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Polychlorinated biphenyl (PCB) and dichlorodiphenylethane (DDE) methyl sulfone (MSF) metabolites possess high affinities for binding two homologous 16,000 Da homodimeric receptor proteins in the lung (Clara cell secretory protein, CCSP) and the uterus (uteroglobin, UG), leading to selective bioaccumulation of MSFs in these tissues. As marine mammals are highly exposed to organochlorines, concentrations of PCBs,

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*PCB MSFs, DDT, and DDE MSF were analyzed in blubber, lung, and uterus samples from harbor seal (*Phoca vitulina*) and striped dolphin (*Stenella coeruleoalba*) morbillivirus epizootic victims to investigate uterine and lung MSF accumulation. Mean uterus concentrations of PCB MSFs and DDE MSF in harbor seals were 0.61 and 0.04 μg/g lipid weight and in striped dolphins 0.05 and 0.01 μg/g lipid weight. Mean lung concentrations of PCB MSFs and DDE MSF in harbor seals were 0.96 and 0.02 μg/g lipid weight and in striped dolphins 0.16 and 0.01 μg/g lipid weight. To ascertain whether uterine and lung bioaccumulation of MSFs is possible due to the presence of CCSP and UG in seals, CCSP and UG proteins in uterine flushings and in uterine and lung and epithelial tissue from Baltic gray and ringed seals were characterized using gel electrophoresis and Western blotting techniques. UG- and CCSP-like proteins with molecular weights of 16,000 Da were resolved in all samples. This is the first demonstration of this protein in any marine mammalian species. The toxicological implications of MSF binding with UG and CCSP in marine mammals are discussed.*

Polychlorinated biphenyls (PCBs) and DDT are nonpolar, highly lipophilic and persistent ubiquitous environmental organochlorine pollutants. Methyl sulfones (MSFs) are the stable metabolites with intermediate lipophilicity. DDE (primary DDT metabolite) and PCB congeners with 2,5-dichloro or 2,3,6-trichloro substitution are converted into 3- and 4-MSF metabolites by enterohepatic metabolism following phase III detoxification (Letcher et al., 2000). The number and relative proportion of MSF metabolites in marine mammal tissues varies between and within species due to inter- and intra-specific variation in hepatic detoxification capacity (Troisi et al., 1996). Marine mammals are apex predators and thus are exposed to biomagnified concentrations of PCBs and DDT in their diet (Simmonds & Hutchinson, 1994; Colborn & Smolen, 1996). Consequently, MSFs are also ubiquitous contaminants present in marine mammals. They are found bioaccumulated in both lipid-rich tissues such as blubber, due to the lipophilic nonpolar biphenyl skeleton, and in nonlipid rich tissues such as liver, due to the ability of the polar sulfone group to bind proteins (Letcher et al., 2000).

In laboratory animals MSFs bind two homologous (16 kD) homodimeric intracellular proteins (Miele et al., 1987), Clara-cell secretory protein (CCSP) in the lung and uteroglobin (UG) in the uterus, leading to selective bioaccumulation of MSFs in these tissues (Brandt & Bergman, 1987; Stripp et al., 1996). CCSP is secreted by Clara cells of nonciliated bronchial epithelium under the regulation of glucocorticoid steroids. Following pathogenic infection or injury in the lung, CCSP suppresses inflammation by sequestering phopholipase A_2 (PLA_2), which signals inflammatory response and also acts as an immunomodulator (Miele et al., 1987; Muckerjee et al., 1999). UG is a progesterone receptor secreted by epithelial cells under the control of progesterone and estrogen in the preimplantation uterus. This protein functions to ensure successful attachment of the embryo in the preimplantation uterus (Miele et al., 1987; Muckerjee et al., 1999). In vitro studies have shown that MSFs can bind CCSP and UG, leading to displacement of endogenous ligands and impairment of protein function (Lund et al., 1985; Gillner et al., 1988).

Environmental exposure to PCBs and DDT has been shown to cause immunosuppression, reproductive failure, and endocrine disruption in marine mammals (Reijnders, 1986; de Swart et al., 1994; Lahvis et al., 1995; Colborn & Smolen, 1996). Population-level effects have been reported as a result of organochlorine exposure. For example, during the 1988 phocine distemper virus (PDV) epizootic in northern Europe over 18,000 seals died, with highest mortality reported in populations from polluted waters, where elevated PCB and DDT burdens compromised immune function (Olsson et al., 1994). Similarly, reduced reproductive output in Wadden Sea seal populations has been associated with high organochlorine burdens (Reijnders, 1986). Considering the capacity of MSFs for CCSP/UG binding, it is plausible that the mechanisms for these toxic effects more likely involve the MSF metabolites rather than the parent molecules. Due to the toxicological importance of MSFs, it was of interest to determine blubber, lung, and uterus PCB and DDE MSFs concentrations in seal and dolphin morbillivirus epizootic victims from populations inhabiting polluted areas. The presence of UG- and CCSP-like proteins in seal lung and uterus was also investigated, since to date these proteins have so far only been characterized in laboratory animals and humans.

