Comparative Responsiveness of Hypothyroxinemia and Hepatic Enzyme Induction in Long-Evans Rats Versus C57BL/6J Mice Exposed to TCDD-like and Phenobarbital-like Polychlorinated Biphenyl Congeners

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Numerous mechanisms have been postulated to explain how polyhalogenated aromatic hydrocarbons alter thyroid homeostasis with almost all data derived from studies using the rat. This study compared the sensitivity of rats and mice to polychlorinated biphenyl (PCB)-induced hypothyroxemia. Male and female C57BL/6J mice and Long-Evans rats were dosed orally for 4 consecutive days with either PCB126 (0.03–300.0 μg/kg/day) or PCB153 (0.3–300.0 mg/kg/day). Trunk blood and livers were collected 24 h after the last dose and used to determine total serum thyroxine (T4) and hepatic microsomal T4 glucuronidation activity. Hepatic microsomal ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-deethylase (PROD) activities were also determined as markers for Ah receptor or phenobarbital response unit activation, respectively. PCB126 did not affect T4 in the mouse but decreased T4 (up to 50%) in the rat. PCB153 decreased T4 (up to 80%) in both the rat and the mouse. PCB126 increased EROD in both rats (12- to 22-fold) and mice (15- to 20-fold). PCB153 induced hepatic PROD activity in both rats (30-fold) and mice (4-fold). T4 glucuronidation was increased approximately 2- to 3-fold in both rats and mice treated with PCB153. PCB126 increased T4 glucuronidation 13-fold in rats but only marginally (20%) in mice at the highest doses. Western blot analysis confirmed the PCB126-induced changes in expression of UGT1A1 in rats and the minimal increase in mice. These data suggest that species differences in response to chemicals that induce hypothyroxinemia are due to differential induction of hepatic UGT enzymes.

Key Words: polychlorinated biphenyls; PCBs; thyroid hormones; C576J/BL mice; Long-Evans rats.

Polychlorinated biphenyls (PCBs) are thought to interfere with thyroid hormone (TH) homeostasis by displacement of hormones from serum transport proteins, altering deiodinase activity and increasing thyroid hormone catabolism via glucuronidation (Barter and Klaassen, 1992a, 1994; Brouwer, 1989; Hood and Klaassen, 2000b). In addition, PCBs have been hypothesized to interfere with thyroid hormone homeostasis by binding directly to thyroid receptors, although recent data suggest that this does not occur in vitro at biologically relevant concentrations (Cheek et al., 1999).

Induction of uridine diphosphate glucuronosyltransferase (UGT) isozymes is another mechanism by which PCBs interfere with TH (Barter and Klaassen, 1992b; Beetstra et al., 1991; Hood and Klaassen, 2000a; Saito et al., 1991). PCBs induce hepatic UGTs, which conjugate the phenolic hydroxyl group of T4 with glucuronic acid, a metabolic prerequisite for its excretion into the bile (Beetstra et al., 1991; Saito et al., 1991; Visser, 1990). A number of prototypic enzyme inducers, such as 3-methylcholanthrene (3-MC), pregnenolone-16a-carbonitrile (PCN), and phenobarbital (PB), also increase the glucuronidation of TH through this pathway (Barter and Klaassen, 1994; De Sandro et al., 1992; Hood and Klaassen, 2000a; Saito et al., 1991).

The effects of prototype hepatic enzyme inducers, such as dioxins and PCBs, on circulating concentrations of TH in rats have been well characterized. Dioxins and dioxin-like PCBs decrease TH in rats by activation of the aryl hydrocarbon (Ah) receptor, which results in the induction of UGT and increases the glucuronidation and elimination of T4 (Schuur et al., 1997). Some of the non-dioxin-like PCBs are classified as PB-like because they induce induce phase I and phase II metabolizing enzymes through the PB response unit (PBRU) pathway (Ganem et al., 1999; Parkinson et al., 1983; Sueyoshi and Negishi, 2001). PCB153 (2,2’,4,4’,5,5’-hexachlorobiphenyl) is a PB-like congener that induces UGT (Ganem et al., 1999; Honka-
kosi et al., 1998) and decrease serum T_4 concentrations in rats (Desaulniers et al., 1999; van Birgelen et al., 1994b).

