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Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*

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ABSTRACT: PCB contamination was measured in the milk and serum of grey seal *Halichoerus grypus* mothers and in the serum of their pups sampled from 2 to 5 times between parturition and weaning on the Isle of May, Scotland, in 1998 and 2000. Blubber biopsies were also taken from the lactating females at early and late lactation in 2000. Concentrations of PCBs in milk stayed constant during the first part of lactation (0.31 ± 0.17 µg g milk^{-1}) and then increased at late lactation (0.67 ± 0.42 µg g milk^{-1}). Curiously, it did not follow the changes of milk lipid content, which increased at early lactation and then stayed constant until the end of the nursing period. As a result, even when expressed per unit of milk lipids, PCBs underwent a rise at the end of lactation. The changes in milk PCBs were accompanied by similar dynamics in maternal serum as well as in pup serum. Increased concentrations of PCBs at late lactation in serum and milk may be explained in part by the changes observed in maternal blubber. PCB levels increased significantly between early and late lactation in inner blubber, suggesting that PCBs are less easily mobilised from blubber than lipids. At late lactation, the retention capacity of the reduced blubber layer for PCBs might have reached its maximum. The mobilization of less polar lipids from blubber might also occur at this stage. In both cases, this could result in a higher mobilization of PCBs at this time. While inner blubber was significantly less concentrated than outer blubber at early lactation (1.26 ± 0.72 µg g lipids^{-1} in inner blubber vs 3.16 ± 1.34 µg g lipids^{-1} in outer blubber), these variations disappeared at late lactation (3.24 ± 2.60 µg g lipids^{-1} in inner blubber vs 3.59 ± 1.46 µg g^{-1} lipids in outer blubber). Newborn pups already had significantly higher serum levels of PCBs than their mothers, revealing an important placental transfer (11.9 ± 7.0 ng ml serum^{-1} in pups vs 6.7 ± 3.5 ng ml serum^{-1} in mothers). These differences were even greater in late lactation, due to the ingestion of milk (27.9 ± 18.1 ng ml serum^{-1} in pups vs 12.2 ± 7.2 ng ml serum^{-1} in mothers). As lactation progressed, PCB levels in pup serum increased exponentially as compared to the levels in the serum of their mothers.

KEY WORDS: Grey seal · *Halichoerus grypus* · PCBs · Lactation · Milk · Serum · Blubber

INTRODUCTION

The oceans are threatened by human-generated over-fishing, noise and pollution. Persistent organochlorine pollutants such as polychlorinated biphenyls (PCBs) tend to accumulate up food chains in the marine ecosystems. Ocean and atmospheric currents have distributed these chemicals throughout the biosphere and they are encountered even in remote areas such as the Arctic (Muir et al. 1992). Because they are

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highly lipophilic and resistant to biodegradation, PCBs biomagnify in the fatty tissues of the marine fauna, their concentrations increasing with the trophic level (Tanabe et al. 1981, Muir et al. 1995, Mössner & Ballschmiter 1997, Bard 1999, Bang et al. 2001). Marine mammals, like seals, are particularly exposed to contamination by PCBs because they are top predators, characterized by relatively long life spans and large deposits of fatty tissue reserves.

Declines of several marine mammal populations, due to virus-related mass mortalities as well as to lowered reproductive success, have been attributed in part to the contamination by PCBs and other organochlorine pollutants (Helle et al. 1976a,b, Martineau et al. 1988, Aguilar & Borrell 1994, Nakata et al. 1995). Several field and semi-field studies on marine mammals have shown that reproductive failure, immune function impairment, developmental abnormalities, endocrine disruption, carcinogenicity, and vitamin A homeostasis disorders are linked to high concentrations of organochlorine pollutants, and particularly PCBs, in the tissues of the animals (Reijnders 1986, Martineau et al. 1988, Brouwer et al. 1989, Zakharov & Yablokov 1990, De Swart et al. 1994, Jenssen et al. 1995, Ross et al. 1995, 2000, Beckmen et al. 1997, Bernhoft et al. 2000, Roland 2000, Ross 2000, Simms et al. 2000, Troisi & Mason 2000).

During lactation, marine mammals transfer high amounts of PCBs from mother to offspring through the milk. This phenomenon leads to a partial detoxification in females. Indeed, they appear to accumulate PCBs in their tissues until they reach sexual maturity. Afterwards, their PCB body burdens remain stable or decline with age due to the transfer of PCBs to offspring during gestation, and particularly during lactation. In contrast, the PCB contamination of males generally increases continuously with age. Levels in adult males thus often exceed those reported in adult females (Tanabe et al. 1994, Kleivane et al. 1995, Nakata et al. 1995, Bernhoft et al. 1997, Westgate et al. 1997, Aguilar et al. 1999, Jepson et al. 1999).

In several seal species, females fast during lactation. Therefore milk constituents as well as the xenobiotics in milk are derived exclusively from maternal body stores. For example, in grey seals *Halichoerus grypus*, where the milk fat content ranges from 30 to 60% (Pomeroy et al. 1996), fasting lactating females appear to excrete about 15% of the PCB body burden during the 18 d nursing period (Addison & Brodie 1977). Thus pups ingest a considerable quantity of PCBs through the milk at a time when they are particularly sensitive to any deficiency of essential nutrients, such as vitamin A, for their growth and development, as well as to any dysfunction of their endocrine or immune system. Total exposures of up to 58.9 mg of PCBs for the whole lactation period have been reported in grey seal pups, which could represent a danger during the earliest stage of life (Pomeroy et al. 1996).

