGT activity toward T₄ and enhanced excretion of T₄-glucuronide. There appear to be two major effects of PCBs on serum T₄. One is increased glucuronidation and increased biliary excretion, which is associated with binding to the

AhR, and the second is enhanced tissue uptake, which is associated with the PB-like characteristics of the congeners. Increased glucuronidation facilitates biliary excretion of T₄-glucuronide; however, it appears that reductions in serum T₄ are more dependent on uptake, probably through transport proteins such as Oatp1a4.

Authorship Contributions

Participated in research design: Martin and Klaassen.

Conducted experiments: Martin and Wilson.

Performed data analysis: Martin and Wilson.

Wrote or contributed to the writing of the manuscript: Martin, Reuhl, Gallo, and Klaassen.

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