Numerous studies have demonstrated that PCBs decrease circulating THs in rats (Barter and Klaassen, 1992b; Brouwer et al., 1998; van Birgelen et al., 1994b), whereas little work has been done in mice. Many studies of TH-disrupting xenobiotics have focused on the male rat because of its sensitivity to the development of thyroid neoplasias (Contrera et al., 1997; Hurley, 1998). Findings in mice treated with PCBs and dioxins are conflicting. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exposure in mice leads to decreases in both T_3 and T_4 (Weber et al., 1995). Work comparing the sensitivity of the rat and mouse to TH modulation by xenobiotics suggests that THs in mice are insensitive to a wide variety of chemicals, including the Ah agonist β-naphthoflavone (Viollon-Abadie et al., 1999). Preliminary work from Kato et al. (2001) suggests that mice and rats show similar suppression of T_4 concentrations after exposure to the non-Ah agonist, di-or-tho-substituted PCBs.

The current research examined potential species differences in response to TH glucuronidation inducers in C57Bl/6J mice and Long-Evans rats. This work tested the hypothesis that species differences in the effects of polyhalogenated aromatic hydrocarbons (PHAHs) on T_4 concentrations are due to differences in induction of T_3 glucuronidation. Because PCBs are often characterized as dioxin-like and non-dioxin-like, 2 specific PCB congeners were used in this study. PCB126 (3,3′,4,4′,5-pentachlorobiphenyl) was chosen as the prototypical dioxin-like PCB because its dioxin-like effects are well characterized (van den Berg et al., 1998). The second PCB chosen was the prototypical PB-like congener, PCB153 (Ganem et al., 1999). Alterations in serum T_4 concentrations and metabolism were measured via radioimmunoassay and T_4 glucuronidation in liver microsomes. T_3 and thyroid-stimulating hormone (TSH) were not measured because work has shown them to be either unaffected or less sensitive than T_4 following exposure to PCBs (Barter and Klaassen, 1994; Desaulniers et al., 1999; Hallgren et al., 2001; Hood and Klaassen, 2000a; van Birgelen et al., 1994a, 1994b). Western blot analysis was used to verify induction of UGT protein. Liver microsomal CYP1A1 (using ethoxyresorufin-O-deethylase [EROD] activity) and CYP2B (using pentoxyresorufin-O-deethylase [PROD] activity) were determined as biomarkers for AhR and PB-like activities, respectively.

MATERIALS AND METHODS

Chemicals. PCB126 and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were obtained from Accustandard Corporation (New Haven, CT) at a purity greater than 99%. PCB153 was obtained from Radian Corporation (lot no. 970728R-AC, Austin, TX). PCB153 was custom synthesized for 99.9% purity. Gas chromatographic/mass spectrophotometric analyses of the PCB153 demonstrated that dibenzo-furan and dibenzodioxin concentrations were below 10 ppb (Radian Corporation, Austin, TX). The stock solutions were prepared by dissolving the PCB in acetone, mixing the acetone solution with corn oil, and then removing the acetone by evaporation (DeVito et al., 1993). All dosing solutions were prepared by dilution of the stock solutions with corn oil (Sigma Chemical Co., St. Louis, MO). Unless stated, all other chemicals were obtained from either Sigma Chemical Co. or Bio-Rad Laboratories (Hercules, CA).