Numerous studies have focused on PCB contamination in tissues such as blubber, liver and, to a lesser extent, blood of non-lactating pinnipeds (e.g. Law et al. 1989, Skaare et al. 1990, Nakata et al. 1995, Wolkers et al. 1998, Kleivane et al. 2000, Severinsen et al. 2000). By contrast, reports on concentrations of PCBs in the milk and/or serum of lactating mothers and pups are rare (Green et al. 1996), and often represent single points during lactation or result from the collection of samples from dead animals (Addison & Brodie 1977, 1987, Bacon et al. 1992, Beckmen et al. 1999). The dynamics of transfer of PCBs from mother to pup over the course of lactation in seals are poorly understood. The present study is the first to describe this process in detail, through longitudinal sampling.

Grey seal mother-pup pairs from the pupping colony of the Isle of May were regularly captured throughout lactation in order to characterize the changes of PCB contamination levels in different compartments (maternal blubber → maternal blood → milk → pup blood). The characteristics of grey seal lactation in the UK (relatively short lactation period, maternal fasting, high lipid transfer from mother to pup) make this species a good model to study the transfer of xenobiotics from mother to pup.

MATERIALS AND METHODS

Field techniques. The study was conducted on the Isle of May, Scotland (56° 12′ N, 2° 32′ W), in November 1998 and 2000. A total of 22 mother-pup pairs were captured repeatedly, from 2 to 5 times between birth and weaning, to collect samples at different lactation stages. Blood (mothers and pups) and milk samples were taken in both 1998 and 2000. During the breeding season of 2000, a blubber biopsy extending the full depth of the blubber layer in the dorso-lateral pelvic area was taken from mothers at early and late lactation. Four mothers in 2000 (Seals 6J, 7J, 6L, 41) had also been sampled in 1998. One mother from 2000 (Seal 7J) was sampled before she gave birth. The dates of birth were recorded by observing the breeding areas on each day. In 2 cases (pups of Seals 41 in 1998 and 1H in 2000), the date of birth could not be reported and was determined using extrapolation (Pomeroy et al. 1999). All animal handling as well as milk and blood collection were carried out as described in Debier et al. (2002) under the UK Home Office licence. The data on mother-pup pairs and number of samples collected are summarized in Table 1.

Table 1. *Halichoerus grypus.* Number of samples obtained from the grey seal mother-pup pairs followed during the longitudinal study

	Seal code	Age of mothers (yr)	Blubber samples (n)	Mother serum samples (n)	Milk samples (n)	Pup serum samples (n)
1998	7J	8	0	2	3	2
	6L	>8	0	3	5	3
	6J	8	0	2	3	2
	41	?	0	2	4	2
	D0	21	0	4	4	4
	68	8	0	2	3	2
	59	?	0	3	4	3
	46	?	0	2	4	2
	44	8	0	0	4	0
2000	7J	10	2	3	3	3
	6L	>10	2	3	3	3
	6J	10	2	3	3	3
	41	?	0	2	2	2
	H7	28	2	0	2	0
	51115	?	2	3	3	3
	51113	?	2	3	3	3
	4B	16	2	2	2	2
	418	?	2	3	3	3
	2B	16	2	0	2	0
	1H	?	0	0	3	0
	OH	?	2	0	3	0
	D8	>20	2	0	2	0

Chemical analyses. ***Chemicals:*** All solvents were of pesticide grade; n-hexane and acetone (Burdick & Jackson brand) were purchased from Fluka (Buchs, Switzerland). The Mirex (Dodecachloropentacyclo-[5.3.0.0.0.0]decane) used as internal standard, the pure PCB congeners (IUPAC nos. 8, 18, 28, 44, 52, 66, 70, 87, 95, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206, 209) and the congener used as surrogate (IUPAC no. 112), were obtained from Ultra Scientific® and Dr Ehrenstorfer®. All other chemicals used were of analytical grade.

Sample preparation: *Milk samples.* Milk samples were thawed and homogenised with an Ultra-Turax (Ika-Werk 18/10 Janke & Kunfel). A 2 g sample was then lyophilized over 20 h and dry matter was determined gravimetrically. Milk lipids were extracted using an accelerated solvent extractor (ASE) (Dionex ASE 2000, Dionex Corporation). A 650 to 750 mg sample of lyophilized milk with 0.5 g of anhydrous sodium sulphate was extracted 3 times with a mixture of hexane, dichloromethane and methanol (5:2:1, v:v:v) at 80°C and under a pressure of 1500 Psi. The solvent with the extracted fat was collected in pre-weighed vials and was evaporated at 40°C under nitrogen flow (Turbovap LV Zymark). The fat content of milk samples ('hexane-extracted fat') was determined gravimetrically. Lipids were then dissolved into 3 ml of hexane and collected into a test tube. The mixture was homogenized by vortexing during 1 min.

Blubber samples. Blubber biopsies were cut at their inner and outer extremities and 1 cm of each extremity (inner blubber and outer blubber) was transferred into a test tube. Fat (~30 mg) was then extracted from the tissue by heating it 3 times in a microwave for 20 s at 650 W.

Serum samples. The sample preparation was set up by adapting a method established by the Laboratory of Food Analysis (University of Liège, Belgium) according to Singh et al. (1998), Janak et al. (1999), Pauwels et al. (1999) and Frenich et al. (2000). Serum was first deproteinised by adding 100 µl of triethylamine and 10 ml of formic acid to a precisely known volume of sample (from 2.5 to 10 ml, depending on the amount of sample available). The mixture was stabilized for 30 min in an ultrasound bath (Julabo USR 05). PCBs were then extracted by SPE (solid phase extraction), using a C18 micro-column (Baker). The column was first conditioned with 10 ml of methanol followed by 10 ml of distilled water using a Supelco elution device (Visiprep DL) and a vacuum pump (ABM). The deproteinized sample was then added to the column. After the sample had passed through, the column was rinsed with 3 ml of distilled water and then dried for 20 min using the vacuum pump. The PCBs retained on the column were then eluted with 5 ml of hexane.

