EFFECTS OF VARIOUS FRACTIONS OF PCB ON MINK REPRODUCTION. PRELIMINARY RESULTS FROM EXPERIMENTAL STUDIES WITHIN THE SWEDISH SEAL PROJECT

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Background.

The Baltic is inhabited by three species of seals, Ringed seal (*Phoca hispida*), Grey seal (*Halichoerus grypus*) and Common seal (*Phoca vitulina*). The population of all three species have declined severely during this century. PCB has been suspected to have caused the reproductive impairment and disease complex found in the populations. (Helle et al. 1976, Bergman and Olsson 1986). The findings of pathological changes were interpreted as a hyperadrenocorticism most probably caused by a primary lesion of the adrenals followed by secondary changes in many other organs. The disease complex included metabolic disorder, immunosuppression and hormonal imbalance.

A study of museum collections of Baltic grey seal skulls showed that the frequency of one of the observed pathological changes (severe bone lesions in the mandibulas) increased after 1950. Similar severe lesions was not found in a comparison with skull collections from the British Isles. The result indicated that the findings were specific for the Baltic and appeared during the 50s. (Bergman et al. 1989). This is a time when the levels of organochlorines increased in the Baltic (Olsson and Reutergårdh 1986).

At this time also European otter (*Lutra lutra*) disappeared from large areas of Europe. Otter is another fishconsumer which was common in Baltic archipelagoes but which is now almost extincted in the archipelagoes and also in many freshwaters in Sweden. The levels of PCB are found to be as high in otters as in Baltic seals and are suspected to be responsible for the decline. (Sandegren et al 1980).

Declining common seal populations in the Dutch Wadden sea lead to experimental works on common seal which were fed fish from the Wadden sea and as a control group seals were fed Atlantic fish. Also these results strongly indicated that PCB was the responsible factor (Reijnders, 1986).

The many indications that environmental pollution to a large extent is responsible for the decline of fishconsumers in Swedish waters lead to a research project financiated jointly by the Swedish environment protection board and WWF. The project started in 1988 and shall be finalized in 1991. The project is a multidisciplinary cooperation between ecologists, chemists, toxicologists and pathologists. The project include following items:

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-Census of the different populations and estimates of the reproductive outcome.
-Pathology in Swedish seals.
-Bycatches of seals.
-Food choice of Swedish seals in relation to contaminant burden.
-Studies on effects of fractions of commercial PCB on mink.
-Comparative *in vitro* and *in vivo* studies on effects of various organochlorines on the adrenals. Species used are mice, mink, otter and seal.
-Analyses of contaminant levels in various Swedish seal populations and their food.
-Chemical synthesis of reference and test substances.

This presentation gives some preliminary results from the studies of effects of various fractions of PCB on mink reproduction. Mink was used of biological and ecological reasons and since it is sensitive to polychlorinated biphenyls (Platonow and Karstad, 1973, Jensen et al. 1977, Bleavins et al. 1980, Aulerich et al. 1977).

Material and methods.
Clophene A 50 was separated into four fractions (Jensen & Athanasiadou, 1990). Three fractions contained the chlorobiphenyls with 0, 1,and 2-4 *ortho* chlorines, respectivly. The fourth fraction contained mainly tricyclic chlorinated substances such as polychlorinated naphtalenes and dibenzofurans. The first year 70 mink bitches, 2 years old, were separated into 7 experimental groups each consisting of 10 individuals. The

Table 1. Reproductive outcome of the various groups of mink treated with different fractions of Clophene A 50. The outcome given as percentual number of whelps compared to the control group. Significant differencies between treated groups and the controlgroup is indicated. $p<0.01$ **, $p<0.05$ *. Mann-Whitney U-test.

Group	Fraction	% welps
1	Control	100
2	Clophene A 50 (100%)	1**
3	2-4 *ortho* fraction (77%)	96
4	1 *ortho* fraction (22%)	74*
5	0 *ortho* fraction	77*
6	Synthezised 0 *ortho* fraction	69
7	Tricyclic fraction	105

experiment started in early February and ended five days after parturition or, if no whelps were borne, 5 to 10 days after expected parturition in the middle of May. During the experiment bitches of the various groups recieved a daily dose of 2 mg Clophene A 50 or fractions from a corresponding dose of the commercial product. One group recieved a synthesized mixture of the coplanar PCBs (3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl) at concentrations and proportions as found in the non-*ortho* fraction obtained from Clophen A 50. The exposure of the different groups can be seen in Table 1. Figures within brackets indicate the relative amount of the commercial mixture found in the various fractions.

