Reduction of Thyroid Hormone Levels by Methylsulfonyl Metabolites of Tetra- and Pentachlorinated Biphenyls in Male Sprague-Dawley Rats

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Received July 6, 1998; accepted October 14, 1998

Male Sprague-Dawley rats received four consecutive intraperitoneal (ip) doses of five kinds of methylsulfonyl (MeSO₂) metabolites of tetra- and pentachlorinated biphenyls (tetra- and pentaCBs) to determine their effects on thyroid hormone levels. The five MeSO₂ metabolites, which were the major MeSO₂-PCBs detected in human milk, liver and adipose tissue were 3-MeSO₂-2,2',4',5-tetraCB (3-MeSO₂-CB49), 3-MeSO₂-2,3',4',5-tetraCB (3-MeSO₂-CB70), 3-MeSO₂-2,2',3',4',5-pentaCB (3-MeSO₂-CB87), 3-MeSO₂-2,2',4',5,5'-pentaCB (3-MeSO₂-CB101), and 4-MeSO₂-2,2',4,5,5'-pentaCB (4-MeSO₂-CB101). All five tested MeSO₂ metabolites (20 μmol/kg once daily for 4 days) reduced serum total thyroxine levels 16–40% on days 2, 3, 4, and 7 (after the last dosage). The total triiodothyronine level was reduced 37% by treatment with 3-MeSO₂-CB49 at day 7, but was increased 35% and 38% by 3-MeSO₂-CB70 and 4-MeSO₂-CB101 at days 3 and 4, respectively. The reductions in thyroid hormone levels led to an increase in thyroid stimulating hormone (TSH) levels by 3-MeSO₂-CB49, 3-MeSO₂-CB87 and 3-MeSO₂-CB101. A 30% increase in thyroid weight was produced by 3-MeSO₂-CB101 treatment. Thus, it is likely that all five tested MeSO₂ metabolites could influence thyroid hormone metabolism. The results show that the tested 3- and 4-MeSO₂ metabolites of tetra- and pentaCBs reduce thyroid hormone levels in rats, suggesting that the metabolites may act as endocrine-disrupters.

Key Words: methylsulfonyl (MeSO₂) metabolites; polychlorinated biphenyls (PCBs); total thyroxine (T₄); thyroid stimulating hormone (TSH); rats.

Polychlorinated biphenyls (PCBs) are widely distributed throughout the ecosystem. PCBs are both highly lipophilic and persistent and, therefore, accumulate readily in the food chains. Their bioaccumulations have led to detectable amounts in the human milk, liver and adipose tissue of several mammalian species from Canada and Sweden (Letcher et al., 1995a,b, 1997), we reported that nine 3-MeSO₂ metabolites such as 3-MeSO₂-2,3',4',5-tetrachlorobiphenyl (3-MeSO₂-2,3',4',5-tetraCB; 3-MeSO₂-CB70), 3-MeSO₂-2,2',3',4',5-pentaCB (3-MeSO₂-CB87) and 3-MeSO₂-CB101 were strong inducers of hepatic microsomal drug-metabolizing enzymes, and that 3-MeSO₂-2,2',4',5'-pentachlorobiphenyls (3-MeSO₂-2,2',4',5'-pentachlorobiphenyls; 3-MeSO₂-CB101 and 4-MeSO₂-CB101) (Bergman et al., 1992, 1994; Haraguchi et al., 1992; Letcher et al., 1995; Norén et al., 1996; Weistrand and Norén, 1997). The biological activities and toxicological significances of MeSO₂ metabolites have not been clarified.

In our preceding papers (Kato et al., 1995a,b, 1997), we reported that nine 3-MeSO₂ metabolites such as 3-MeSO₂-CB49, 3-MeSO₂-CB87 and 3-MeSO₂-CB101. A 30% increase in thyroid weight was produced by 3-MeSO₂-CB101 treatment. Thus, it is likely that all five tested MeSO₂ metabolites could influence thyroid hormone metabolism. The results show that the tested 3- and 4-MeSO₂ metabolites of tetra- and pentaCBs reduce thyroid hormone levels in rats, suggesting that the metabolites may act as endocrine-disrupters. Additionally we suggested that some 3- and 4-MeSO₂ metabolites may act as liver tumor promoters, based upon the results from an in vitro intercellular communication assay (Kato et al., 1998b). We also showed that four MeSO₂ metabolites of hexachlorobiphenyls (hexaCBs) possess the ability to decrease serum total thyroxine (T₄) level in rats (Kato et al., 1998a).