Sample clean-up: All prepared samples (milk, blubber and serum) were then purified by acid and Florisil clean-ups. A 2 ml volume of sulphuric acid mixture (fuming sulphuric acid 30% and concentrated sulphuric acid 95%, 1:3, v:v) was added to the sample and the mixture was homogenized by vortexing before being centrifuged for 3 min at 1810 × *g* at 10°C (Jouan). The organic phase was transferred to another tube and the acidic phase was extracted with 3 ml of hexane, vortexed and centrifuged for another 3 min. The organic phases were pooled and reduced to 1 ml under a nitrogen flow. The second clean-up was performed with Florisil® solid phase cartridges (Supelco, Envi-Florisil). The cartridges were first conditioned with 5 ml of acetone, 5 ml of an acetone-hexane mixture (50:50, v:v) and 12 ml of hexane, successively. The sample was then added at the top of the column. Polar molecules were retained on the Florisil® (magnesium-silicate mixture). The test tubes containing the sample were rinsed with 3 ml of hexane and added to the cartridge. Another 3 ml of hexane were finally directly

added to the column. The eluate was then evaporated just to dryness under a gentle nitrogen flow.

Analysis: The dried residue of milk samples was reconstituted in 500 µl of hexane. Two levels of dilution were then realized, leading to theoretical volumes of 1 and 5 ml, in order to quantify the least and most concentrated congeners, respectively. Mirex (100 pg μl^{-1}) (Dr Ehrenstorfer®) was added as an internal standard at a final concentration of 50 pg μl^{-1}. Concerning blubber and serum samples, the dried residue was reconstituted with 125 µl of hexane and 125 µl of Mirex (100 pg μl^{-1}).

The purified extracts were then analysed by gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a ^{63}Ni ECD detector (Thermo Quest, Trace 2000) and an automatic injector. From 1 to 5 µl of each purified extract was injected by means of a cold 'on column' injector. PCB congeners were separated on a 30 m × 0.25 mm (0.25 µm film) DB-XLB capillary column (J&W Scientific). The temperature program was as follows: 2 min at 60°C, gradual heating from 60 to 140°C at the rate of 20°C min^{-1}, 3 min at 140°C, gradual heating from 140 to 270°C at the rate of 25°C min^{-1} and 12 min at 270°C. The carrier gas was hydrogen with a flow rate of 4 ml min^{-1} and a pressure of 130 kPa, and the make-up gas was Ar:CH_4 (95:5) at a flow rate of 30 ml min^{-1}. The injector was at ambient temperature and the detector was kept at 300°C. PCBs were identified according to their retention times. Twenty-six congeners, mostly present in Aroclor 1242, 1254 and 1260 mixtures, were measured (IUPAC 8, 18, 28, 44, 52, 66, 70, 87, 95, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206, 209). Data were recorded using ChromCard 1.19 software. Quantification was performed by comparison with external standards of the 26 pure PCB components in a certified calibration mixture (Ultra Scientific and Dr Ehrenstorfer®), using a linear calibration curve for each PCB congener whose concentration ranged from 2 to 100 pg μl^{-1}. PCB concentrations are expressed as the sum of the congeners measured.

Quality assurance. Blanks were run with each sample series to control the clean-up procedures. Concerning the milk samples, blanks were also used to control lyophilization and ASE steps. For each type of sample (milk, blubber or serum), a quality control (QC) was also analysed in parallel. Milk cream and bovine serum, enriched with a defined concentration of PCBs, as well as cod liver oil of known PCB concentration (BCR RM 349) were used as a QC for milk, serum and blubber analysis, respectively.

The PCB recovery was calculated on the basis of the concentration of the surrogate standard (IUPAC 112, Dr Ehrenstorfer®) (50 pg μl^{-1}). It was added to the sample at the beginning of the clean-up for blubber and milk samples and at the beginning of the sample preparation for serum samples. All results were corrected to obtain 100% recovery. However, the results of the PCB analyses were accepted only if the recoveries were between 70 and 130%.

Data analyses. Results were analyzed using the GLM procedure (SAS/STAT 1990).

Blubber samples: Blubber biopsies were taken only at early and late lactation for all females and were divided into 2 layers (inner and outer blubber, see 'Chemical analyses' subsection). PCB concentration variations in the blubber were analyzed using a 3-way mixed ANOVA, crossed design, with the following factors: individual, stage of lactation (early or late), and blubber layer (inner or outer). Stage of lactation and blubber layer were combined into a single 4-level factor, whose levels could then be compared pairwise, using Bonferroni's *t*-test for paired samples.

Milk and serum samples: The variations of PCB concentrations among individuals and as a function of the age of pups (a continuous regressor variable) were analyzed using a 2-way mixed analysis of covariance (ANCOVA). Both PCB concentrations and their logarithms (log_e) were analysed, the latter transformation intending to lower the variance heterogeneity observed between early and late lactation. The logarithmic model was chosen.

Milk samples were taken for each female at different stages of lactation. We compared the fit of a quadratic function of the age of pup with that of a linear (first order) model. The model was chosen on the basis of the significance of the quadratic term. Only the linear model was considered in serum samples because there were fewer longitudinal data.

In order to facilitate the presentation of the results, we will refer to 'early lactation' for the period from Day 0 to Day 5 and to 'late lactation' for the period from Day 11 to Day 20.

