Reproductive failure in common seals feeding on fish from polluted coastal waters

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The population of common seal *Phoca vitulina* in the westernmost part of the Wadden Sea, The Netherlands, has collapsed during the past few decades[1,2]. Between 1950 and 1975 the population dropped from more than 3,000 to less than 500 animals. Comparative studies of common seal populations from different parts of the Wadden Sea reveal that pup production has declined sharply only in the western (Dutch) part[2–4]. A comparative toxicological study[5] on the levels of heavy metals and organochlorines in tissues of seals from the western and northern parts of the Wadden Sea shows that only the polychlorinated biphenyl (PCB) levels differ significantly. This is predominantly a result of PCB pollution from the river Rhine[6–9], which mainly affects the western (Dutch) part. PCBs are thought to be responsible for the low rate of reproduction in Dutch common seals on the basis of epidemiological and experimental data on the ability of PCBs to interfere with mammalian reproduction[5,10]. Here I report that reproductive failure in common seals from the Dutch Wadden Sea is related to feeding on fish from that polluted area. This is the first demonstration of a causal relationship between naturally occurring levels of pollutants and a physiological response in marine mammals.

An experiment with two groups of 12 female common seals was carried out to test the detrimental effects of PCBs on seal reproduction. Each group, consisting of 7 seals from the east coast of the United Kingdom and 5 seals from the Museum of Natural History at Texel, was fed a diet containing different levels of pollutants. Group 1 received fish (predominantly plaice, flounder and dab, with some eelpout and hooknose) caught in the western part of the Wadden Sea. Group 2 received fish (mainly mackerel) from the north-east Atlantic. The fish were maintained at −28° C and thawed before being fed to the seals. The diets are comparable with respect to nutritional quality, except for fat levels; this was compensated for by a high daily intake. Residue analysis for aldrin, dieldrin, endrin, heptachlor, hepox, α,β,γ-hexachlorocyclohexane, pentachlorobenzene, hexachlorobenzene, *pp'*-dichlorodiphenyl-dichloroethylene (DDE), *op'*-dichlorodiphenyl-dichloroethane, *pp'*-dichlorodiphenyl-dichloroethane and PCBs showed statistically significant differences between the two diets for PCBs and *pp'*-DDE. The average daily intake (during ~2 years) was 1.5 mg PCBs and 0.4 mg *pp'*-DDE for group 1, and 0.22 mg and 0.13 mg for group 2 (J. P. Boon, P.J.H.R., J. Dols, and P. F. Wensvoort, unpublished data). Three males receiving Atlantic fish were alternated between both groups during the mating period. To detect whether hormonal regulation was affected by pollutants, blood samples were taken regularly and serum concentrations of progesterone and oestradiol-17β were determined. Because the timing of oestrus differs for individuals, all profiles have been synchronized by taking the day of previous delivery as day zero and adjusting the profiles of non-pregnant animals accordingly. Although there is some published information about the hormonal changes during reproduction in seals[11,13], none of these studies reported the changes during a complete annual reproductive cycle in a specific group of females. The oestradiol-17β and progesterone concentration profile of the control group (Fig. 1*e,g*) illustrate the normal annual cycle. The number of non-pregnant animals in the control group is too small to consider their reproductive cycle as 'normal' for non-pregnant females.

No statistically significant differences (Spearman, $p<0.005$) were found in the progesterone and oestradiol-17β profiles of pregnant seals from groups 1 and 2. Identical tests for the non-pregnant animals in both groups gave the same result. This is important as it implies that the differences in diet do not influence hormone patterns. The patterns resemble those for eutherian mammals in general[14,15]. Pinnipeds are classified as having an obligate and seasonal embryonic diapause[16,17]. In common seals in this study implantation probably occurred at

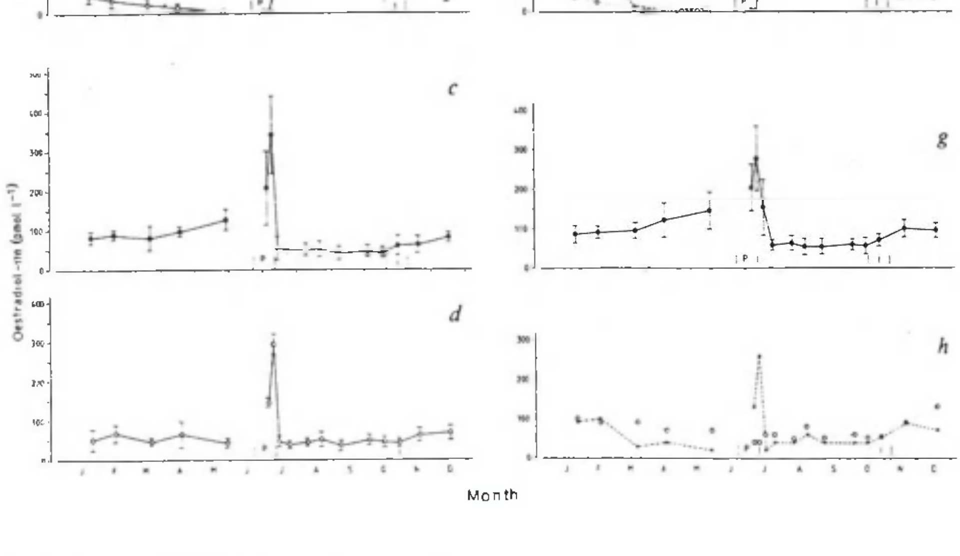

Fig. 1 Circulating serum concentrations of progesterone and oestradiol-17β during the reproductive season 1983–84, in common seals feeding on fish from the Wadden Sea (group 1) and on Atlantic fish (group 2). *a–d*, Group 1 (*a,b,c,d*): (●---●), pregnant seals ($n=4$); (○—○), non-pregnant seals ($n=8$). *e–h*, Group 2: (●---●), pregnant seals ($n=10$); (×---×) and (○—○) non-pregnant seals. Vertical bars, 1 s.d.; p = period over which previous parturitions occurred; i = implantation period. Blood samples were obtained from veins in the hind flippers after restraining the animals on a V-shaped bench. The blood (~8 ml) was collected in vacutainers, centrifuged and the serum stored frozen at −20 °C. Serum concentrations of progesterone and oestradiol-17β were determined by radioimmunoassay. The intra run coefficients of variation for progesterone and oestradiol-17β were consistently below 3.0% and 3.05% respectively.

the end of October or beginning of November, and was characterized by an increase in oestradiol-17β levels (Fig. 1, *c, g*).