METHODS

For contaminant analysis, 2–15 g of blubber, liver, lung, and uterus was sampled from 10 Schleswig-Holstein (Germany) harbor seal (*Phoca vitulina*) victims of the 1988 PDV epizootic and from 12 West Mediterranean striped dolphin (*Stenella coeruleoalba)* victims of the 1990–1992 dolphin morbillivirus (DMV) epizootic. Samples were stored in hexane-washed foil at –20°C until analysis. Tissue lipid content and the concentrations of 13 PCB MSF congeners (PCB-CH_3SO_2), DDE MSF (3-DDE-CH_3SO_2), 20 PCB congeners, and DDT (*p,p*-DDD, *o,p*-DDT, and *p,p*-DDE) were determined according to published methodology (Troisi et al., 1998). To each sample extract, 960 ng of the internal standard 3-CH_3SO_2-4-CH_3-2′,3′,4′,5,5′-pentachlorobiphenyl was added to monitor recovery. Reference standards were obtained from Supelco and Cambridge Isotope Laboratories (U.S). Recovery of MSFs, PCBs and DDT was 80–95%. The limit of detection was 0.01 μg/g.

For characterization of UG/CCSP, uterine flushings (taken with 25 ml phosphate buffer, pH 7.4) and 5–15 g of uterine and bronchial epithelium samples from adult female Baltic gray (*Halichoerus grypus*) and ringed (*Phoca hispida*) seals were donated by the Finnish Game and Fisheries Research Institute (Helsinki, Finland) from its annual research expedition in the Bothnian Bay region of the Baltic. Unfortunately, it was not possible to obtain similar fresh tissue samples from cetacean species for this study. All samples were stored at –80°C until use. Tissue samples were homogenized in 4 volumes of Tris-HCl buffer (pH 7.4) on ice, using a blender and Potter homogenizer. Tissue homogenates and uterine flushings

were all centrifuged at 105,000 × g to obtain the soluble protein fraction. Protein concentrations were determined using the Lowry assay (Lowry et al., 1951). Samples, human urine protein-1 reference standard containing >70% UG/CCSP protein (UP-1; Scipac, UK), and sodium dodecyl sulfate (SDS) molecular weight markers (Sigma) were separated under nonreducing conditions by gel electrophoresis and Western blotted on nitrocellulose with polyclonal rabbit anti-human UP-1 antibody (Dako). Two-tailed Student *t*-tests were carried out using the data to determine whether any differences in tissue/species MSF burdens were statistically significant.

RESULTS AND DISCUSSION

As found in several other studies (Letcher et al., 2000), concentrations of parent compounds were greater than MSF metabolites for all tissues in harbor seal and striped dolphin (Table 1). Mean ΣPCB and ΣPCB-MSF concentration in blubber of Schleswig-Holstein harbor seals, were in the same range as those detected in other harbor seal PDV epizootic victims from the Swedish Coast (Haraguchi et al., 1992). Mean ΣPCB, ΣPCB-MSF, DDE MSF, and DDT concentrations in striped dolphin blubber and liver were in the same range as those reported by other studies of epizootic victims from the Mediterranean population (Kannan et al., 1993; Troisi et al., 1998). No comparison of uterine and lung contaminant levels could be made due to the lack of comparative studies.

