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HYDROXYLATED AND METHYLSULFONYL POLYCHLORINATED BIPHENYL METABOLITES IN ALBATROSSES FROM MIDWAY ATOLL, NORTH PACIFIC OCEAN

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Abstract—Concentrations of hydroxylated metabolites of polychlorinated biphenyls (PCBs) (OH-PCBs) and methylsulfonyl metabolites of PCBs ($MeSO_2$-PCBs) were determined in plasma and liver of albatrosses collected from the Midway Atoll in the central North Pacific Ocean. The mean total concentrations of OH-PCBs in plasma of Laysan albatrosses (*Diomedea immutabilis*) and black-footed albatrosses (*Diomedea nigripes*) were 11.5 and 27.1 ng/g wet weight, respectively. Total concentrations of OH-PCBs were only one- to fivefold less than those of total PCBs. 4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorinated biphenyl and 4-hydroxy-2,2′,3,4′,5,5′-hexachlorinated biphenyl were the predominant polychlorinated biphenylols, constituting 70 to 90% of the total OH-PCBs. Concentrations of $MeSO_2$-PCBs in liver were between 10.6 and 77 ng/g, lipid weight, approximately 250 times less than those of total PCBs. The $MeSO_2$-PCBs congeners retained in the liver were dominated by those having the methylsulfonyl group in the 3-position.

Keywords—Methyl sulfones Dichlorodiphenyldichloroethylene methyl sulfone Hydroxy-polychlorinated biphenyls Blood plasma Polychlorinated biphenyl metabolites

INTRODUCTION

Polychlorinated biphenyls (PCBs), once produced for applications such as flame resistant oils in transformers and capacitors, as heat transfer medium, and as plasticizers in paint and sealants, are among the most widespread environmental contaminants known. As members of the group of halogenated aromatic hydrocarbons, PCBs have received much attention over the last 25 years because of their great potential for bioaccumulation and biological effects [1–3]. Although ample evidence suggests toxicity of the parent compounds, information regarding the toxicity of PCB metabolites is scarce. In animals, metabolism of the individual components of PCBs proceeds via CYP450-mediated formation of arene oxide intermediates, which results in both hydroxylated products [2,4], and mercapturic acid pathway (MAP) metabolites including products after C-S lyase cleavage of the alkyl sulfur carbon bond in the cysteinyl moiety, methylation to PCB methyl sulfides, and oxidation to PCB methyl sulfoxides and finally sulfones [2,5,6].

Hydroxylated metabolites of PCBs were reported more than 20 years ago from experimental work with individual PCB congeners (for review see [7]) and also as found naturally in excreta of gray seal and common murre [8]. Polar hydroxylated PCB metabolites have been detected in excreta as free polychlorinated biphenylols or conjugated to sulfate or glucuronic acid [7]. Hydroxy-PCBs (OH-PCBs) have been found in the intraluminal uterine fluid of pregnant mice dosed with 2,4′,5,-trichlorobiphenyl (chlorobiphenyl [CB]-31) [9]. Recently, OH-PCBs have been identified and quantified in blood from wildlife and humans [10,11]. Concentrations of OH-PCBs in human and animal blood plasma have been found to be only slightly less than the total PCB concentrations [10]. Hydroxy-PCBs found in blood have two structural elements in common: either a 4-hydroxy-3,5-dichlorophenyl ring (or more chlorine atoms in the ring) or a 3-hydroxy-2,4-dichlorophenyl ring (or more chlorine atoms in the ring) and chlorine atoms in at least 3- and 4-positions in the other phenyl ring. Hydroxy-PCBs are thus, in part, structurally similar to thyroxine and several OH-PCBs can compete with thyroxine for a binding site on the transport protein, transthyretin (TTR) [12]. Hydroxy-PCB congeners determined in gray seal and human blood are therefore most probably bound to TTR [12]. The toxicologic relevance of the observed binding of OH-PCBs to TTR is still unknown.