Dosing protocol. Male and female Long-Evans rats (23–25 days of age) were obtained from Charles River Laboratory (Raleigh, NC) and male and female C57Bl/6J mice (60 days of age) from Jackson Laboratory (Bar Harbor, ME). They were allowed to acclimate for a minimum of 4 days before use. Younger rats were used to save on chemical synthesis costs for the PCB153. Effects on thyroid hormones and constituent expression of UGT, EROD, and PROD are similar in these younger rats compared with adults (Zhou et al., 2002; unpublished data). All animals were housed in standard plastic hanging cages containing sterilized pine shavings (Beta Chips, Northeastern Products, Inc., Warrensburg, NY). Rats were housed 2 per cage, and mice were housed either 3 or 4 per cage. Food (Lab Diet 5001, PMI, Brentwood, MO) and tap water were provided ad libitum. The animal facilities had an ambient temperature of 22°C, a relative humidity of 55 ± 5%, and a 12-h light-dark cycle.

Rats and mice were dosed by oral gavage for 4 consecutive days with PCB126 (0.03–100 μg/kg/day for rats and 0.003–300 μg/kg/day for mice), PCB153 (0.3–300 mg/kg/day and 0.9–90 mg/kg/day), or TCDD (0.003–10 μg/kg/day and 0.03–30 μg/kg/day). Short-term (2–4 day) dosing regimens are effective in altering THs for a variety of PHAHs (Zhou et al., 2001; Khan et al., 2002). The dosing volume was 1.0 ml/kg body weight for the rats and 10.0 ml/kg body weight for the mice. Control animals were dosed with the vehicle only. Because some experiments involved a large number of doses, these experiments were done in 2 replicate blocks. On the day after the last dose, animals were randomly killed by decapitation between 0800 and 1000 h. All animal husbandry was done in accordance with AAALAC regulations. All experiments with animals were approved in advance by the Animal Care Committee of the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency.

Sample collection and preparation. Blood was quickly collected from the neck (after decapitation) into clean funnels that drained into serum separator tubes and placed on ice and allowed to clot for up to 2 h. After centrifuging the blood for 30 min at 4°C in a Sorvall refrigerated centrifuge (model no. RT6000B) at 1257 × g (3K rpm), serum aliquots were stored at −80°C. Livers were dissected, weighed, and placed in sample vials and immediately frozen in liquid nitrogen and then later stored at −80°C. Liver microsomes were prepared from thawed samples according to the method described in DeVito et al. (1993).

Thyroxine assay. Total serum T_4 was measured using standard assay kits (Count-a-Count Kit, TKT41, Diagnostic Products, Inc., Los Angeles, CA). The tubes were decanted and counted on a gamma counter (model BS050, Packard Instruments, Downers Grove, IL). T_4 concentrations in the samples were determined from a standard curve created using calibration standards included in the kit. All samples were run in duplicate.

EROD and PROD assays. EROD activity was used as a marker for CYP2B activity. Microsomal protein concentrations were calculated photometrically using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard. All samples were run in duplicate. EROD and PROD values were calculated as picomoles of resorufin per milligram of protein per minute.

UGT assay. UGT activity toward T_4 was determined according to the method of Beestra et al. (1991) as modified by Zhou et al. (2001). 125I-labeled T_4 (NEN Life Science Products, Inc., Boston, MA) was purified by rinsing it on a Sephadex LH-20 column (bead volume, 2 ml) with a series of HCl and NH_4OH solutions and eluting the purified T_4 according to the method of Beestra et al. (1991). Reaction mixtures contained 0.2 ml 100 mM Tris/HCl (pH 7.8) and 5 mM MgCl_2, 0.4 mg protein, 125I-labeled T_4 (50,000 cpm), 1 mM cold T_4, and 0.1 mM of 6-propyl-2-thiouracil to inhibit outer-ring deiodinase activity. The reaction was initiated with 5 mM uridine-diphosphoglucuronic acid (UDPGA). Sample blanks not containing UDPGA were ana-
lyzed concurrently. After 30 min, reactions were stopped with the addition of 200 μl ice-cold methanol. After centrifugation, 200 μl of the supernatant was removed and added to 750 μl of 0.1 M HCl. The samples were then placed on a Sephadex LH-20 column (bead volume, 2 ml) and 1.0 ml water-eluted fractions containing the conjugate were combined and counted on a gamma counter (Model BS05, Packard Instruments, Downers Grove, IL). All samples were run in duplicate.