TABLE 1. Concentrations and Ranges of PCB, DDT, and MSFs in Seal and Dolphin

		Concentration (μg/g lipid weight)			
Tissue	HEL (%)	ΣPCB	ΣPCB – MSF	ΣDDT	DDE – MSF
		Harbor seals (n = 10)			
Blubber	60.12 ± 11.4	18.91 ± 3.85	1.24 ± 0.53	1.78 ± 0.4	0.04 ± 0.01
Range	6.33 – 80.1	6.4 – 38.8	0.21 – 4.32	0.82 – 3.87	0 – 0.06
Lung	1.52 ± 0.47	21.89 ± 5.67	0.96 ± 0.35	1.13 ± 0.25	0.02 ± 0.01
Range	0.36 – 4.51	10.7 – 60	0.19 – 3.3	0.4 – 2.38	0 – 0.06
Uterus	0.75 ± 0.08	17.17 ± 3.82	0.61 ± 0.17	1.60 ± 0.56	0.04 ± 0.01
Range	0.43 – 0.97	8.5 – 34.6	0.15 – 1.38	0.79 – 4.38	0.01 – 0.06
		Striped dolphins (n = 12)			
Blubber	40.83 ± 4.93	75.11 ± 14.06	0.52 ± 0.2	61.10 ± 12.26	0.06 ± 0.01
Range	24.7 – 58	34.6 – 142.8	0.2 – 1.63	22.8 – 94.9	0.03 – 0.11
Uterus	0.42 ± 0.11	21.07 ± 6.7	0.05 ± 0.02	9.71 ± 2.89	0.01 ± 0.001
Range	0.11 – 0.82	6.2 – 41.9	0.01 – 0.1	3.78 – 19.7	0 – 0.01
Lung	0.78 ± 0.001	24.71 ± 5.21	0.16 ± 0.01	15.07 ± 3.5	0.01 ± 0.001
Range	0.54 – 0.95	7.2 – 51	0.01 – 0.42	3.74 – 27.1	0 – 0.04

Note. Data are mean ± SE. HEL (%), percent of hexane-extractable lipid in sample; ΣPCB, sum of 20 PCB congeners; ΣPCB MSF, sum of 13 PCB MSF congeners; ΣDDT, sum of *o,p*-DDT, *p,p*-DDE, and *p,p*-DDD.

Blubber concentrations of PCBs and DDT were significantly greater in striped dolphins compared with harbor seals (Table 1). In contrast, there was no significant difference in blubber PCB MSF and DDE MSF concentrations between these species. However, this is probably an artifact of the lower percent lipid in striped dolphin blubber (41%) compared with seals (60%), resulting in concentration of lipophilic PCBs and DDT. Furthermore, this explains the significantly greater (three- to sixfold) levels of all the contaminants studied in blubber compared with uterus and lung in striped dolphin, which was not observed in the harbor seals. Another explanation for PCB MSF concentrations being significantly lower than PCBs in striped dolphins but similar in harbor seals is the lower capacity of cetaceans for cytochrome P-450-mediated metabolism and excretion of PCBs compared with pinnipeds (Boon et al., 1994; Troisi et al., 1998). Despite lower blubber concentrations, uterine levels of MSFs in harbor seals were also significantly higher than in striped dolphins, which may be suggestive of a greater potential for uterine MSF metabolite bioaccumulation in seals. However, further investigation is warranted since these observations may also be artifacts of low blubber lipid content in the striped dolphins.

In both harbor seal and striped dolphin tissues, PCB congeners 153, 138, 180, 187 and 170 were most strongly accumulated (>60% of total). These PCBs typically constitute the bulk of total PCB burdens, as congeners with *ortho* chlorine substitution are poorly metabolized by marine mammals due the absence/inactivity of hepatic cytochrome P-4502B isozymes (Boon et al., 1994). The PCB MSF pattern was similar for all tissues studied in harbor seal, with 3-101 and 4-101 congeners most strongly accumulated (29 and 21% of total) and to a lesser extent congeners 4-110 and 4-149 (10 and 7% of total). There was a similar PCB MSF pattern in the striped dolphin tissues studied again with 3-101 and 4-101 but also 3-49 and 3-87 being the most strongly accumulated congeners (24, 19, 20, and 17% of total). The accumulation of 3-49 and 3-87 in striped dolphins may be explained by a lower capacity for metabolism and excretion of these compounds compared with harbor seals (Boon et al., 1994; Troisi et al., 1998).

Gel electrophoresis and Western blotting highlighted 16,000-Da proteins in gray seal uterine flushing and ringed seal uterine and lung epithelial samples. Rabbit anti-human UP-1 antibody resolved proteins in all of the samples adjacent to the human UG standard, and these were concluded to be UG/CCSP-like proteins (Figure 1). Although the data suggest the presence of UG/CCSP like proteins in two species of seal, further confirmation of UG/CCSP characterization will be undertaken in the future using specific antibodies raised against seal UG/CCSP proteins. Preliminary binding studies with progesterone suggest that these seal proteins are functional and likely to play the same role in reproductive and immune function as that described for other mammalian species (Miele et al.,1987; Muckerjee et al., 1999).