In this study, we have investigated the potential of 3- and 4-MeSO₂ metabolites of tetra- and pentaCBs, 3-MeSO₂-CB49, 3-MeSO₂-CB70, 3-MeSO₂-CB87, 3-MeSO₂-CB101, and 4-MeSO₂-CB101, to reduce thyroid hormone levels. These metabolites have been identified in human milk, liver, and adipose tissue and the tissues of several mammalian species (Bergman et al., 1992, 1994; Haraguchi et al., 1992; Letcher et al., 1995, Norén et al., 1996; Weistrand and Norén, 1997). The thyroid is a common target organ of toxicity of metabolites such as these which are enzyme inducers. This study evaluated the acute endocrine-disrupting effects by the MeSO₂ metabolites of tetra- and pentaCBs. Figure 1 shows the chemical
structures of MeSO₂ derivatives of PCB congeners used in this paper.

MATERIALS AND METHODS

Chemicals. The MeSO₂-PCBs were prepared as described elsewhere (Haraguchi et al., 1987). The purity of these compounds was >99% when analyzed by gas chromatography (GC). Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo). All other chemicals were obtained commercially in appropriate grades of purity.

Animal treatments. Male Sprague-Dawley rats weighing approximately 180–200 g (Charles River Japan Inc.), were housed three or four per cage in the laboratory, with free access to commercial chow and tap water, and were maintained on a 12-h dark/light cycle (8:00 A.M.–8:00 P.M. light) in a room with controlled temperature (24.5 ± 1°C) and humidity (55 ± 5%).

Rats received four consecutive intraperitoneal (ip) injections of 20 μmol/kg MeSO₂-tetra- and pentaCBs dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. This is equivalent to about 8 mg/kg in about 1 ml vehicle/rat.

Analysis of thyroid hormones. On days 2, 3, 4, and 7, after last dosing, 0.2 ml of blood was drawn from the tail vein. Blood was collected from animals between 10:30 and 11:30 A.M. After clotting at room temperature, serum was separated by centrifugation and stored at −50°C prior to determination of total thyroxine (T₄), total triiodothyronine (T₃), and thyroid stimulating hormone (TSH) levels by radioimmunoassay using Amerlex-MT4, Amerlex-MT3 and Biotrak rTSH [¹²⁵I] assay system purchased from Amersham Life Science, Ltd. (Little Chalfont, U.K.). All rats were killed by decapitation on day 7, and the thyroid glands were removed and weighed.

Determination of MeSO₂-PCBs in tissues. The concentrations of MeSO₂-PCBs present in thyroid glands and liver were determined by analyzing the n-hexane extracts from these samples by GC (Haraguchi et al., 1997).

Statistics. The results were statistically analyzed using Student’s t-test.

RESULTS

Serum total T₄ concentrations were reduced by all treatments (Figs. 2 and 3). 3-MeSO₂-CB49, 3-MeSO₂-CB70, 3-MeSO₂-CB87, 3-MeSO₂-CB101, and 4-MeSO₂-CB101 (20 μmol/kg given on 4 consecutive days) significantly reduced serum total T₄ levels by 16–40% at days 2, 3, and 4, and through to day 7. The greatest T₄ depression was produced by 3-MeSO₂-CB101 and 4-MeSO₂-CB101 from day 2, and the depression continued through day 7.

Serum concentration of total T₃ was decreased by 37% by 3-MeSO₂-CB49 treatment at day 7, but increased by 3-MeSO₂-CB70 and 4-MeSO₂-CB101 at days 3 and 4 (35% and 38% increases), respectively. Serum concentrations of TSH were increased over 2-fold by 3-MeSO₂-CB101 at days 3 and 4. 3-MeSO₂-CB49 and 3-MeSO₂-CB87 treatments increased serum TSH levels at day 4.

A significant increase in thyroid weight was observed with 3-MeSO₂-CB101 treatment (Table 1). After the dosing of all five MeSO₂ derivatives, they were present in the thyroid glands and livers. The concentrations of methyl sulfones in the thyroid glands were as much as one half to one third of those in the liver.

FIG. 1. Chemical structures of methyl sulfone derivatives of PCB congeners.