The effects of detergent (Brij 56) concentrations on T4 glucuronidation activity were determined in control and treated rat and mouse microsomal fractions. Initial studies indicated that in rat microsomes the presence of detergent increased basal activity of T4 glucuronidation by about 4-fold. In the rat, PCB153-treated microsomes showed a 2- to 3-fold induction of UGT activity in the absence of Brij 56; in the presence of Brij 56 there was no induction apparent. TCDD induction of UGT was decreased about 50% in rat microsomes in the presence of Brij 56. Therefore, in subsequent assays, detergents were not used with rat microsomes. In mouse microsomes, Brij 56 also increased basal activity. However, in contrast to rats, the greatest fold induction for both PCB126 and PCB153 in mouse microsomes was observed in the presence of 0.25 mM Brij 56. Therefore, all assays with mouse microsomes included 0.25 mM Brij 56.

Immunoblotting analysis. Aliquots of liver microsomes from control, low-, and high-dose animals treated with PCB126 were thawed and diluted to 15 mg/ml for the mouse and 5 mg/ml for the rat. These concentrations demonstrated the best linearity in density with respect to protein concentration (data not shown). The samples were loaded onto a precast 10% acrylamide Tris-Glycine gel (Novex, San Diego, CA) at a sample volume of 15 ml and then incubated with a secondary anti-rabbit immunoglobulin G (1:1000 with a Tris buffer (30 mM Tris, 0.3 M NaCl, and 0.2% Tween, pH 7.4) (TBST) MA) at a 1:5000 dilution. This antibody recognizes human UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A9, and 1A10 isoforms. The membrane was washed twice with a Tris buffer (30 mM Tris, 0.3 M NaCl, and 0.2% Tween, pH 7.4) (TBST) and then incubated with a secondary anti-rabbit immunoglobulin G (1:1000 dilution) (Amersham Life Science, Buckinghamshire, England). After 2 washes with TBST, the membrane was incubated with a streptavidin horseradish peroxidase solution (1:1000 dilution) (Amersham Life Science). Membranes were again rinsed with TBST followed by a rinse with Tris-buffered saline (30 mM Tris and 0.2 M NaCl, pH 7.4) and then soaked for 5 min with SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL) to visualize proteins. Protein band density was quantified from the chemiluminescent images using Fluor-S Multimager and Quantity One software (Bio-Rad, Hercules, CA) after a 500 s exposure period. A total of 3 to 4 samples were run for each treatment group.

Statistical analysis. The data for T4 concentrations, EROD, PROD, and UGT-T4 activities were analyzed independently using a one-way analysis of variance (SAS Institute, Cary, NC). Duncan’s multiple range tests were used to compare differences among treatment groups, with acceptable significance levels set at p < 0.05. For comparative purposes, T4, EROD, and PROD data are presented as percentage of control. UGT data were presented as actual values to demonstrate the difference in basal expression between rats and mice.

RESULTS

PCB126

Body and liver weights. There were no treatment-related mortalities of either the rats or mice exposed to PCB126. Body weight gain was affected in female rats but not male rats or mice. In female rats body weight gain was decreased by about 10–20% at doses of 30 μg/kg/day and higher (p < 0.05). Liver-body weight ratios were increased by PCB126 in both rats and mice (data not shown). In rats, maximal increases of 20% were observed at 3 and 10 μg/kg/day or higher in males and females (p < 0.05), respectively. Liver-body weight ratios were increased in both male (30% increase) and female mice (20% increase) at 30 μg/kg/day or higher (p < 0.05).

Tyroxine concentrations. Figure 1 illustrates the effects of PCB126 on serum T4 in rats and mice. Actual control T4 values are presented for both species in Table 1. Both male and female rats demonstrated a 50% decrease in serum T4 concentrations with respect to controls. There were significant main effects of treatment for both male and female rats, F(4, 62) = 22.00, p < 0.0001, and F(8, 71) = 23.50, p < 0.0001, respectively. Group mean contrast tests revealed statistically significant decreases (p < 0.05) beginning at the 3.0 μg/kg dose in female rats and beginning at the 10.0 μg/kg dose in male rats. Serum T4 concentrations were not significantly altered at any PCB126 dose in either the male or female mouse.