RESULTS

Maternal blubber

PCB levels in blubber layers are presented in Table 2 and Fig. 1. Concentrations showed significant differences between females ($p < 0.01$, df = 9,27). At early lactation, inner blubber was significantly less contaminated than outer blubber ($p < 0.01$, df = 3,27). These differences disappeared at late lactation ($p > 0.05$, df = 3,27). Concentrations in inner blubber increased significantly between early and late lactation ($p < 0.01$, df = 3,27). In outer blubber, no significant difference of concentration was noted between early and late lactation ($p > 0.05$, df = 3,27).

Table 2. *Halichoerus grypus.* PCB (µg g lipid^{-1}) concentrations in the inner and outer blubber layers of mothers at early (Days 0–5) and late (≥11 d) lactation

Seal code	Early lactation		Late lactation	
	Inner	Outer	Inner	Outer
7J	1.11	2.33	1.43	2.65
6L	0.86	2.28	2.48	2.83
6J	0.58	2.37	1.16	2.61
15	0.94	3.56	2.49	3.87
13	0.93	1.52	2.39	2.71
4B	2.82	5.88	10.09	6.78
18	1.83	5.00	4.33	5.59
OH	0.73	3.00	3.93	2.77
H7	1.99	3.10	2.31	2.53
2B	0.82	2.53	1.78	3.57
Mean	1.26	3.16	3.24	3.59
SD	0.72	1.34	2.60	1.46

Maternal serum

In blood, PCBs are mainly associated with proteins and lipoproteins. The lipid fraction of serum is composed of more polar lipids when compared to subcutaneous fat or other organs (Henderson et al. 1994), and contains non-polar lipids (i.e. hexane-extractable fat) at a very low rate. Expressing PCB concentrations in serum on a lipid weight basis (i.e. hexane-extractable fat) would thus lead to an overestimation. As a consequence, and in order to compare the PCB levels in serum with data in the literature, PCB concentrations were expressed on a per serum volume basis.

PCB concentrations in maternal serum were 6.69 ± 3.45 ng ml^{-1} and 12.18 ± 7.15 ng ml^{-1} at early and late lactation, respectively.

The results of the ANCOVA for PCBs in maternal serum are summarized in Table 3. PCB concentrations differed between individuals, especially at late lactation (Fig. 2A). PCB concentrations in maternal serum were higher at late lactation compared to early lactation (Table 3). The magnitude of the increase varied from one individual to the other. It was for example very slight for 6J-00 and 7J-00 while for 4B, concentrations more than doubled between early and late lactation (Fig. 2A).

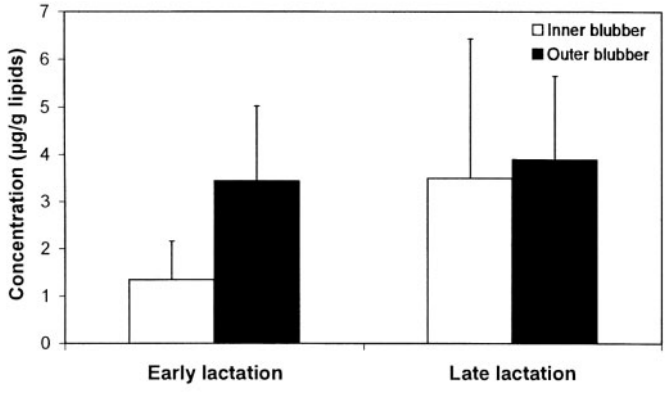

Fig. 1. *Halichoerus grypus.* PCB contamination in inner and outer blubber layers of lactating mothers at early (Days 0–5) and late (≥11 d) lactation

Table 3. *Halichoerus grypus.* Effect of time (age of the pup) and mother (individual) on the PCB content in the serum of mothers (ANCOVA)

Source	df	Mean square	*F*-value	p > *F*
Age of pup (A)	1	2.23	16.86	0.0017
Individual (I)	15	0.34	2.55	0.0611
Interaction I–A	15	0.13	1.00	0.5104

Milk

Milk lipid content increased as lactation progressed, from 33.8 ± 5.0% in colostrum to 56.3 ± 6.2% in milk after Day 12 (Fig. 3) ($p < 0.01$, df = 1,23). There were slight but significant differences in milk lipid content among mothers ($p = 0.02$, df = 21,23).

In whole milk, mean PCB levels were 0.31 ± 0.17 µg g^{-1} and 0.67 ± 0.42 µg g^{-1} at early and late lactation, respectively. In milk lipids, mean concentrations were 0.76 ± 0.43 µg g^{-1} and 1.22 ± 0.78 µg g^{-1} at early and late lactation, respectively. The levels of PCBs did not follow the same dynamics as lipids in milk (Fig. 3).

The results of the ANCOVA for PCBs in milk are summarized in Table 4. Milk PCB concentrations were very different from one female to another. As in maternal serum, the differences among mothers were greater at late lactation compared to early lactation (Fig. 2B). There was a significant quadratic change in concentration of PCBs in milk throughout lactation (Table 4): levels appeared to stay relatively constant until Day 10 and then underwent an increase towards the end of lactation. As in maternal serum, the increase was not as important in all females. PCB concentrations more than tripled during lactation in Seals 4B and D0, while they increased only slightly in Seals 1H and H7 (Fig. 2B). These variations were reflected in the covariance analysis (ANCOVA) by the significant interaction between the individual and the time of lactation (Table 4). When PCB concentrations were expressed on a lipid weight basis, their concentrations also appeared to differ between individuals ($p < 0.01$, df = 21,23) and to increase significantly at the end of lactation ($p < 0.01$, df = 1,23).