Polychlorinated biphenyls are also metabolized to methylsulfonyl-substituted compounds, neutral metabolites of somewhat lesser hydrophobicity than their parent compounds. Methylsulfonyl-PCBs ($MeSO_2$-PCBs) are readily accumulated in adipose tissue of mammals [13–15] and birds [16]. On average, 20 to 30 different $MeSO_2$-PCBs occur in mammals, originating from PCB congeners with at least one phenyl ring substituted with chlorine atoms in the 2,5-or 2,3,6-positions and with the other phenyl ring at least substituted in the 4-position [17]. In contrast to the OH-PCBs retained in blood, the $MeSO_2$-PCBs are mainly accumulated because of their

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great lipophilicity. However, $MeSO_2$-PCBs have also been found to bind noncovalently to proteins in lung and liver. Methysulfonyl-PCBs were first observed to have a strong affinity for a uteroglobinlike protein [18], today known as PCB-binding protein [19] but have also been shown to bind to fatty acid-binding protein [20]. The affinity for proteins results in $MeSO_2$-PCBs being retained with great selectivity in mammals. Depending on the species, $MeSO_2$-PCBs are found in lung bronchial mucosa [18,21], kidney cortex [21], in intraluminal uterine fluid, and in the liver [9,14]. The toxicologic potential of $MeSO_2$-PCBs is not known, but they have been linked to respiratory effects observed among Yusho victims [22] and more recently, some 3-$MeSO_2$-PCBs have been reported to strongly induce P450 enzymes [23–25]. In fact, some of these sulfones are up to three orders of magnitude more potent than their parent PCB congener. Unfortunately, no information is presently available for the toxicity of $MeSO_2$-PCBs in birds.

Biotransformation of PCBs in avian species occurs via CYP450-mediated insertion of a single molecule of oxygen [26–29]. The ability of birds to metabolize PCBs and the extent of CYP450 enzyme activities in bird tissues has been shown to be species-dependent [28,30,31]. The available evidence indicates that xenobiotic drug-metabolizing enzymes of procellariiform birds, including albatrosses, can be induced by exposure of these birds to halogenated aromatic hydrocarbons [32].

Because of the previously observed selective tissue retention of OH-PCBs in blood and 3-$MeSO_2$-PCB in liver, PCB and PCB metabolites were analyzed in albatross blood and liver samples. Hydroxylated PCBs were quantified in blood plasma in black-footed albatrosses (*Diomedea nigripes*) and Laysan albatrosses (*Diomedea immutabilis*). Polychlorinated biphenyl methyl sulfones were quantified in Laysan albatross livers. Total PCBs were quantified in all samples. The bird samples were collected from Midway Atoll, central North Pacific Ocean, to provide information on the metabolism of PCBs in albatrosses and to determine concentrations of PCBs and PCB metabolites in these birds. The aim of this study was to determine concentrations of PCBs and DDT (1,1,1-trichloro 1,1,1-trichloro 2,2′-bis-*p*-chlorophenyl ethane (DDT) metabolites in birds at a remote location that had no local sources of PCBs or DDT.

MATERIALS AND METHODS

Collection of samples

Livers were collected from 10 individual adult male Laysan albatross (≥11 years old) on Sand Island, Midway Atoll, in the central North Pacific Ocean (28°11′N, 177°22′W) during January and February 1994. Blood (~3 ml) was collected from both Laysan (n = 10) and black-footed (n = 10) albatrosses during April and May 1993. Blood was drawn from the brachial vein of each bird and placed into vacutainers containing 0.015 ml ethylenediaminetetraacetic acid (EDTA) anticoagulant and stored at −20°C until analyses.

Chemicals

2,3,3′,4,4′,5,5′-Heptachlorobiphenyl (CB-189) [33], 4-hydroxy-2,3,3′,4′,5,5′,6-heptachlorobiphenyl (4-OH-2,3,3′,4′,5,5′,-6-heptaCB) [34], and 4-methyl-3-methylsulfonyl-2′,3′,4′,5,5′-pentachlorobiphenyl [35] were used as internal standards in the analysis of PCBs, OH-PCBs, and aryl methyl sulfones, respectively. A commercial PCB product, Clophen A50, was used as a standard for quantification of PCBs in the blood and liver samples [36]. Methoxy-PCB standards synthesized as described elsewhere [10,34] were used for qualitative and quantitative determinations of this group of PCB metabolites. The PCB and dichlorodiphenyldichloroethylene (DDE) methyl sulfone congeners [35,37,38] were also used as standards in the present work.