To confirm the lack of effect of Ah receptor agonists on serum T4 concentrations in mice, both female rats and mice were treated with TCDD (Fig. 2). In female rats, TCDD decreased serum T4 concentrations in a dose-dependent manner at doses as low as 0.1 μg/kg/day, F(4, 45) = 11.57, p < 0.0001. Maximal decreases of approximately 50% were observed at doses of 1 μg/kg/day or higher (p < 0.05). Serum T4 concentrations in female mice treated with doses as high as 30 μg/kg/day for 4 days were not significantly different from controls, F(4, 24) = 1.02, p < 0.4156. It should be noted that mice in the highest dosage group received a total of 120 μg TCDD/kg, which is slightly lower than the LD50 of TCDD in mice of approximately 150 μg/kg (Pohjanvirta and Tuomisto, 1994). No mice died before the terminal sampling on day 5.

Cytochrome P450 activity. Hepatic EROD activity was used as a marker of Ah receptor activation by PCB126 and is
Experimental Values Obtained in Control Male and Female Long-Evans Rats and in Control Male and Female C57BL/6J Mice

<table>
<thead>
<tr>
<th>PCB126 (μg/kg/day)</th>
<th>T4 (μg/dl ± SE)</th>
<th>EROD (pmol/mg protein/min ± SE)</th>
<th>PROD (pmol/mg protein/min ± SE)</th>
<th>UGT (pmol/mg protein/min ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male Rat</strong></td>
<td>50.0 ± 2.1</td>
<td>259.5 ± 18.2</td>
<td>—</td>
<td>0.5 ± 0.10</td>
</tr>
<tr>
<td><strong>Male Mouse</strong></td>
<td>54.8 ± 2.9</td>
<td>585.6 ± 39.2</td>
<td>—</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td><strong>Female Mouse</strong></td>
<td>47.2 ± 2.7</td>
<td>449.3 ± 29.9</td>
<td>—</td>
<td>2.4 ± 0.13</td>
</tr>
<tr>
<td><strong>Male Mouse</strong></td>
<td>34.3 ± 3.5</td>
<td>337.8 ± 57.6</td>
<td>—</td>
<td>2.1 ± 0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCB153 (μg/kg/day)</th>
<th>T4 (μg/dl ± SE)</th>
<th>EROD (pmol/mg protein/min ± SE)</th>
<th>PROD (pmol/mg protein/min ± SE)</th>
<th>UGT (pmol/mg protein/min ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male Rat</strong></td>
<td>51.5 ± 2.8</td>
<td>16.6 ± 2.6</td>
<td>0.4 ± 0.1</td>
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<tr>
<td><strong>Male Mouse</strong></td>
<td>57.5 ± 2.1</td>
<td>12.4 ± 3.0</td>
<td>0.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Female Mouse</strong></td>
<td>59.6 ± 3.1</td>
<td>33.4 ± 2.4</td>
<td>2.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td><strong>Male Mouse</strong></td>
<td>35.1 ± 2.6</td>
<td>21.6 ± 3.7</td>
<td>2.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Note. T4 concentrations are expressed as ng/dl ± SE (n = 6–9/group). EROD activities as pmol/mg protein/min ± SE, PROD activities as pmol/mg protein per minute ± SE (n = 4–9/group), and UGT activity by amount of T4 glucuronide produced per mg protein per min ± SE (n = 4–6/group).

Also significant induction of EROD in female mice exposed to TCDD, F(4, 24) = 42.41, p < 0.0001. EROD was significantly increased 11-fold and 13-fold in the 3 μg/kg/day and 30 μg/kg/day dose groups, respectively (data not shown).