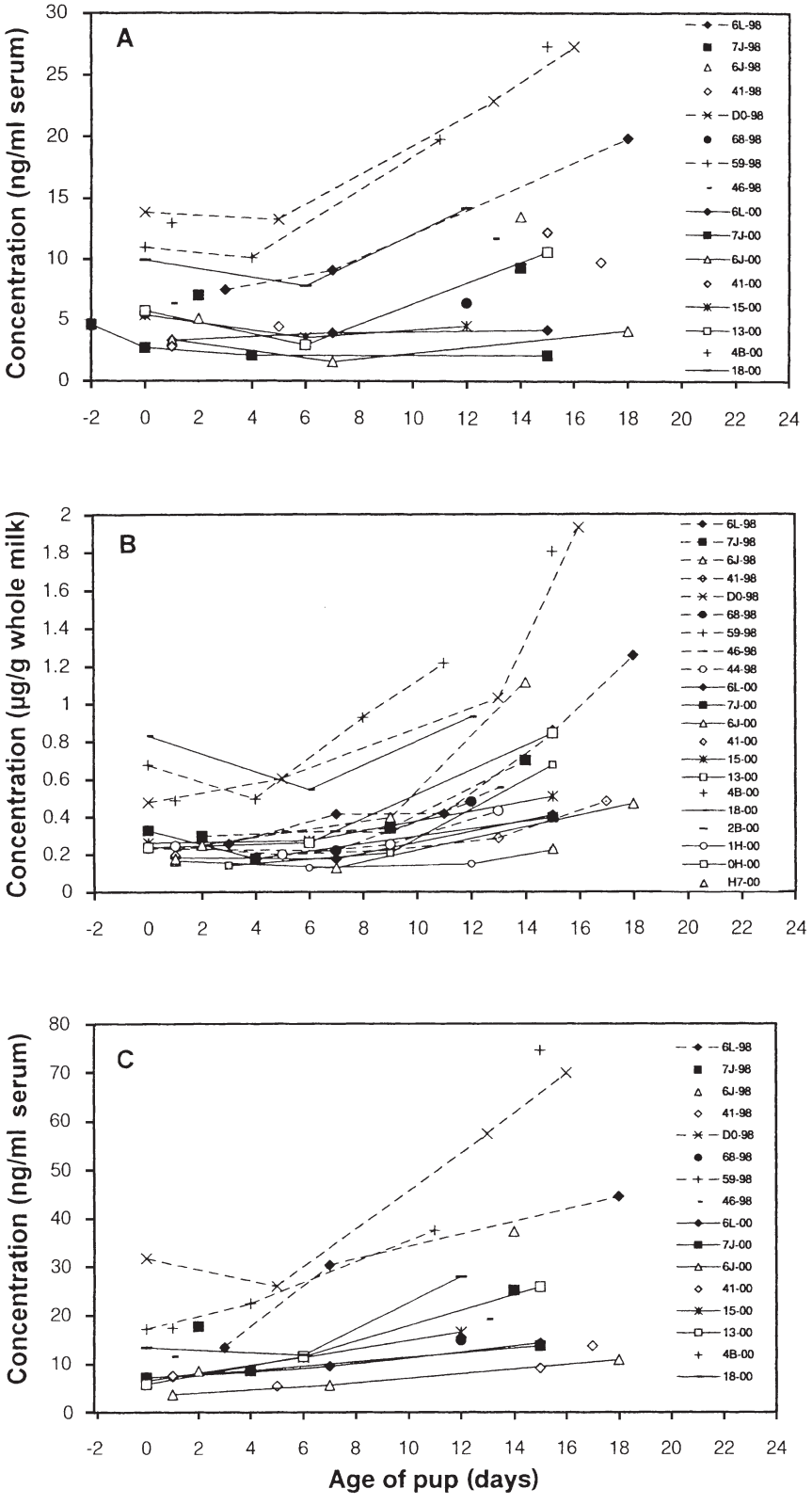

Fig. 2. *Halichoerus grypus.* PCB contamination of (A) mother serum, (B) milk, and (C) pup serum throughout the lactation period. Dashed lines: pairs from 1998; straight lines: pairs from 2000. Isolated points are from pairs captured twice only.

Pup serum

Mean PCB levels in pup serum were 11.86 ± 7.00 ng ml^{-1} and 27.89 ± 18.13 ng ml^{-1} at early and late lactation, respectively.

The results of the ANCOVA of PCB concentrations in pup serum during nursing are shown in Table 5. Contamination levels highly differed from one pup to another and were greater at late lactation compared to early lactation (Fig. 2C). The differences between individuals appeared once again to be more important at late lactation compared to early lactation, and the increase in concentration varied between individuals (Fig. 2C).

Relationships

Positive correlations were observed between inner blubber and maternal serum ($r = 0.93$, $p < 0.01$), maternal serum and milk ($r = 0.92$, $p < 0.01$) as well as milk and pup serum ($r = 0.90$, $p < 0.01$). These correlations are the result of comparable changes of concentration occurring in each of the compartments investigated throughout lactation. Fig. 4 illustrates this phenomenon by presenting 2 mother-pup pairs: one showing slight variations with time (Seal 6J-00) and the other showing large variations with time (Seal 4B).

When considering the entire lactation period, contamination levels appeared to be significantly higher in the serum of pups compared to the serum of their mothers ($p < 0.01$, df = 1, 15). The PCB concentration in pup serum as a function of the PCB concentration in mother serum shows an exponential relationship (Fig. 5).