All solvents used were either of pro-analysis or pesticide-grade quality. Diazomethane was synthesized according to the procedure by Fieser and Fieser [39]. Other chemicals and materials are as described previously [10,40].

Instruments

Gas chromatography was performed on a Varian GC 3400 (Varian Associates, Palo Alto, CA, USA), equipped with an electron capture detector kept at 360°C, and a fused silica capillary column (XTI-5, 30 m × 0.25-mm inner diameter; 0.25-μm film thickness, Resteck, Bellafonte, PA, USA). Injections were made in the splitless mode at a temperature of 280°C with a Varian 8200 autosampler. The carrier gas was hydrogen and the make up gas was nitrogen. For PCBs and OH-PCBs, the oven temperature was programmed as follows: 80°C for 2 min, a linear increase of 10°C/min up to 300°C, where the temperature was held for 10 min. For analysis of $MeSO_2$-PCBs, the temperature was kept at 80°C for 2 min, the temperature linearly increased by 20°C/min up to 230°C and then by 3°C/min to 300°C, where it was held for 10 min. Data collection and processing were made with a lab data system ELDS-Pro (Chromatography data system AB, Svartsjö, Sweden).

Extraction and clean-up of plasma

Internal standards, CB-189, and 4-OH-2,3,3′,4′,5,5′,6-heptaCB were added to the plasma prior to extraction. The extraction and clean-up procedures are described elsewhere [10]. Briefly, plasma proteins were denaturated by methanol and hydrochloric acid, organic compounds were extracted with hexane:methyl *tert*-butyl ether. The extract was partitioned between potassium hydroxide and organic solvent to separate phenolic metabolites from neutral compounds. After acidification and reextraction, the phenolic compounds were derivatized with diazomethane. Lipids, both in the neutral and the phenolic extract, were removed by partitioning with concentrated sulfuric acid. The neutral compounds were further purified on a column with silica:sulfuric acid (2:1, 1 g) and hexane (13 ml) as mobile phase.

Extraction and clean-up of liver

Samples of Laysan albatross liver samples were extracted as previously described [40]. 2,3,3′,4,4′,5,5′-Heptachlorobiphenyl and 4-methyl-3-methylsulfonyl-2′,3′,4′,5,5′-pentachlorobiphenyl were added as internal standards. The extracts were partitioned between hexane and dimethylsulfoxide to separate PCBs from $MeSO_2$-PCBs. Lipids were removed as described elsewhere [40].

Analysis

Identification and quantification of OH-PCBs, as methyl derivatives, and $MeSO_2$-PCBs was based on comparison to authentic standards. Concentrations of individually resolved peaks of PCB congeners were summed to estimate a total PCB concentration. Clophen A50 of known mole fraction of chlo-

Table 1. Concentrations (ng/g, wet wt.) of *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), total polychlorinated biphenyls (PCBs), and hydroxylated PCBs (OH-PCBs) in adult albatross blood plasma

Compound	Laysan albatross (n = 5)				Black-footed albatross (n = 5)			
	Median	Mean	SD	Range	Median	Mean	SD	Range
p,p'-DDE	2.6	2.6	0.38	2.0–3.0	7.6	9.0	5.2	4.8–18
PCBs[a]	18.1	18.3	3.37	13.7–22.3	65.5	83.8	45.9	42–160
OH-PCBs	10.8	11.5	6.38	5.9–18.5	25.9	27.1	5.65	20–33
OH-PCB to PCB ratio	0.53	0.57	0.27	0.33–0.89	0.33	0.39	0.22	0.21–0.78

[a] Sum of chlorinated biphenyls 118, 153, 105/132, 138, 183, 128/167, 156/171, 180, and 170.

robiphenyl content was used as a standard to quantify individual CBs [36]. Hydroxy- and $MeSO_2$-PCBs were quantified from individually resolved peak areas with the corresponding peak area of authentic standards. The quantifications were corrected for internal standard recoveries.