FIG. 2. Effects of MeSO₂ derivatives of tetraCBs on serum total thyroxine, triiodothyronine and thyroid stimulating hormone concentrations in rats. MeSO₂-tetraCBs (20 μmol/kg) were given ip to rats once daily for four days. Control; ——, 3-MeSO₂-CB49; ——, 3-MeSO₂-CB70. Each point represents the mean ± SE (vertical bars) for 4–6 animals. *p < 0.05, significantly different from the control.
DISCUSSION

We previously reported that some MeSO₂ metabolites of hexaCBs reduced thyroid hormone levels in rats (Kato et al., 1998a). In the present study, four 3-MeSO₂ metabolites and one 4-MeSO₂ metabolite of tetra- and pentaCBs were shown to reduce serum thyroid hormone levels in rats. The extent of reduction of serum total T₄ concentrations after the administration of five different methyl sulfonyl metabolites of tetra- and pentaCBs (20 μmol/kg once daily for 4 days), was almost the same as that after the administration of MeSO₂ metabolites of hexaCBs at the same dose. Each of the metabolites reduced total T₄ concentrations at all time points. The reason might be due to prolonged retention of the metabolites in the thyroid glands and liver, but the irregular time course in some cases suggest multiple actions as well.

Some PCBs have been reported to interfere with endocrine levels as well as the immune system (Birnbaum, 1994). For example, in pregnant women and in newborn children, elevated levels of PCB congeners have been reported to alter thyroid hormone status (Koopman-Esseboom et al., 1994). It is well known that some PCB congeners, such as 2,2′,4,4′-tetraCB (CB47), 3,3′,4,4′-tetraCB (CB77), 2,3′,4,4′,5- and 3,3′,4,4′,5-pentaCBs (CB118 and CB126), and 2,2′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexaCBs (CB153 and CB156), alter thyroid hormone levels and metabolism (Chu et al., 1995; Ness et al., 1993; Saeed and Hansen, 1997; Van Birgelen et al., 1995). The thyroid effects of the parent PCB congeners yielding the 3- and 4-MeSO₂ metabolites tested in this study have not yet been reported; however, the metabolites, which all reduced serum T₄ levels, are the major MeSO₂-PCBs detected in human liver and milk (Norén et al., 1996; Weistrand and Norén, 1997), and they produced continuous reductions of total T₄ at each time point examined. Thus, these MeSO₂ metabolites may play an important role in alterations in thyroid hormone levels on exposure to the PCB congeners, and MeSO₂ metabolites should be given attention in reference to the influence of PCB congeners on thyroid hormone metabolism.

In normal thyroid physiology, reduction of circulating levels of T₄ and T₃ is compensated for by increased release of TSH from the anterior pituitary, resulting in increased production of thyroid hormones by the thyroid glands. Increases in TSH at 3 and/or 4 days following 3-MeSO₂-PCBs were not maintained by the 7th day, even though serum T₄ was still reduced. Two

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative thyroid weight (% × 100)</th>
<th>Methyl sulfone concentration (nmol/g)</th>
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<tr>
<td>Control</td>
<td>0.81 ± 0.07</td>
<td>Thyroid glands 23.03 ± 5.62</td>
</tr>
<tr>
<td>3-MeSO₂-CB49</td>
<td>0.79 ± 0.05</td>
<td>Liver 23.58 ± 0.66</td>
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<tr>
<td>3-MeSO₂-CB70</td>
<td>0.94 ± 0.05</td>
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<tr>
<td>3-MeSO₂-CB87</td>
<td>0.93 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>3-MeSO₂-CB101</td>
<td>1.05 ± 0.06*</td>
<td></td>
</tr>
<tr>
<td>4-MeSO₂-CB101</td>
<td>0.88 ± 0.01</td>
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Note. MeSO₂-PCBs (20 μmol/kg) were given ip to rats once daily for four days and the rats were killed seven days after final administration. Results are expressed as the mean ± SE for 3–6 animals.

* Significantly different from control, p < 0.05.
Compounds (3-MeSO₂-CB70, the only tested compound with a single ortho-chlorine, and 4-MeSO₂-CB101, the only tested para-MeSO₂) caused no significant increases in TSH and were the only tested compounds that caused a significant increase in T₃. This suggests that different mechanisms or combinations of mechanisms of thyroid hormone disruption may be possible since thyroid and liver residues were similar to the other compounds.

In conclusion, all 3- and 4-MeSO₂ metabolites of tetra- and pentaCBs possess the ability to reduce serum thyroid hormone levels in rats. The results suggest the endocrine-disrupting potential of these compounds and that risk assessment should be done for the toxicity of MeSO₂ metabolites of tetra- and pentaCBs. Further studies are needed to reveal the details of their mechanism(s) of reduction.

ACKNOWLEDGMENTS

We acknowledge Kiyoe Taketani and Miwa Nakamura for their technical assistance. The work was partially supported by a Grant-in-Aid for Scientific Research (C) (no. 09680531) from the Ministry of Education, Science, Sports and Culture of Japan and grants from the Showa Shell Sekiyu Foundation for Promotion of Environmental Research and from the Shizuoka Research Institute.

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