UGT induction. Treatment with PCB126 increased T4-UGT activity approximately 13-fold in female rats and approximately 7-fold in male rats (Fig. 4). Induction became significant at the 10 μg/kg/day dose in both the male and female rat, F(4, 25) = 31.18, p < 0.0001 and F(8, 62) = 23.29, p < 0.0001, respectively. Only slight increases in T4 glucuronidation were observed in both male, F(5, 24) = 5.45, p < 0.0017, and female, F(4, 28) = 3.34, p < 0.0234, mice (see Fig. 4). There was significant induction of 15% (above control) only in 90 μg/kg/day group for the female mice; there was no significant increase in the 300 μg/kg/day group. In male mice, significant induction of about 20% was found only in the 300 μg/kg/day group.

Western blot analyses clearly demonstrated a large PCB126-induced increase in protein expression in female rats at approximately 52 kDa (Fig. 5). Data from male rats showed the same pattern of expression (data not shown). Quantitative estimates of protein induction are illustrated in Figure 5 (inset). Results from the Western blots are consistent with those obtained in the UGT activity assays. The Western blot also verified the results seen in the mice in the UGT activity assay. Figure 5 illustrates a very minimal increase in protein expression in the highest dose group in mice compared with controls. Note that the mouse Western blots contained 3 times the amount of protein as used in the rat Western blots. Importantly, this slight increase was not consistently observed in all samples (see Fig. 5 insert).
PCB153

Body and liver weights. There were no treatment-related mortalities of either the rats or mice exposed to PCB153. Body weight gain was affected in female rats but not in male rats or mice (data not shown). In female rats, body weight gain was decreased in female rats (30%) only in the 90 mg/kg/day group ($p < 0.05$). In mice, body weights were not altered by any of the PCB153 dose levels examined ($p > 0.05$). Liver-body weight ratios were increased by PCB153 in both rats and mice (data not shown). In male rats, increases of 20–30% were observed at 10 mg/kg/day or higher. In female rats, increases of up to 30% were observed at doses of 3 mg/kg/day or higher ($p < 0.05$). Liver-body weight ratios were increased in both male (up to 30%) and female mice (up to 20%) at 30 mg/kg/day or higher ($p < 0.05$).

Thyroxine concentrations. Figure 6 illustrates the similar effects of PCB153 on serum $T_4$ in both rats and mice. Actual $T_4$ control values are presented for both species in Table 1. Both male and female rats demonstrated dose-related decreases in serum $T_4$ concentrations and achieved an ~80% maximal suppression with respect to controls. Statistical decreases were apparent at doses as low as 9.0 mg/kg in female rats and 10.0 mg/kg in male rats, $F(4, 27) = 48.66, p < 0.0001$, and $F(6, 34) = 43.13, p < 0.0001$, respectively. Both male and female mice also exhibited a statistically significant decrease in serum $T_4$, at doses as low as 3.0 mg/kg in the female and 20.0 mg/kg in the male, $F(4, 24) = 55.51, p < 0.0001$, and $F(5, 84) = 48.21, p < 0.0001$, respectively.

Cytochrome P450 activity. Hepatic PROD activity was used as a marker of PBRU activation by PCB153 and is presented in Figure 7. Increased PROD was noted in both genders of rat. Male rats reached significant induction levels beginning at the 0.9 mg/kg dose and reached 50-fold induction at the highest dose administered, $F(4, 15) = 62.52, p < 0.0001$. Female rats followed a similar pattern, with significant induc-
The decreases in serum T₄ in the rat after PCB126 or TCDD exposure are consistent with findings from other studies (Henry and Gasielwicz, 1987; Saito et al., 1991; Schuur et al., 1997; van Birgelen et al., 1994b). PCB126 and TCDD exposure in female Sprague-Dawley rats caused a 50% decrease in serum T₄ concentrations compared with controls, and this decrease was associated with increased hepatic T₄ glucuronidation activity (van Birgelen et al., 1994a). The present data showing no effects of PCB126 and TCDD on serum T₄ concentrations in mice are consistent with Viollon-Abadie et al. (1999), who failed to alter T₄ concentrations in OF-1 mice treated with the AhR agonist β-naphthoflavone. In contrast, decreased T₄ was reported in C57 mice after TCDD exposure (Weber et al., 1995). The reasons for this discrepancy remain to be determined.

