PCBs and their Methylsulphonyl Metabolites in the Maternal Blubber, Milk, Pup Blubber and Faeces of Grey Seals

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Introduction

Female sea mammals transfer a large part of their organochlorine burden to their offspring during lactation. With the growing recognition that methylsulphonyl metabolites of PCBs constitute an abundant class of persistent organochlorine contaminants we analysed a series of samples of milk, adipose tissue and faeces from individual grey seals (*Halichoerus grypus*) to address the transfer of these compounds from mother to pup. PCBs were analysed in concert, including the precursor congeners for those metabolites identified. Both classes of compound were found in all samples. Σmsf-PCB ranged 57-84ng/g lipid, 49-116ng/g lipid and 65-110ng/g lipid in mother blubber, milk and pup blubber respectively. Ratios of Σmsf-PCB:ΣPCBs were ~1%, 4-10% and 4-9% for the same compartments.

Materials

We were interested in looking at the fate of PCBs and their persistent methylsulphonyl metabolites in the lactating mother seal in respect of their transfer to her pup.

Individual grey seals (*Halichoerus grypus*) on the Isle of May, Scotland (56°12'N, 2°32'W) were observed daily during the pupping season of 1993. Mothers were sedated with an intramuscular injection of Zoletil 100 (Vibrac UK Ltd.) to allow measurement of length, girth and weight, in addition to sampling of adipose by biopsy and milk. Let-down of milk was stimulated by an intravenous injection of oxytocin. Mothers were captured twice during the period of lactation, on each occasion their pups were weighed and if either animal defecated a sample of faeces was taken.

The weaning of grey seals is abrupt, when the mother abandons her pup after *circa* 19 days of milk feeding. Pups remain ashore alone for a further two weeks before venturing into the sea. On abandonment, each pup was restrained for 24 hours in a bar-floored cage and all urine and faeces was collected in a tray fitted beneath the cage. At the end of 24 hours the pup was weighed and a blubber biopsy taken. The procedure was repeated for each pup seven days later. All samples were frozen to -20°C on the day of sampling and remained at that temperature until being thawed prior to analysis.

Experimental

Milk samples were homogenised after thawing and a 1g subsample taken for analysis. The skin was removed from the adipose samples and the remainder used for analysis (0.1 - 0.7g). The entire samples of excrement were used. Samples were spiked with a solution containing PCBs #40, #155 and #185 and 3-methylsulphonyl-4-methyl-5,2',3',4',5'-pentachlorobiphenyl. Extraction was by blending with acetone/hexane followed by diethyl ether/hexane. The solvents were filtered, washed with a solution of sodium chloride in dilute phosphoric acid and dried. A portion was taken for lipid determination, and the remainder was washed with a mixture of aqueous KOH in ethanol before being concentrated and subjected to gel permeation chromatography. PCBs were separated from their methylsulphonyl metabolites by adsorption chromatography on 10g phosphoric acid impregnated silica

gel. The PCB fraction was further cleaned by elution through a column containing 3.0g alumina on 2.5g silica. The methylsulphone fraction was partitioned between hexane and DMSO, then back extracted into MTBE/hexane following dilution 1:1 with water. Final clean-up was by elution through 1g silica gel. PCBs were analysed by splitless injection onto a 50m DB-5 column in a Fisons MD8000 GC with MD800 MS detection using SIM. Methylsulphonyl-PCBs were analysed by on column injection onto a DB-5 column in a Carlo Erba GC with electron capture detection. Peak characterisation of msf-PCBs was confirmed by GCMS. Quantitation of msf-PCBs was confirmed by reanalysis on a DB 1701 column.

Results and discussion

The constraints inherent in working with live wild animals meant that the sampling regime could not be carried out to its optimum. The quality of some samples was therefore not ideal, the adult adipose tissue biopsies generally were small and so gave unfortunately high limits of detection. This did not impair the quality of those data that were obtainable however, and PCB results agreed well with similar samples from the same animals taken in previous years. The faecal samples gave considerable analytical problems and to date only one has yielded acceptable metabolite data and four have given acceptable PCB data.

The ranges of concentrations of PCBs and their methylsulphonyl metabolites found in the four types of sample analysed are summarised in table 1. The identity and relative concentrations of the methylsulphonyl PCBs in this study were similar to those found by Bergman *et al.* in grey seal blubber[1]. The ratios for ∑msf/∑PCB found here also echo those reported previously[2-4] which are generally quoted in the range 3-20% for grey seals. The low ratio exhibited by the adult adipose samples relative to the milk is indicative of the differences in the extent of transfer of the two classes of compound from the mothers' fat to their milk. Figure 1 shows the metabolite congener patterns for the same four sample types, normalised to 4-methylsulphonyl-2,2',3,4',5',6-hexachlorobiphenyl (4-149).

