Methods for Assessing the Effects of Chemicals on Reproductive Functions
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Appraisal of Reproductive Failure in Wildlife

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1 FIELD OBSERVATIONS

A number of reports indicate that the seal populations (*Halichoerus grypus, Pusa hispida* and *Phoca vitulina*) in the Baltic area has decreased seriously during the last decades (Bergman, 1956; Hook and Johnels, 1972; Söderberg, 1975). Other observations also suggest decreasing population sizes of some terrestrial species, e.g., pheasants (Göransson, 1980). Simultaneously there has been increasing pollution of our environment, resulting in higher residue levels of DDT, PCBs, heavy metals and other toxic contaminants in the biotic and abiotic components of the ecosystem. Often the reproductive processes of mammals and birds are very sensitive to these toxic chemicals. Therefore, one possible explanation of the decreasing wildlife populations could be the increased incidence of reproductive failure caused by some of these substances.

2 FINDINGS IN INDIVIDUALS FROM DECREASING POPULATIONS

Helle *et al.* (1976a), found that only 27% of the adult female ringed seals (*Pusa hispida*) in the Baltic area are pregnant compared to 80–90% in other areas. Moreover, the non-pregnant individuals demonstrate higher concentrations of DDT and PCBs than the pregnant ones (Helle *et al.*, 1976a, b). About 40% of the studied animals show unilateral or bilateral occlusions of the uterine horns, preventing any passage between the oviduct and the body of the uterus (Helle *et al.*, 1976b). Animals with these pathological changes show even higher levels of DDT and PCBs than the non-pregnant females with normal uteri (Helle *et al.*, 1976b). Therefore, it is tempting to assume the high concentrations of PCBs and/or DDT interfere with fertilization, implantation of ova, fetal growth and/or the maintenance of pregnancy.

Because of some physiological and ecological similarities to the seals (a fish-eating species with delayed implanation), the mink (*Mustela vison*) has been used as animal 'model' in order to verify or falsify the above hypothesis experimen-

tally. PCBs (a mixture of Clophen A 50 and Clophen A 60, prepared in order to obtain a PCB mixture similar to that extracted from Baltic herrings) and/or DDT/DDE (1/1) was given via the food daily for 666 days, including the mating season and the gestation period. The doses varied from 0.02 to 33 mg/kg food corresponding to 6–9900 μg/day per individual.

The reproduction of the mink was not influenced by DDT, even at the highest doses used, in spite of increased liver weights (+ 12%), indicating a stimulation of the activity of the liver (Jensen *et al.*, 1977). On the other hand, there was a close dose–response relationship between the PCB residues in the tissues of the mothers and the number of whelps born. At a mean PCB residue of 48 mg/kg body fat, corresponding to a dose of 330 μg/day per individual (1.1 mg/kg food), the number of whelps significantly decreased; and at 280 mg PCB/kg fat (corresponding to 11 mg/kg food), no whelps were born. The fertilization rate and the duration of the gestation were not influenced by PCBs. Post-mortem examinations revealed normal numbers of implantations, even at the highest doses of PCBs used, but also showed a high frequency of dead and macerated fetuses (Jensen *et al.*, 1977). Later it was demonstrated that limiting the administration of PCBs (2700 μg/day per individual) to 10 days during the later part of the gestation period also resulted in fetal death (Kihlström *et al.*, 1983). Moreover, controls and experimental animals showed the same concentrations of progesterone throughout the pregnancy (Kihlström *et al.*, 1983).

It is generally believed that the persistent chemicals in the environment influence the reproduction via an increased catabolic activity of the liver, thus reducing the blood levels of the steroid hormones regulating the sexual processes. One important conclusion of the studies reviewed above is that this explanation cannot be the entire explanation. Alternative explanations may be an accumulation of the toxicants in the fetuses or a decreased transport of nutrients or excretion products across the placenta.