RESULTS AND DISCUSSION

Mean concentrations of total PCBs in the blood plasma of Laysan and black-footed albatrosses were 18.3 ng/g, wet weight (13.7–22.3 ng/g) and 83.8 ng/g, wet weight (42–160 ng/g), respectively (Table 1). Concentrations of PCBs and DDE in plasma of black-footed albatrosses were approximately fourfold greater than in Laysan albatrosses but the PCB to DDE ratio was similar for both albatross species, which indicates that both species are exposed from a similar source of DDE and PCBs, but at a different magnitude. The greater concentration of PCBs and DDE observed in the black-footed albatross than in the Laysan albatross is similar to the trends in concentrations observed for organochlorine pesticides, polychlorodibenzo-*p*-dioxins, and polychlorinated dibenzofurans [40,41]. This phenomenon can be explained by the different ecological niches and diets of the two species [41]. The diet of the Laysan albatross consists primarily of ommastrephid squids (~68% of the diet) whereas the black-footed albatross feed mostly on flying fish (50%) and squids constitute a relatively lesser proportion (32%) of the diet. Details regarding the sources of environmental contaminants for these two species of albatrosses have been reported previously [41,42].

About 90% of the total mass of PCBs in albatross plasma was due to 12 individual PCB congeners (Table 2). The congener CB-153 was the most abundant PCB congener, comprising 26% of the total PCBs, followed by CB-128, and notably CB-118 was the third most abundant PCB congener in both Laysan and black-footed albatrosses. Thus, if similar toxic responses occur in albatrosses as in mammals it cannot be excluded that CB-118 may give a major contribution to the dioxin-type toxicity in the birds. The relatively small concentrations of less chlorinated PCB congeners in the birds indicate uptake of weathered PCBs or rapid metabolism of low-chlorinated PCBs. No significant differences in the composition of the PCB congeners were observed between the two albatross species. The sample number was too small to permit a robust statistical evaluation of the results.

Hydroxy-PCBs were detected in all the plasma samples analyzed. Concentrations of OH-PCBs, corresponding to authentic reference compounds, ranged between 5.9 and 18.5 ng/g, wet weight, in Laysan albatrosses and between 20 and 33 ng/g, wet weight, in black-footed albatrosses (Table 1). As observed for total PCBs, concentrations of total OH-PCBs were two- to threefold greater in black-footed albatrosses (mean: 11.5 ng/g, wet weight) than in Laysan albatrosses (mean: 27.1 ng/g, wet weight). Generally, the total OH-PCB concentrations were about 50% of the concentrations of PCBs. The ratios of one of the major OH-PCB congeners, 4-OH-2,2',3,4',5,5',6-heptaCB, to CB-153 was 0.3 to 1.1 for the Laysan albatross blood samples and 0.4 to 1.1 for the black-footed albatross samples.

Among several congeners detected in plasma, 4-OH-2,2',3,4',5,5',6-heptaCB and 4-OH-2,2',3,4',5,5'-hexaCB accounted for the greatest mass, 70 to 90% of the total OH-PCBs in the plasma. These two OH-PCBs were also major OH-PCBs in human blood [10]. The parent PCB congeners for these two

Table 2. Concentrations (ng/g, wet wt.) of polychlorinated biphenyl congeners in plasma of Laysan and black-footed albatrosses

Sample	Chlorobiphenyl									Total
	118	153	105/132	138	183	128/167	156/171	180	170	
Laysan albatross										
LA1	2.9	6.0	0.6	0.9	0.5	0.7	1.6	1.5	1.7	16
LA2	4.2	6.9	0.8	1.9	0.6	1.4	1.7	1.6	1.2	20
LA3	4.2	7.8	0.7	1.8	0.5	1.4	1.7	1.8	1.3	21
LA4	3.0	5.2	0.7	1.2	0.4	1.0	1.6	1.2	1.1	15
LA5	3.0	4.8	0.6	1.2	0.4	1.1	1.4	1.1	0.9	15
Mean	3.5	6.1	0.7	1.4	0.5	1.1	1.6	1.4	1.3	17.6
Black-footed albatross										
BF1	12.9	35.9	2.5	6.7	3.0	5.6	4.5	10	5.5	89
BF2	11.4	22.2	2.4	6.5	2.2	4.2	4.1	5.5	3.8	62
BF3	13.8	34.8	2.7	7.8	2.7	5.7	4.8	9.1	5.3	87
BF4	35.5	66.9	6.6	19.2	4.9	12.6	10.2	14.7	9.3	180
BF5	8.1	16.7	1.6	3.9	1.6	3.1	3.0	4.4	2.8	45
Mean	16.3	35.3	3.2	9.2	2.9	6.2	5.3	8.7	5.3	92.5