There are a number of possible explanations why, in the present study, PCB126 and TCDD would decrease T₄ in rats and not in mice. First, mice may be insensitive to the effects of dioxins. This is unlikely to be the case, however, because in this study EROD activity, a marker for CYP1A1 and responsiveness to Ah receptor agonists, was clearly induced. This is consistent with numerous studies demonstrating that mice are responsive to PCB126 and TCDD (DeVito et al., 2000; Pohjanvirta and Tuomisto, 1994; Safe, 1994). Another explanation for the lack of effect on T₄ concentrations in mice is that T₄ UGT activity may not be inducible in mice exposed to PCB126 and other dioxin-like chemicals. There is ample evidence that UGT activity toward many substrates other than T₄ (e.g., p-nitrophenol) are induced in mice exposed to Ah receptor agonists (e.g., Nemoto and Takayama, 1980; Owens, 1977; Shen et al., 1989). In the current study, a very small increase of UGT1A proteins, as demonstrated by Western blot analysis, was observed at the highest dose examined. Consistent with this small induction of protein was the small, approximately

**DISCUSSION**

The present study examined species differences in response to PCB126 and PCB153 in rats and mice. In rats, PCB126 and PCB153 decreased serum T₄, and these decreases were associated with increased hepatic microsomal T₄ glucuronidation activity. In mice, PCB153 decreased serum T₄ and induced significant T₄ glucuronidation activity. PCB126 did not alter serum T₄ concentrations in mice, and induction of T₄ glucuronidation was very minimal. To confirm this species difference in response to an Ah receptor ligand, rats and mice were also exposed to TCDD, the most potent Ah receptor agonist. Similar to PCB126, exposure to TCDD induced a dose-dependent decrease in serum T₄ in rats, but did not alter serum T₄ concentrations in mice at doses as high as the LD₅₀. Finally, the degree of induction of UGT activity in rats and mice exposed to PCB126 was confirmed with Western blot analyses.

**FIG. 7.** PROD activity in male and female rats and mice after exposure to PCB153; n = 4–9/group, except n = 3/group for the 300 mg/kg male mouse; ′ indicates significantly different from respective rat control group (p < 0.05); ″ indicates significantly different from respective mouse control groups (p < 0.05).

**FIG. 8.** T₄ glucuronide production in male and female rats and mice after exposure to PCB153; n = 4–6/group; ′ indicates significantly different from respective rat control group (p < 0.05); ″ indicates significantly different from respective mouse control group (p < 0.05).
20%, increase of T₄ glucuronidation in mice. This small induction of UGTs in mice would not result in changes in serum TH. Although higher doses of PCB126 may have resulted in greater elevation of UGT, it should be noted that the dose administered was 300 μg/kg/d for a total of 1.2 mg/kg, which is approximately the LD₅₀ for this chemical in mice (Pohjanvirta and Tuomisto, 1994). The human UGT1A antibody is capable of detecting increases in mouse UGT proteins; there is an 8- to 10-fold increase in the density of the ~52-kD band in mice exposed to PCB153 at 300 mg/kg/day (unpublished data). Thus, the effects of Ah-agonists on T₄ glucuronidation activity appear to be species dependent.

PCB153 dramatically decreased serum T₄ concentrations and increased hepatic T₄ glucuronidation in both rats and mice. These effects are consistent with other reports examining the effects of either non-coplanar PCBs or A1254 in rats (Desaulniers et al., 1999; Hood et al., 1999; Hood and Klaassen, 2000a; Kato et al., 2001; Khan et al., 2002; van Birgelen et al., 1994b). Viollon-Abadie et al. (1999) suggested that THs in mice are insensitive to the effects of hepatic enzyme inducers. The present study indicates that the insensitivity in mice may be chemical specific. Other PHAHs decrease T₄ in mice, including polybrominated diphenyl ethers, di-ortho-substituted PCBs, and PHAH contaminated fish (Cleland et al., 1987; Hallgren et al., 2001; Kato et al., 2001). The presence of Ah agonists (dioxins and coplanar PCBs) and other contaminants in the fish examined in the Cleland study makes it difficult to ascribe the T₄ decrease to any one type of contaminant. The present data suggest that the nondioxin-like PCBs could be responsible for the effects on serum T₄ in the studies by Cleland et al. (1987).

