ORGANOCHLORINES AND LACTATION : MOBILIZATION AND TRANSFER TO OFFSPRING IN NORTHERN ELEPHANT SEALS

Debier C[1], Crocker D[2], Houser D[3], Xhonneux V[4], Thomé JP[4]

[1.] Unité de Biochimie de la Nutrition. Institut des Sciences de la Vie. Université catholique de Louvain. Croix du Sud 2/8. B-1348 Louvain-la-Neuve. Belgium
[2.] Department of Biology, Sonoma State University, Rohnert Park, California 94928, USA
[3.] BIOMIMETICA, 7951 Shantung Drive, Santee, CA 92071. USA.
[4.] Laboratoire d'Ecologie animale et d'Ecotoxicologie. Institut de Zoologie. Université de Liège. Quai Van Beneden, 22. B-4020 Liège. Belgium

Abstract
The transfer of PCBs and DDTs from mother to pup was studied in 20 Northern elephant seal *Mirounga angustirostris* mother-pup pairs. Organochlorine concentrations in milk increased from 127.3 ± 25.0 ng/g to 200.9 ± 39.6 ng/g for PCBs and from 278.2 ± 92.4 ng/g to 494.9 ± 163.4 ng/g for DDTs between day 4 and day 21 of lactation. A similar longitudinal rise was observed in the serum of mothers (5.4 ± 0.8 to 7.7 ± 1.4 ng/ml for PCBs and 7.8 ± 2.4 to 11.9 ± 4.1 ng/ml for DDTs) and, to a lesser extend, in the serum of their pups (5.8 ± 1.4 to 6.1 ± 2.5 ng/ml for PCBs and 8.3 ± 2.5 to 9.9 ± 4.3 ng/ml for DDTs) between early and late lactation. The rise of contaminants in serum and milk might originate from the changes observed in maternal inner blubber in which PCB and DDT levels increased significantly throughout lactation (604.2 ± 142.8 to 1292.5 ± 421.6 ng/g lipids for PCBs and 1223.9 ± 460.0 to 2395.9 ± 737.3 ng/g lipids for DDTs). By contrast, contaminant levels remained constant in outer blubber (933.2 ± 271.8 to 999.7 ± 223.5 ng/g lipids for PCBs and 2010.7 ± 698.3 to 2061.9 ± 603.5 ng/g lipids for DDTs).

Introduction
Pollutant levels in marine mammals are influenced by various factors such as age, diet and physiological status. In particular, periods of voluntary fast that imply a mobilization of body lipid stores will have an impact on the dynamics of fat-soluble contaminants. During such periods, the animal is particularly vulnerable because the contaminants that are mobilized from the blubber may reach target organs and exert negative effects. In addition, during lactation, the contaminants may also be transferred to the offspring through the milk. The northern elephant seal provides an excellent model to study the dynamics of mobilization of organochlorine pollutants from body lipid stores during periods of natural fasting. Mothers fast during the entire nursing period (~ 25 days), secreting a fat-rich milk (up to 55% fat) synthesized from their body stores.[1] They lose almost 60% of their body fat.[2] The mass transfer to the pup is efficient, excess of 50%. During the nursing period, the pup gains around 90 kg, more than half of it being composed of lipids.[2] In the present study, free-ranging northern elephant seal mother-pup pairs were followed and longitudinally sampled in order to characterize the dynamics of mobilization of PCBs and DDTs from maternal body stores and their transfer to offspring.

Material and Methods
- *Sample collection*. The study was conducted on the colony of Año Nuevo, CA, USA (37°06'30''N, 122°20'10''W) in January and February 2005. Twenty mother-pup pairs were captured at day 4 and day 21 of lactation. Dates of birth were recorded by marking the mothers and observing the colony each day. Mothers were captured after intramuscular injection of Telazol (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA) at a dose of 1 ml per 100kg of estimated body mass. At both captures, blood, milk and blubber were taken from the mothers. Blood samples were collected from the extradural vein. Blubber samples were taken after subcutaneous injection of Lidocaïne (4 ml) for local anaesthesia. A blubber biopsy extending the full depth of the blubber layer was taken in the lateral pelvis area using a 6mm biopsy punch (Uni-Punch, Premier Medical, Plymouth, PA, USA). Blubber biopsies were placed in aluminium foil. Milk was collected from the teat using a clean cut-off syringe after a subcutaneous injection of 40 IU of oxytocin (American Pharmaceuticals Partners, Los Angeles,

CA, USA) near the mammary gland. Concerning the pups, blood samples were collected at both captures whereas blubber was taken only at late lactation. At day 4, the pups were hand captured in order to collect a blood sample from the extradural vein. At day 21, pups were first anaesthetized with Telazol, as for mothers, before collecting a blood sample from the extradural vein. The blubber biopsy was collected as for mothers. Mothers and pups were reunited after the procedure. At the end of each day, whole-blood samples were centrifuged for 30 min and serum was aliquoted into 1.5 ml Nunc tubes (Nalge Nunc International, Rochester, NY, USA). Samples were stored at -20°C until analyses.