During the experiment metabolites of hormones were followed in the urine and 5 days after parturition the bitches were killed and the number of implantation scars in the uterine wall was determined. The bitches were autopsied and histologically examined and blood samples were taken for clinical blood chemistry. Vitamine A was determined in blood, liver and lung tissue. Five days old whelps were weighed and killed. The induction of some liver enzymes was measured and t-cells in thymus were studied. Tissues were analysed for presence and amount of the various PCB fractions as well as individual congeners, PCB methylsulphones, dioxines and polychlorinated naphtalenes. A complete presentation of all results is planned to the end of 1991. This presentation will only give some data on reproduction in the various groups.

Table 2. Reproductive outcome of the different groups of mink treated with various combinations of fractions from Clophene A 50. The outcome given as the percentual number of whelps compared to the control group. Significant differencies beween treated groups and the controlgroup are indicated. $p < 0.01$ **, $p < 0.05$ *. Mann-Whitney U-test.

Group	Fraction	% whelp
8	Control	100
9	Clophene A 50	21**
10	2-4, 1, 0 *ortho* and tricyclic fractions	21**
11	2-4, 1 and 0 *ortho* fractions	12**
12	2-4 and 1 *ortho* fractions	51*
13	2-4 and 0 *ortho* fractions	36*
14	1 and 0 *ortho* fractions	19**
15	2-4, 1 *ortho* and tricyclic fractions	74

Results and discussion

The results presented in table 1 show the reproductive outcome measured as the percentual number of whelps in the different groups compared to the controlgroup. The results clearly indicate the importance of 0 and 1 *ortho* congeners but do not indicate a significant influence from the tricyclic fraction. None of the single fractions could itself explain the lowered reproduction found in group 2. In order to study the combined effects of different fractions a second experiment was conducted following year. Mink bitches, 1 year old, were treated in a similar way as the first year and recieved the same daily dose of Clophene A 50 as the year before or combinations of the different fractions. Each experimental group consisted of 10 individuals. The exposure of the different groups are indicated in Table 2.

The reproductive outcome is presented in Table 2. The results show that the 1 and 0 *ortho* fractions are the active fractions to explain the lowered reproductive rate in minks treated with Clophene A 50. The results also vizualize the importance of 1 *ortho* substituted chlorinated biphenyls in the environment.

References

Aulerich, R.J. and Ringer, R.K.: Arch. Environ. Contam. Toxicol. 6, 279 (1977).

Bergman, A. and Olsson, M.: Finnish Game Research 44, 47,(1986).

Bergman, A., Reiland, S. and Olsson, M.: In Proc. Soviet-Swedish Symposium, Influence of Human Activities on the Baltic Ecosystem. Moscow, April 14-17, 1986. 87,(1989).

Bleavins, M.R., Aulerich, R.J. and Ringer, R.K.: Arch. Environ. Contam. Toxicol. 9, 627 (1980).

Helle, E., Olsson. M. and Jensen, S.: Ambio, 5, 261, (1976).

Jensen, S. and Athanasiadou, M.: Ambio, in press. (1990).

Jensen, S., Kihlström, J.E., Olsson, M., Lundberg, C. and Örberg, J.: Ambio, 6, 239, (1977).

Olsson, M. and Reutergårdh, L.: Ambio, 15, 103, (1986).

Reijnders, P.J.H.: Nature, 324, 456, (1986).

Platanow, N.S. and Karstad, L.K.: Canad. J. Comp. Med. 37, 391 (1973).

Sandegren, F., Olsson, M. and Reutergårdh, L.: In Der Fischotter in Europa - Verbreitung, Bedrohung, Erhaltung. Selbstverlag, Oderhaus & Göttingen. 107,(1980).