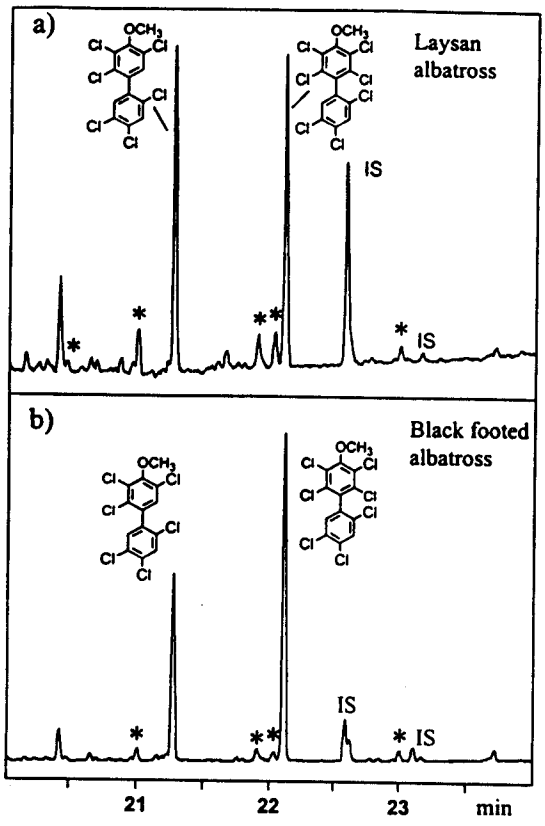

Fig. 1. Gas chromatograms of methylated hydroxy-polychlorinated biphenyls (OH-PCBs) in blood plasma from Laysan albatrosses (**a**) and black-footed albatrosses (**b**). The structures of the two major OH-PCB congeners (as methyl derivatives) are shown. Minor OH-PCBs are indicated by an asterisk. IS = internal standard.

OH-PCBs are most likely CB-180/CB-187 and CB-138/CB153, respectively.

The relatively great concentrations of OH-PCBs observed in the albatross plasma and the limited number of OH-PCB congeners (Fig. 1a and b), indicate that these metabolites are selectively retained. The albatrosses were found to strongly retain primarily two OH-PCBs, a situation different from the mammalian species studied so far. However, the number of studies to which our data can be compared are too few to draw any conclusions about the interspecies differences in patterns. The retention of OH-PCB congeners may be influenced by the exposure profile of PCBs, metabolism rate, and protein binding specificity. Further work needs to be done to verify specificity and concentration levels of the OH-PCBs in albatrosses and in other bird species at higher trophic levels.

Concentrations of total PCBs in the liver of Laysan albatrosses ranged between 1.6 and 8.2 μg/g, lipid weight (mean: 4.3 μg/g) (Table 3). In plasma, CB-153 was the most prominent PCB congener, accounting for 30% of the total PCB concentration, followed by CB-118, CB-138, and CB-180, accounting for 17, 6.5, and 5% of the total PCBs, respectively (Table 4). The presence of greater concentrations of CB-153 than other PCB congeners has been shown in several avian species [28]. Chlorobiphenyl-153 does not contain adjacent unsubstituted carbon atoms susceptible to metabolic reactions and therefore has a long half-life in biota.