The reproductive success was significantly lower in group 1 than in group 2 (Table 1; $p<0.02$, Fisher's exact probability test). The hormone profiles of the non-pregnant animals in group 1 show that the effect occurs at a stage of the reproductive process around implantation; the follicular, luteal and post-implantation phases until the end of gestation are not affected. These findings corroborate the results from experiments with mink[18,19] where PCBs impaired reproduction: ovulation, mating and implantation occurred but were followed by early abortion or resorption. These results may be due to hormonal disturbance, to direct dominant-lethal action or to an embryo lethal effect caused by toxicants. Hormonal disturbance may be caused by disruption of the steroid synthetic pathway resulting in reduced circulating levels of hormones. PCBs are known to cause microsomal enzyme induction, accelerating hydroxylation of body steroids such as oestrogens[5]. To determine if pollutants suppress circulating levels of hormones during the implantation period, the four subgroups (pregnant and non-pregnant seals in groups 1 and 2) are compared. A statistical test revealed no difference between progesterone levels of pregnant animals in group 2 and non-pregnant animals in group 1. It is concluded that progesterone levels were not reduced in group 1. The rise in oestradiol levels of the non-pregnant seals in group 2—indicating follical growth—is lacking in non-pregnant seals of group 1, which suggests that the nature of non-pregnancy differs between the groups. Because there were only 2 non-pregnant animals in group 2, this could not be tested statistically. The results also demonstrate that the levels of oestradiol in all group 1 seals are lower than those in group 2 combined, ($p<0.05$, Wilcoxon), although the initial rise in some of the animals of group 1 (Fig. 1*c*) was apparently sufficient to ensure reproductive success. The mechanisms behind the smaller increase in oestradiol levels and its consequences for the priming effect on the endometrium, as well as the maternal rejection response[20-22], will be discussed in detail elsewhere. Hypotheses about impaired steroid binding capacity by PCBs[23], a dominant-lethal action and an embryo lethal effect cannot be tested with the information available.

I conclude that the reproductive success of the seals receiving the diet with the highest level of pollutants was significantly decreased. No circumannual reduction in levels of circulating hormone levels was observed. The reproductive process is disrupted in the post-ovulation phase. The period around implantation seems to be the most sensitive stage, but no conclusions about the mechanism of action can be drawn. A similar experiment was simultaneously carried out with the American mink *Mustela vison*, a fish-eating mammal with a comparable reproductive physiology. It was designed to test whether pure PCBs had the same effect as the PCB-polluted fish. Three groups of 'Standard' type mink were fed either a basic diet of commercial mink cereal, or the same basic diet supplemented with livers from fish caught in the western part of the Wadden Sea or with Clopen A-60 or A-30. The results show that reproduction is inhibited at very low (25 μg per day) levels of PCB intake and that the effects of the pure PCB diet were identical to those of contaminated fish diet[18].

Irrespective of the precise mechanism involved, the results from this study show that the reproductive failure in common seals from the Dutch Wadden Sea is related to feeding on fish from that polluted area. The available epidemiological experimental data on effects and levels of PCBs in seals and mink fed on fish from this area[5,27] suggest that these organochlorines are the main cause of this failure.

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A common mammalian plan of accessory optic system organization revealed in all primates

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The accessory optic system (AOS), which was described as early as 1870 by Gudden[1], constitutes a distinct midbrain visual pathway in all classes of vertebrates[2]. In non-primate mammals, retinal fibres of this system project to a set of three nuclei[3,4]: the dorsal (DTN), the lateral (LTN) and the medial (MTN) terminal nuclei. Whereas all AOS cells respond to the slow motion of large visual stimuli, the neurons are tuned to complementary directions of movement[5]: horizontal temporo-nasal direction for the DTN, vertical up and down for the LTN and vertical down for the MTN. It has thus been suggested that these nuclei establish a system of retinal coordinates for the detection of whole field motion[6]. As the AOS provides direct and indirect pathways to both oculomotor and vestibular structures[7,8], each of these nuclei is thought to be an essential link in the co-ordination of eye and head movements in relation to movement within the visual field. One problem for the generalization of this theory is that the medial terminal nucleus has never been found in primates. In this report we establish both the existence of this nucleus and its afferent input from the retina in all major groups of primates (prosimians, New and Old World monkeys and apes), indicating a common anatomical plan of organization of the AOS in mammals.

We used both autoradiographic and histochemical anterograde tracing techniques to study the retinal projection to the AOS in the two primate suborders[9] Strepsirhini (prosimians: lemurs and lorises) and Haplorhini (simians: New and Old World monkeys, apes and man). For the Strepsirhines we studied five mouse lemurs (*Microcebus murinus*) and one bushbaby (*Galago demidovii*). Haplorhine species included two marmosets (*Callithrix jacchus*), three macaques (*Macaca fascicularis*) and two gibbons (*Hylobates concolor*). Most animals were injected with a radioactive amino acid mixture (500-