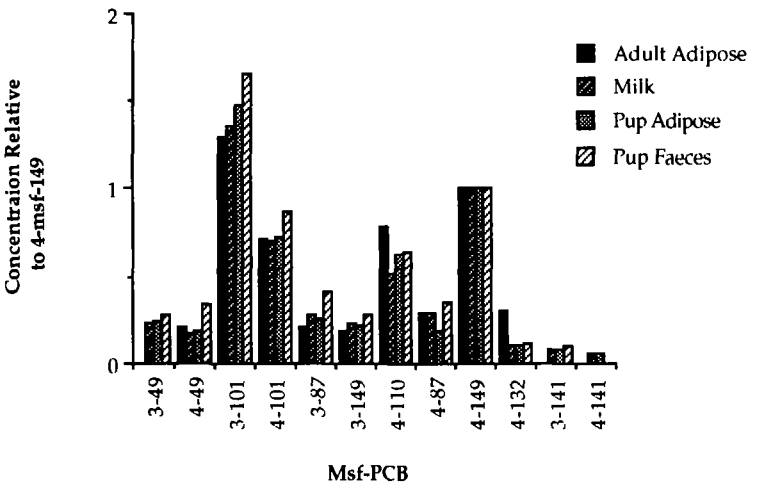
Table 1.
Ranges of sum PCBs and their methylsulphonyl metabolites in four types of grey seal sample. All milk and adipose values are ng/g lipid. The faeces sample was collected from a pup during the milk feeding period. Values for the faeces sample are in ng/g wet weight.

	Adult Adipose	Milk	Pup Adipose	Pup Faeces
∑msf-PCB	57 -84	49 -116	65 - 150	2.4
∑PCB	4000 - 9000	800 - 1300	900 - 1500	134
∑msf/∑PCB	*ca* 1%	4-10%	4-9%	*ca* 2%

It has previously been shown that there is a substantial barrier to the release of PCBs from a seal mother's fat and their incorporation into her milk (Addison and Brodie; Green et al). This barrier is selective, with more highly chlorinated congeners transferring less efficiently to milk. This was also observed in the current sample set. Figure 2 shows the ratios of individual PCB congeners in a milk sample to their concentrations in an adipose sample taken simultaneously from the same mother seal. The milk typically contains *ca* 20% ∑PCB of the adipose on a lipid weight basis. No such barrier is seen to hamper the movement of methylsulphonyl PCB metabolites. Milk lipid contains similar amounts of metabolites to that in the adipose lipid. Furthermore, no selectivity could be concluded for the transfer of metabolites to milk.

Uptake of both sets of compounds from milk by the pup is near quantitative. On average lipid concentrations increase by a factor of 1.2 - 1.4 from milk to pup adipose. This increase is a result of the pup's utilisation of some of its lipid to service its energetic requirements, leading to a concentration of the PCBs in the remaining lipid reserves. Transplacental transfer of contaminants may also account for some of this increase. No congener selectivity is observed for the uptake of msf-PCBs, nor is any selectivity of PCB ingestion discernible from an analysis of congener patterns in milk and pup adipose (fig. 3).

Figure 1.
Methylsulphonyl metabolite congener pattern for four sample types.



Uptake by the pup is not entirely complete, as evidenced by the presence of the compounds in their faeces. The faeces sample included in table 1 was collected from a pup during the period of milk feeding. The contaminants detected in it are therefore likely to represent those egested rather than the excretion of compounds previously ingested. The results obtained show that one gram of faeces contained one tenth of the amount of PCB-msf metabolites that were in one gram of this pup's milk. The pup received approximately 2kg milk per day and may be expected to have excreted of the order of 50-100g per day during the period of milk feeding. This suggests an uptake efficiency of >99.5%. Analysis of relative concentrations of individual metabolite congeners indicates that their uptake by the pup is non-selective.

By the same calculation the uptake of PCBs proved a little less efficient (*ca* 99%), resulting in the Σmsf/ΣPCB ratio in the faeces being 40% of the milk ratio. In contrast to the metabolites, the uptake of PCBs proved to be selective, with the more highly chlorinated congeners less easily absorbed than those of lower molecular weight (fig. 4). The apparent contradiction that the egestion of PCBs is selective whilst their ingestion is non-selective is explicable in terms of scale. 1% egested is twice 0.5% egested while 99% absorbed is indistinguishable from 99.5% absorbed.

Two 24 hour samples of combined urine and faeces collected from pups post-weaning showed no consistent differences in PCB congener pattern from that in the pup's adipose sampled at the end of the 24 hour period. The total PCB content of these samples was approximately equivalent to that in one gram of adipose from the respective pups. This is equivalent to *ca* 0.005% of the pup's total body burden of PCB. No data for the methylsulphonyl metabolites have yet been obtained from these samples.