Table 4. Concentrations (μg/g, lipid wt.) (n = 10) of polychlorinated biphenyl congeners in livers of adult male Laysan albatrosses

Chlorobiphenyl	Concentration[a]
CB-118	0.76 (0.27–1.5)
CB-153	1.3 (0.44–2.4)
CB-105,132	0.17 (0.06–0.33)
CB-138	0.29 (0.1–0.57)
CB-183	0.09 (0.03–0.18)
CB-128,167	0.24 (0.08–0.45)
CB-156,171	0.18 (0.08–0.35)
CB-180	0.25 (0.11–0.63)
CB-170	0.20 (0.09–0.44)

[a] Values in parentheses indicate range.

Concentrations of $MeSO_2$-PCBs, corresponding to authentic reference compounds, in Laysan albatross livers were between 10.6 and 77.4 ng/g, lipid weight (mean: 23.7 ng/g, median: 13.5 ng/g), with a PCB to $MeSO_2$-PCB ratio of 254 (SD ± 170) (median 231), with one sample excluded (ratio 1,235) in the statistical calculation because it was very different from all other samples (Table 3). Concentrations of $MeSO_2$-PCBs in the livers of albatrosses were 25 to 1,000 times less than those reported for polar bear, beluga whale, gray seal, otter, or wild mink from Canada or Sweden [10]. No consistent correlation was observed between the liver concentrations of PCBs and $MeSO_2$-PCBs, probably because the PCB congeners present were the persistent congeners, whereas the sulfones were formed from the more readily metabolized PCB congeners. Furthermore, only a few of the PCB methyl sulfone metabolites formed were selectively retained in the albatross livers.

Concentrations of PCBs and $MeSO_2$-PCBs in other bird species have occasionally been determined [16]. White-tailed eagle (*Haliaeetus albicilla*), Eurasian eagle-owl (*Bubo bubo*), and common murre (*Uria aalge*) have been reported to have $MeSO_2$-PCB concentrations in muscle of 70, 140, and 1,400 ng/g lipid weight, respectively [16]. The PCB to $MeSO_2$-PCB ratios were in these cases shown to be between 1,000 and 3,000, similar to what has been found in white-tailed eagle eggs (A. Olsson, personal communication). Birds seem to be able to metabolize PCBs to $MeSO_2$-PCB, but according to the results obtained so far the rate of formation is less in birds than in mammals or possibly, the excretion rates of $MeSO_2$-

Table 3. Concentrations of methylsulfonyl-*p,p'*-dichlorodiphenyldichloroethylene ($MeSO_2$-*p,p'*-DDE) (ng/g, lipid wt.), total polychlorinated biphenyls (PCBs) (ng/g, lipid wt.), and methylsulfonyl-PCBs (ng/g, lipid wt.) in livers of Laysan albatrosses (n = 9; outlier removed)

Compound	Median	Mean	SD	Range
$MeSO_2$-*p,p'*-DDE	6.0	9.74	9.23	4.12–33.4
PCBs[a]	3,340	4,250	2,270	1,580–8,180
$MeSO_2$-PCBs	13.5	23.7	22.6	10.6–77.4
PCB to $MeSO_2$-PCB ratio	231	254	170	56–388

[a] Sum of chlorinated biphenyls 118, 153, 105/132, 138, 183, 128/167, 156/171, 180, and 170.

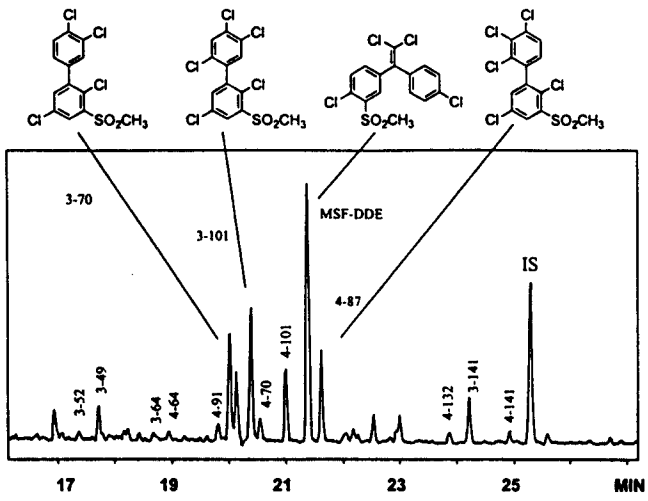

Fig. 2. Gas chromatogram of methylsulfonyl-polychlorinated biphenyls ($MeSO_2$-PCBs) in a representative liver sample from a Laysan albatross. The three major PCB methyl sulfones are indicated by their structures. An additional number of $MeSO_2$-PCBs are indicated by their numbers (e.g., 3-52 = 3-$MeSO_2$-2,2′5,5′-tetrachlorinated biphenyl [CB]). IS = internal standard.