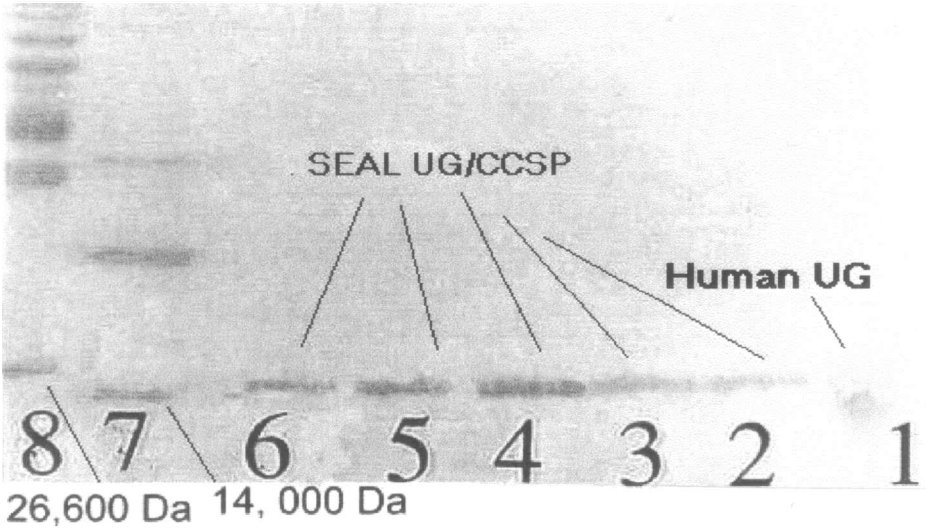

FIGURE 1. Western blot of uteroglobin and Clara-cell secretory protein in uterus, uterine flushings, and lung from gray and ringed seals. Each lane loaded with 5 µl sample (corrected to 20 µg protein), UG (UP-1) or molecular weight markers (14,200 to 66,000 Da) and 10 µl sample buffer (10% v/v glycerol, 10% v/v SDS, 0.2 µg /ml bromophenol blue, 1.25% v/v stacking buffer); lane 1, human UG (UP-1); lane 2, gray seal uterine flushing; lane 3, ringed seal uterine flushing; lane 4, ringed seal uterine epithelium; lane 5, ringed seal lung epithelium; lane 6, gray seal lung epithelium; lane 7, molecular weight markers; lane 8, molecular weight markers.

The toxicological implications of the bioaccumulation of MSFs in the uterus of seal are not known. Binding of progesterone to UG signals endometrial thickening and capillary formation (angiogenesis) vital for successful embryo attachment. This binding also protects the embryo from excessive progesterone. The availability of functional UG also functions to protect the embryo from the maternal immune response, such as uterine inflammation and maternal lymphocyte attack (Miele et al., 1987; Muckerjee et al., 1999). However, MSFs possess a binding affinity for UG that exceeds that for progesterone by orders of magnitude (Gillner et al., 1988).

It is possible, therefore, that MSFs can significantly impair UG function in the uterus, leading to implantation failure or abortion (Miele et al., 1987; Muckerjee et al., 1999). Reproductive failure following PCB and DDT exposure has already been reported in controlled feeding studies with captive harbor seals (Reijnders, 1986). Infertility has also been reported in seals due to the presence of uterine stenosis and occlusions in Liverpool Bay and Baltic populations, which were correlated with tissue PCB and DDT burdens (Helle, 1980; Baker, 1989). It is possible that MSF-associated UG dysfunction may be responsible for reprotoxic effects via implantation failure or embryo abortion. The subsequent accumulation of resorbed embryo tissue is thought to lead to the development of uterine occlusions and stenoses (Helle, 1980; Baker, 1989).

The toxicological implications of the bioaccumulation of MSFs in the bronchial epithelium of seals are also not known. Considering the demonstrated potential of PCB MSFs to bind CCSP, it is feasible that MSFs inhibit PLA_2 (Muckerjee et al., 1999). This would leave inflammation and modulation of phagocytosis as well as monocyte and neutrophil chemotaxis unchecked, thereby compromising lung host defense (Miele et al., 1987). For example, CCSP-deficient mice challenged with an adenovirus were found to show a marked increase in lung inflammation compared with normal mice (Harrod et al., 1998).

Due to the immunosuppressive nature of morbillivirus infection, the seals and dolphins in this study suffered secondary infections, particularly in the lung. Pathological examination of epizootic victims demonstrated a high incidence of pneumonia, alveolar atelectasis, alveolar collapse, and emphysema (Schumacher et al., 1990). Interestingly, similar observations were found in humans poisoned with PCB in the "Yusho disaster" (Shigematsu et al., 1978), and PCB MSFs levels in exposed humans correlated with severity of pulmonary effects (Haraguchi et al., 1986). It is therefore likely, not only that immune function in epizootic victims was compromised by immunotoxic PCBs (de Swart et al., 1994; Lahvis et al., 1995), but also that MSF metabolites bioaccumulated in lung tissue in association with CCSP may be involved in the toxicological phenomena.

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