Conclusion

The barrier to transfer of PCB congeners from a lactating grey seal's fat to her milk does not restrict the same passage of methylsulphonyl PCBs. The ingestion efficiency of both sets of compounds by pups from milk is almost quantitative. Neither egestion nor excretion of PCBs is significant to the lactational transfer of PCBs to the offspring of grey seals.

Figure 2.

Concentrations of individual PCB congeners in seal milk divided by their concentrations in the corresponding mother's adipose tissue.
The boxes group together the congeners into homologues, tetra- through octachlorination.

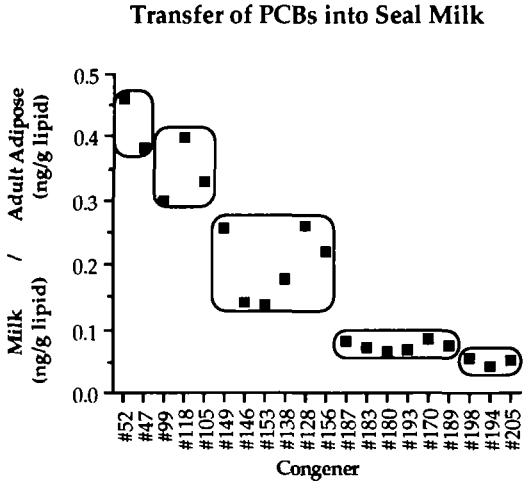


Figure 3.
Concentrations of individual PCB congeners in a seal pup's adipose divided by the concentrations found in the milk on which it fed.

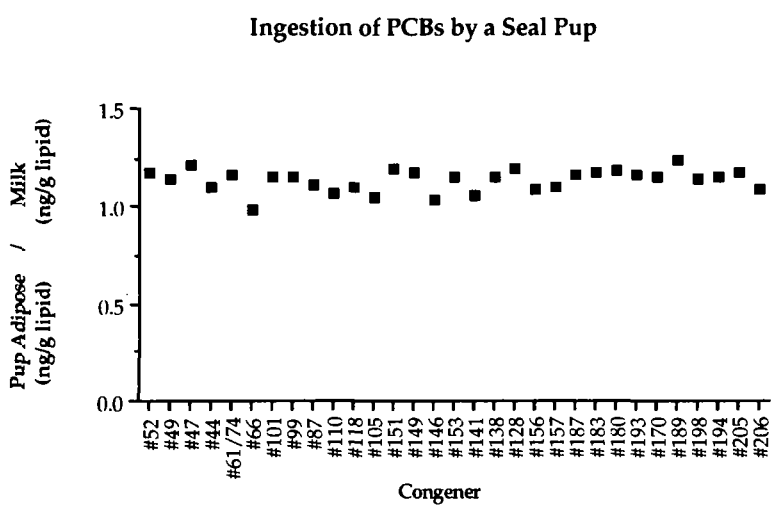
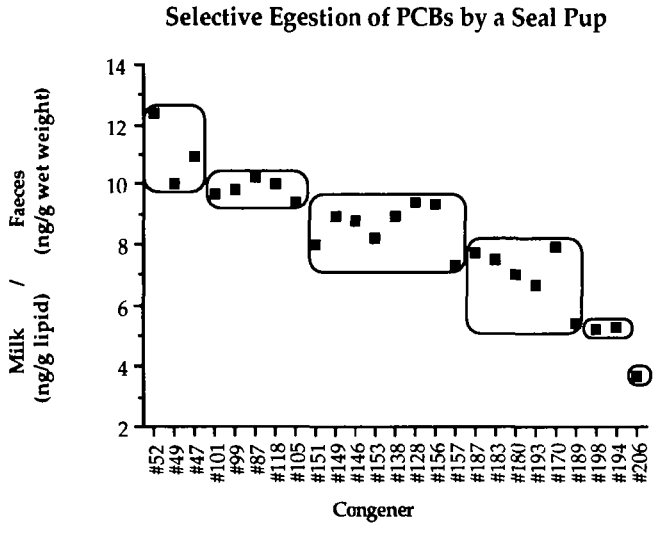


Figure 4.
Concentrations of PCB congeners in seal pup faeces divided by their concentrations in the pup's milk collected at the time of excretion. The boxes group together the congeners into homologues, tetra- through nonachlorination.



Acknowledgements
Field work, seal handling and sampling were organised by the UK NERC Sea Mammal Research Unit. Nicholas Green was funded by a short term fellowship from the European Environmental Research Organisation. The investigation of methylsulphonyl PCB compounds was funded in part by the Rijkswaterstaat, National Institute for Coastal and Marine Management, The Netherlands. Standard chemicals were a kind gift from Åke Bergman.

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