PCBs are greater in birds than in mammals. Experimental studies are needed to understand the mechanisms of formation, retention, and excretion of $MeSO_2$-PCBs in birds.

The dominating individual aryl methyl sulfone identified in samples of liver from Laysan albatross was 3-$MeSO_2$-*p,p′*-DDE (Table 3). Concentrations of 3-$MeSO_2$-DDE in livers from polar bear, otter, and mink have been reported to be greater than in their blubber (on a lipid weight basis) [14]. The importance of 3-$MeSO_2$-DDE in birds is unknown but in mammals (e.g., the mouse), this compound is known to bind covalently to macromolecules in the adrenal cortex and to be strongly toxic in the zona fasciculata of this organ [43]. Further experimental data are needed for 3-$MeSO_2$-DDE in birds to determine proper risk assessments.

Up to 14 individual $MeSO_2$-PCB congeners were identified in samples of albatross liver. These compounds could be divided into six pairs of 3- and 4-$MeSO_2$-PCBs with the same parent PCB congener (Fig. 2). The original PCBs for these PCB methyl sulfones were CB-49, CB-64, CB-70, CB-87, CB-101, and CB-141. Among these, the major $MeSO_2$-PCB congeners were 3-$MeSO_2$-CB-70, 3-$MeSO_2$-CB-87, 3-$MeSO_2$-CB-101, and 4-$MeSO_2$-CB-101; the latter three metabolites were also found to be retained in liver from wild mink, gray seal, otter, and polar bear [14]. It can be speculated that a similar retention specificity is operating in the Laysan albatross as in the mammals mentioned above. So far no protein or other receptor has been identified in liver tissue that can explain this type of $MeSO_2$-PCB congener retention. Nevertheless, it is now important to determine the mechanism of this binding, particularly because 3-$MeSO_2$-CB-87 and 3-$MeSO_2$-CB-101 have been reported to have strong CYP2B-inducing potency [25].

In general, metabolites of PCBs are considered to be less toxic than their parent compounds [44]. However, hydroxylated metabolites of 3,3′,4,4′-tetraCB have a marked structural resemblance to thyroxine, the natural ligand for TTR and, therefore, competitively bind to TTR and can cause reductions in plasma tetraiodothyroxine (T_4) levels and serum transport of vitamin A in rodents [12,45]. Hydroxylated metabolites of PCBs have been shown in vitro to have binding affinities that are 10 times greater for TTR than for T_4 [12]. This results in persistent retention of these metabolites in blood of both humans and seals exposed environmentally to PCBs [10].

This study indicates that albatrosses metabolize PCB congeners to hydroxylated and methyl sulfone metabolites. Information pertaining to the presence of PCB metabolites in biota, including birds, is meagre to make comparisons and to evaluate risks. Toxicologic implications of the presence of detectable concentrations of PCB metabolites in albatrosses are not known. It is hypothesized that some PCB-induced toxic responses in biota may be due to the interaction of PCB metabolites with TTR or other endogenous proteins. For example, toxic symptoms resulting from the imbalance in the synthesis and regulation of thyroid hormones in PCB-exposed birds [46,47] may be caused by hydroxylated PCB metabolites, due to competitive binding to T_4-carrier proteins. Herring gull (*Larus argentatus*) populations in some locations in the lower Laurentian Great Lakes have displayed evidence of thyroid dysfunction related to halogenated hydrocarbon exposure [48]. A significant negative correlation between plasma free T_4 levels and PCB concentrations in yolk was reported in great cormorant (*Phalacrocorax carbo*) hatchlings from the Netherlands [49]. Further studies are needed to understand the toxicologic implications of PCB metabolites in biota.

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