The magnitude of induction of UGT enzymes was not well correlated with the magnitude of decrease in circulating concentrations of serum total T₄. The present data show an 80% maximum reduction in T₄ concentrations and a 2- to 3-fold induction of UGT activity in both rats and mice exposed to PCB153. Maximal decreases in serum T₄ concentrations of only 50% were found in rats exposed to PCB126, yet these animals had up to a 13-fold induction of UGT activity. Consistent with this observation, both De Sandro et al. (1992) and Hood and Klaassen (2000a) found no clear relationship between UGT induction and T₄ concentrations. One possible reason for the discrepancy between levels of induction of UGT activity and serum concentrations of T₄ is that the UGT assay may not accurately measure the true changes in hepatic glucuronidation of T₄. There are a number of different isoforms of UGT, and they require different ex vivo assay conditions to produce maximal activity (De Sandro et al., 1992; Hood and Klaassen, 2000a; Mackenzie et al., 1984; Visser et al., 1993). In the present study, rat hepatic microsomal T₄ glucuronidation activity was assayed without Brij 56 because this detergent completely masked the induction of UGT activity in microsomes from PCB153 rats. In contrast, mouse hepatic microsomal T₄ glucuronidation activity was assayed in the presence of Brij 56 to observe the greatest fold induction. Presently, the relationship between ex vivo measurement of UGT enzyme activity and the in vivo effects on T₄ concentrations is not well understood. It is possible that the enzyme assays used in this report underestimate induction by PCB153 compared with PCB126.

There are other possible explanations as to why serum T₄ concentrations are not directly associated with hepatic T₄ glucuronidation. TH homeostasis is regulated through a variety of mechanisms. Exposure to A1254 has been shown to upregulate type 2 deiodinase in rat brain during development (Morse et al., 1996). Deiodinases convert T₄ to T₃ in tissues, and this could lead to decreases in circulating T₄ concentrations. However, Hood and Klaassen (2000b) demonstrated that a number of microsomal enzyme inducers, including A1254, had only a minimal impact on overall outer ring deiodination activity. That alterations in deiodinase activity impact circulating T₄ concentrations in the present animal model is currently speculative. Another explanation is that PCB congeners and hydroxy-metabolites of PCBs displace T₄ from serum binding proteins in vitro (Brouwer, 1989; Chauhan et al., 2000). Although the exact role of this mechanism in the regulation of serum hormone concentrations in vivo is unknown, displacement from serum binding proteins could lead to greater availability of the hormones for glucuronidation and elimination, resulting in lowered serum concentrations. A combination of the just-mentioned mechanisms may be ultimately responsible for the differences between measured increases in UGT activity and serum T₄ concentrations between chemicals.

In the present study exposure to the di-ortho-substituted congener PCB153 led to significantly increased PROD activity in both species. This data is consistent with the literature on PCB153 exposure in rats and mice (Parkinson et al., 1983; Ganem et al., 1999; Ikegwuonu et al., 1996). The similarity in effects of PCB153 in mice and rats is in contrast to the species-specific effects of PCB126.

Most toxicity studies examining the effects of xenobiotics on TH homeostasis have been completed in the rat. This report demonstrates that there are important species differences in the effects of some environmental contaminants on thyroid hormone homeostasis. Data illustrate the differences in the regulation of thyroid hormone glucuronidation in Long-Evans rats and C57BL/6J mice following exposure to the AhR agonist PCB126. This report also highlights striking similarities in response to PCB153 in these same species. Until more reliable data on endocrine disrupting chemicals is collected in multiple species, any comparison to possible consequences of these xenobiotics on humans should be viewed with caution, as evidenced by the species comparison data presented here.

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