We were unable to show variation in contaminant levels in the blubber, serum and milk of mothers as a function of age. When Seals D0 (21 yr old) and 4B (16 yr old) were compared to the 8 to 11 yr group (Seals 6J, 7J and 6L), a positive relationship seemed to appear with the age of the mother. However, Seal H7 (28 yr old) had contamination levels relatively similar to those

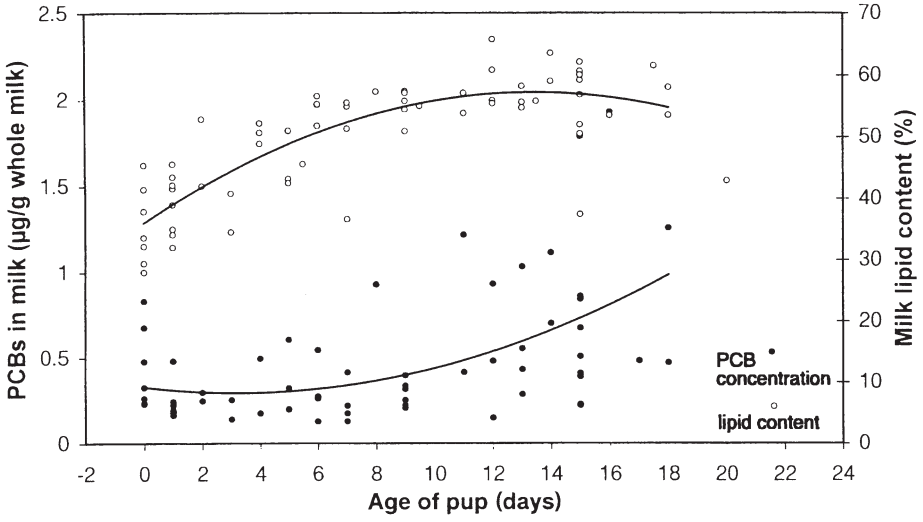

Fig. 3. *Halichoerus grypus.* Overall changes in PCB contamination as well as lipid content in milk throughout lactation

of the younger mothers. Moreover, Seals 4B and 2B (both 16 yr old) showed very different contamination levels.

DISCUSSION

Grey seals, like several other true seal species, fast during lactation. Milk energy and nutrient contents are thus derived entirely from maternal body stores. The lipids of grey seal milk originate mainly from the blubber. This important lipid transfer from maternal blubber stores inevitably induces the mobilization of PCBs and other fat-soluble pollutants from the blubber compartment into the blood and then the milk. These chemicals are known to induce negative effects, like immunotoxicity, reproductive failure and developmental problems in the organisms they contaminate (Helle et al. 1976a,b, Reijnders 1986, Zakharov & Yablokov 1990, De Swart et al. 1994, Ross et al. 1995). The impact may be particularly deleterious in young animals because they rely on adequate metabolism for their growth, development and immune system, and they have a lower ability to detoxify xenobiotics than adults (Timbrell 1991, Bernhoft et al. 1997).

Table 4. *Halichoerus grypus.* Effect of time (age of the pup) and mother (individual) on the PCB content in the milk (ANCOVA)

Source	df	Mean square	*F*-value	p > *F*
Age of pup (A)	1	0.33	14.54	0.0009
Age of pup^2 (A^2)	1	1.50	66.2	0.0001
Individual (I)	21	0.20	8.85	0.0001
Interaction I–A	21	0.09	3.74	0.0014

Table 5. *Halichoerus grypus.* Effect of time (age of the pup) and pup (individual) on the PCB content in the serum of pups (ANCOVA)

Source	df	Mean square	*F*-value	p > *F*
Age of pup (A)	1	6.02	131.02	0.0001
Individual (I)	15	0.40	7.36	0.0015
Interaction I–A	15	0.07	1.52	0.2542

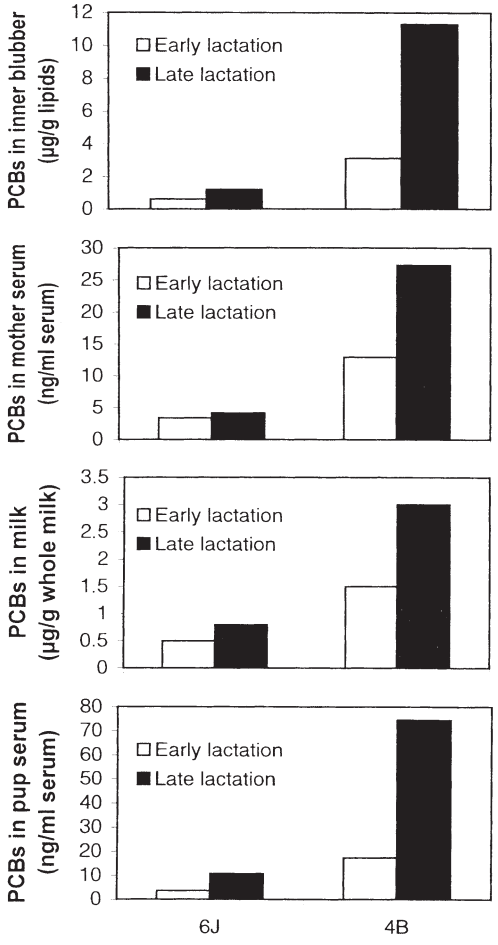

Fig. 4. *Halichoerus grypus.* Changes in the concentrations of PCBs from one compartment to the other for 2 mother-pup pairs (Seals 6J-00 and 4B)