- *Chemical analyses.* Blubber biopsies (approximately 6 cm long) were cut into 3 equal parts. Inner (closest to the muscle) and outer (closest to the skin) layers were analyzed separately. Blubber, serum and milk were analysed for 19 PCB congeners (IUPAC 52, 101, 105, 110, 118, 128, 138, 143, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206, 209) as well as *p,p'*DDT, *p,p'*DDE and *p,p'*DDD by gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a 63Ni ECD detector (Thermo Quest, Trace 2000, Milan, Italy). The details of sample preparation and clean-up as well as quality assurance are provided elsewhere.[3]

- *Data analyses.* Results were analysed using the GLM procedure (Statistica 7.1). In order to lower the variance heterogeneity and normalize the data, all the tests were conducted on the logarithms ($\log_e$) of PCB and DDT concentrations. The effect of sex on the concentrations of PCB or DDT in the serum and blubber of pups was examined with a 1-way ANOVA at each capture. Blubber biopsies were divided into inner and outer blubber. The variations of PCB or DDT concentrations in the blubber of mothers 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 then combined into a single 4 level-factor (stage-layer), whose levels then be compared pairwise, using Bonferroni's t-test for paired samples. The variations of PCB or DDT concentrations in the blubber of pups were analysed using a 2-way mixed ANOVA with the following factors : individual and blubber layer (inner or outer). The variations of PCB or DDT concentrations in the serum (mother or pup) and in the milk were tested using a 2-way mixed ANOVA, crossed design, with the following factors: individual and stage of lactation (early or late).

Results and discussion

In all compartments, hexa-chlorobiphenyls were present in highest proportions (50-60%), followed by hepta- (25-30%) and penta-chlorobiphenyls (20-25%). Concerning DDTs, p,p'DDE accounted for more than 95% of all forms. The ratios DDTs/PCBs in the different compartments of transfer ranged from 1.5 to 2.5.

- *Maternal blubber*

PCB and DDT concentrations showed significant differences among lactating females ($p < 0.01$). PCB and DDT concentrations in inner blubber increased significantly between early and late lactation ($p < 0.01$) (Figs. 1 and 2). On the other hand, no significant difference of PCB and DDT concentrations was noted between early and late lactation in outer blubber ($p = 1.00$). At early lactation, inner blubber was significantly less contaminated by PCB and DDT than outer blubber ($p < 0.01$). As a direct consequence of the increase of contaminant levels in inner blubber with time, the inverse tendency was observed for PCBs at late lactation ($p < 0.01$) and no difference was observed for DDTs at late lactation ($p=0.68$).

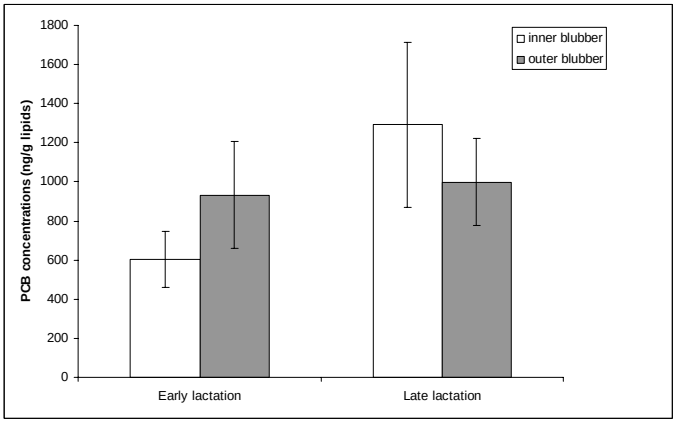

Fig. 1. PCB contamination in inner and outer blubber layers of lactating northern elephant seal mothers at early (day 4) and late (day 21) lactation.

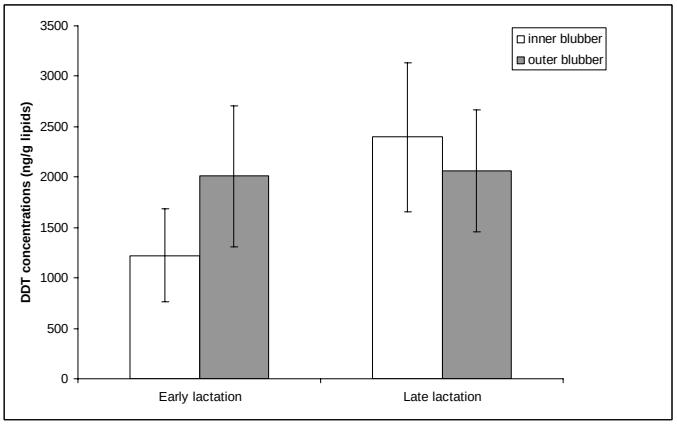

Fig. 2. DDT contamination in inner and outer blubber layers of lactating northern elephant seal mothers at early (day 4) and late (day 21) lactation.

The variation of PCB and DDT concentrations between inner and outer blubber may result from the fact that both layers exert differential roles, with the inner layer being mostly devoted to fat/energy storage whereas the outer layer is more involved in the insulation and is not really affected by the physiological status of the animal.[3,4,5,6] The increase of PCB concentrations in inner blubber between early and late lactation may be explained by the fact that this layer is more metabolically active and is first depleted.[4,7] The fatty acids from inner blubber are therefore first mobilized in case of negative energy balance. As lactation progresses, the freeing of fatty acids into the circulation induces the concentration of PCBs in the remaining blubber layer.

-Maternal serum

PCB concentrations were 5.4±0.8 ng/ml at early and 7.7±1.4 ng/ml serum at late lactation. DDT concentrations were 7.8±2.4 ng/ml at early and 11.9±4.1 ng/ml at late lactation. PCB and DDT concentrations varied among individuals ($p < 0.01$) and increased significantly between early and late lactation ($p < 0.01$).

The increase of PCBs and DDTs in serum most probably results from an increase of their mobilization rate from blubber. At late lactation, the PCB and DDT retention capacity of the reduced blubber layer may have reached its maximum, and the contaminants are no longer able to concentrate in the blubber and start to be released in higher amounts in the circulation, as already suggested for lactating grey seals.[3]

-Milk

PCB concentrations were 127.3±25.0 ng/g milk (wet weight) at early and 200.9±39.6 ng/g milk at late lactation. DDT concentrations were 278.2±92.4 ng/g milk at early and 494.9±163.4 ng/g milk at late lactation. PCB and DDT concentrations did not vary among individuals ($p = 0.25$ for PCBs and $p = 0.15$ for DDTs). Both PCB and DDT increased significantly between early and late lactation ($p < 0.01$).

The increase of PCB and DDT concentrations in milk reflects the rise observed in maternal serum.

-Pup serum

PCB concentrations were 5.8±1.4 ng/ml at early and 6.1±2.5 ng/ml serum at late lactation. DDT concentrations were 8.3±2.5 ng/ml at early and 9.9±4.3 ng/ml at late lactation. PCB and DDT concentrations did not vary among individuals ($p = 0.13$ for PCBs and $p = 0.14$ for DDTs). PCBs increased significantly between early and late lactation ($p < 0.01$) whereas DDT levels remained more constant ($p = 0.12$).

The tendency towards an increase of PCB and DDT as lactation progresses is less obvious in this compartment. This phenomenon is quite surprising as milk contamination as well as the amount of milk ingested by the pups increase with time.

- Pup blubber (at late lactation)

PCB concentrations were 617.3±189.5 ng/g lipids in inner blubber and 823.3±472.9 ng/g lipids in outer blubber. The difference of concentrations was not significant ($p = 0.08$). DDT concentrations were 1438.8±415.5 ng/g lipids in inner blubber and 2069.5±1901.8 ng/g lipids in outer blubber. The difference of concentrations was not significant ($p = 0.10$). PCB and DDT concentrations did not vary among individuals ($p = 0.56$ for PCBs and $p = 0.14$ for DDTs).

The lack of variation of contamination between the two layers most probably results from the fact that the animals are still young. The fatty acid composition of both layers is probably similar. The stratification may take place progressively, as the animal gets older. Fatty acid analyses of blubber would be necessary to confirm this hypothesis.

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