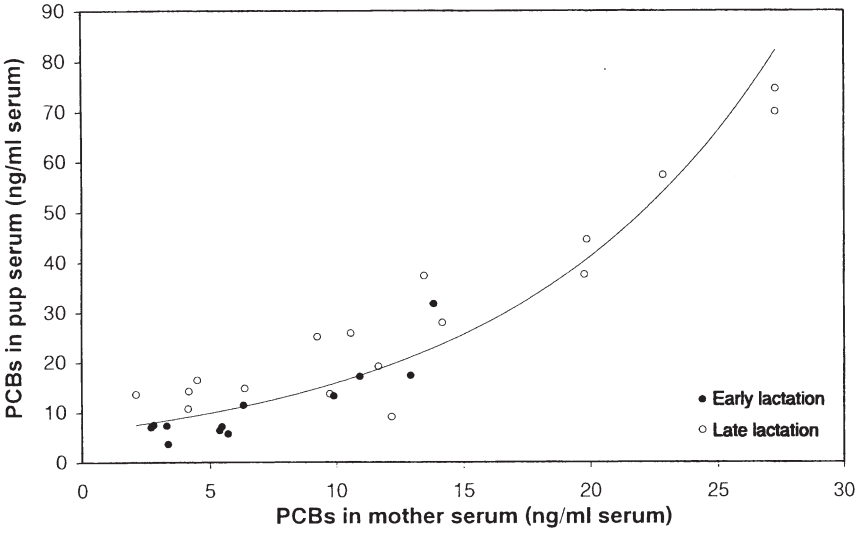

Fig. 5. *Halichoerus grypus*. PCB contamination in pup serum as a function of maternal serum at early and late lactation

Contamination levels of grey seals during lactation

At early lactation, inner and outer blubber PCB concentrations within seals were very different, while these variations were much less clear at late lactation. Differences in blubber-layer concentrations have already been noticed by Severinsen et al. (2000) for male and female ringed seals *Phoca hispida* from Svalbard. Fatty acid composition of the blubber layers was suggested to be one of the causative factors of such differences. The authors pointed out the fact that if the studies reporting PCB levels in blubber of marine mammals are based on biopsies using sampling tools that only penetrate the outer part of the blubber, the PCB burden is probably overestimated. Indeed, most studies focusing on PCB concentrations in marine mammal blubber do not make the difference between inner and outer blubber or the condition of the animal. Comparisons of contamination within the literature are therefore unlikely to be standardized.

PCB levels observed in the blubber of lactating grey seals from this study were lower than those reported for pinnipeds such as harbour seals *Phoca vitulina*, Baikal seals *P. sibirica*, California sea lions *Zalophus californianus* and grey seals from highly polluted areas (Law et al. 1989, Schweigert & Stobo 1994, Nakata et al. 1995, Bernt et al. 1999, Kajiwara et al. 2001). Conversely, the blubber concentrations observed in this study were higher than those reported in Arctic species such as the ringed seal (Addison & Zinck 1986, Cleeman et al. 2000, Severinsen et al. 2000). Concentrations encountered in the blubber of lactating UK grey seals were below the threshold of 17 mg kg^{-1} at which direct health problems have been reported (Ross et al. 1996).

Few studies report PCB concentrations in the serum and milk of marine mammals. PCB concentrations in the serum of grey seals presented in this study were respectively slightly higher and 1 order of magnitude higher than the levels reported in the serum of ringed seal and bearded seal *Erignathus barbatus* females from Svalbard (Bang et al. 2001). The levels found in grey seal milk from the present study were lower than the ones reported in grey seal milk from Sable Island (Schweigert & Stobo 1994). Comparable PCB concentrations were seen in the milk of northern fur seals *Callorhinus ursinus* from Alaska and in the milk of northern elephant seals *Mirounga angustirostris* from California, when expressed on a wet weight basis (Bacon et al. 1992, Beckmen et al. 1999). Levels of up to 2 orders of magnitude lower were noticed in the milk of Antarctic fur seals *Arctocephalus gazella* (Bacon et al. 1992).

The geographic location thus seems to play an important role on the contaminant burden of pinnipeds. However, factors such as the age and sex of the animals, their feeding habits, physiological status (lactating, fasting, ill …), the blubber layer considered, the total body blubber content, the inter-specific metabolism capacities, as well as the sampling and analytical techniques used might also have an impact on the PCB levels reported in the different surveys (Addison & Zinck 1986, Skaare et al. 1990, Nakata et al. 1995, Mössner & Ballschmiter 1997, Wolkers et al. 1998, Bernt et al. 1999, Kleivane et al. 2000, Severinsen et al. 2000, Addison & Stobo 2001).

PCB concentrations in all compartments (blubber, serum and milk) differed among mothers, consistent with previous observations in grey seal milk from the same colony (Pomeroy et al. 1996). While we did not detect an effect of age on the variation in PCB concentrations in these lactating females, age and reproductive history may nonetheless influence contaminant concentrations. Indeed, gestation and lactation constitute major ways of elimination of PCBs and, in several species, a decrease in the concentrations of PCBs with age has been noticed in the tissues of sexually mature females. By contrast, some studies reported a steady state in the tissue PCB concentrations, the elimination through milk being compensated for by food ingestion (Addison & Brodie 1977, Nakata et al. 1995, Aguilar et al. 1999, Bernt et al. 1999). The foraging habits and sites used can also be responsible for the inter-individ-

ual differences of contamination. Indeed, variations in the foraging behaviour of individual grey seals occur in the North Sea (Hammond et al. 1993). Regional and seasonal variations in the diet of grey seals have also been reported (Prime & Hammond 1990). Other factors such as the reproductive history (females sometimes miss some reproductive years), total blubber amount, as well as temporal trends in the contamination levels of the marine biota would also need to be taken into account to explain patterns of contamination.

The variations of the contamination status in mothers were reflected by varying levels in the serum of the nursing pups. Very young pups had higher serum PCB levels than their respective mothers, revealing an important transplacental transfer as described by Beckmen et al. (1999) for Northern fur seal pups. This phenomenon was greatly amplified during lactation with the ingestion of milk. The higher PCB levels may be in part explained by the higher lipid content in the serum of pups compared to the mothers, reflecting the pup's intense feeding on milk (Addison & Brodie 1987). Moreover, from a contaminant perspective, nursing pups can be regarded as feeding at a higher trophic level than their mother (Beckmen et al. 1999).

PCB transfer from mother to pup

In all the successive transfer compartments studied with regard to PCB transfer (maternal blubber → maternal blood → milk → pup blood), an increase in the concentration of PCBs was noted in the course of lactation. An increase of PCB levels in grey seal milk throughout lactation was also observed by Schweigert & Stobo (1994) but, as only 2 measures were made, at early and late lactation, the dynamics of this increase could not be reported. In the present study, longitudinal data were able to describe how these changes occurred in milk over the course of lactation, and a curious phenomenon could be observed. It appeared that concentrations stayed relatively stable until Day 10, and then started to increase towards the end of the nursing period. Curiously, this dynamic was not similar to the one observed in the present study for milk lipid content which increased at early lactation and then stayed stable after Day 10. Thus, even when expressed per kg of milk lipids, PCBs underwent a rise at the end of lactation.

The increase in milk lipid PCBs at late lactation is probably because of a mobilization from a more contaminated site during that period. Data reported in blubber may explain this phenomenon. Concentrations in inner blubber, the layer probably the most in contact with the blood circulation, appeared to increase sharply between early and late lactation, while in the outer blubber, PCB content stayed much more stable. The increase in the inner blubber levels from early to late lactation possibly resulted from the fact that the PCBs are less easily mobilized from blubber than triglycerides, the dominant lipid type (Henderson et al. 1994), and are thus redistributed in the remaining blubber layer which becomes more and more reduced as lactation proceeds. This pathway was part of the hypotheses stated by Aguilar (1985) concerning the destination of pollutants from the blubber, when fat is mobilized from this tissue during fasting. An increase in the concentrations of organochlorine pollutants like PCBs has been documented in harp seals during the moulting season, when feeding was at a minimum or non-existent (Kleivane et al. 1997). However, these authors did not analyse the differences between blubber layers. Sequestration of contaminants to outer blubber layers could occur along lactation. Qualitative variations in lipids between the different layers (polarity) may play a role in the distribution of PCBs in the blubber. At late lactation, the mobilization of less polar triglycerides from blubber could be one explanation of the increased mobilisation of PCBs. It is also possible that, at late lactation, as most of the lipids have disappeared, the retention capacity of the reduced blubber layer for PCBs has reached its maximum. At this stage, PCBs no longer accumulate in the blubber and start to be mobilized in higher amounts at the end of lactation. Fewer transfers, however, seemed to happen in the outer blubber layer than in the inner blubber. This layer thus appeared to be more 'inert' in a physiological sense. Inner blubber reflects the contaminant burden brought via the female's diet during the year. The contaminant burden of this more 'active' layer is more susceptible to have an effect on the metabolism of the animal and to be transferred to offspring.

The increased mobilization of PCBs from blubber at late lactation was coupled to an increase in the levels observed in the serum of mothers. As for milk, the rise seemed to occur at late lactation. Similarly, the dynamics of change of PCBs in milk was accompanied by an increase in the serum of pups, which also occurred at late lactation. The increase of PCB concentrations in the milk ingested at late lactation was even amplified by the increase of daily milk intake at that moment (2.4 kg at early lactation vs 3 kg at late lactation, Pomeroy et al. 1996). Indeed, in the study of Pomeroy et al. (1996), the daily PCB exposure of grey seal pups increased during the lactation period. PCBs accumulated exponentially in pup serum compared to maternal serum, as lactation progressed. This phenomenon reflects the increase of PCBs in milk at late lactation coupled with the increase of daily milk intake as lactation progresses. Other factors, such as a better intestinal absorption of lipids at late lactation, may also favour such a relationship.

CONCLUSION

Most of the PCB transfer from mother to pup appeared to occur during the latter part of the nursing period. Indeed, contamination levels increased in the different compartments of transfer (maternal inner blubber, maternal serum, milk, pup serum) at late lactation. PCB levels in pup serum increased exponentially compared to those in maternal serum, as lactation progressed. This curious phenomenon poses the question of how a greater maternal investment of some grey seal mothers, with higher body-store mobilization and a longer lactating period, may induce more adverse effects on the nursing pup, as greater amounts of pollutants could then be transferred. At early lactation, blubber layers appeared to have different PCB levels, outer blubber being more contaminated than inner blubber. This variation was much less evident at late lactation, due to the rise of concentrations in the inner blubber layer.

From a molecular point of view, a qualitative study focusing on the composition of the PCB mixture throughout lactation will bring complementary information on the dynamics of transfer of these pollutants from mother to pup in grey seals (Debier et al. 2003, this issue). From a physiological point of view, the distribution of PCBs among lipoproteins in serum, as well as among blubber lipids, should be examined in order to understand the mechanisms of transfer as well as the increase of concentrations observed at late lactation. From an eco-toxicological point of view, body composition changes would allow the calculation of PCB concentrations per unit of body mass and allow a better assessment of the contamination burden of the animals. Total PCB body burden combined with milk output should also enable an estimation of the decontamination rate of individuals per lactation cycle to be made. Finally, from an immuno-toxicological point of view, a study of the survival rate of pups during their first year of life, related to their PCB intake during lactation, should bring answers to the possible adverse impact of PCB exposure during the critical stages of development.

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