3 EXPERIMENTAL ANALYSIS

3.1 Placental Perfusion

Fetal death after administration of PCB substances to pregnant animals has been observed in the mink (Jensen *et al.*, 1977, technical PCBs), the rat (Örberg, 1978, 2, 2′, 4, 4′, 5, 5′-hexachlorobiphenyl), the mouse (Merson and Kirkpatrick, 1976, technical PCBs; Török, 1978, 2, 2′-dichlorobiphenyl), and the guinea pig (Brunström *et al.*, 1982, technical PCBs). In order to evaluate the reproductive disturbances caused by environmental pollutants, we have developed a technique for perfusion of the fetal part of the guinea pig placenta *in situ* (Kihlström and Kihlström, 1981). This technique provides viable conditions for the placenta as demonstrated by an active stereoselective transfer of alanine from the mother and by placental impermeability to trypan blue.

Using this method the transfer of [^{14}C] 2, 2′, 4, 4′, 5, 5′-hexachlorobiphenyl

from the maternal circulation to the fetal part of the placenta was shown to proceed rapidly when blood or an albumin solution was used as perfusion medium (Kihlström, 1982). Identical concentrations of the chlorobiphenyl in the maternal and fetal compartments are obtained in 20 minutes. At that time, concentration of the chlorobiphenyl in the perfusion medium exceeds that of the maternal plasma and continues to rise throughout the perfusion (Kihlström, 1982). This placental transfer of hexachlorobiphenyl is highly dependent on the albumin concentration in the medium and fails to occur when albumin is excluded (Kihlström, 1982). In addition, there is an exponential relationship between the accumulation of PCBs in the perfusion medium and albumin concentrations (Kihlström, 1982). This fast penetration of hexachlorobiphenyl through the placenta may be one of the serious hazards to the fetuses.

The same method has been used to study the placental transfer of amino acids in mothers treated with environmental toxicants. Experiments with pregnant guinea pigs given triethyllead chloride (used as an antiknock agent in gasoline) indicate a decreased placental transfer of alanine (Kihlström and Odenbro, 1980). Similar experiments with PCB treated animals using a non-metabolizable amino acid are in progress. This method may become a useful tool for rapid screening of potentially fetotoxic substances.

3.2 Embryotoxic studies of embryonated hens' eggs

Injection of substances into embryonated hens' eggs is an inexpensive and simple method of studying embryotoxicity. However, a major problem is to find a proper vehicle for injection of lipophilic substances into the eggs. In order to find an appropriate vehicle, several different solvents containing a fat-soluble dye were injected on day 4 of incubation and the distribution of the dye studied. On the basis of these studies, an emulsion of lecithin, peanut oil and water has been chosen as the most suitable vehicle. Using this vehicle, a labelled lipophilic substance ([^{14}C] 2, 2′, 4, 5′-tetrachlorobiphenyl) was injected on day 4 of incubation. By means of autoradiography the chlorobiphenyl was detected in the embryos one day later. Five and 25, but not 1, mg Aroclor 1248/kg egg injected into hens' eggs significantly decreased their hatchability. Eggs injected with 25 mg Aroclor 1248/kg exhibited anomalies (clearly visible at candling on day 7) localized in areas around the vessels of the yolk-sac (Brunström and Örberg, 1982). No teratogenic effects have been observed thus far.

Preliminary experiments using the same technique demonstrate that 2, 2′, 4, 5′-tetrachlorobiphenyl is, in this test, at least 100 times more toxic than 3, 3′, 4, 4′-tetrachlorobiphenyl (Brunström and Örberg, 1982), while toxaphenes and chlorinated paraffins seem to demonstrate low embryotoxicity (Brunström and Örberg, 1981). Therefore, this method may be a useful tool for an inexpensive and rapid screening of potentially embryotoxic contaminants.

4 CONCLUSIONS AND RECOMMENDATIONS

The PCB story (Jensen, 1972) and similar toxic chemical releases into the environment point out the necessity of assessing the potential impact of new chemicals before they become widespread in the environment. The reproductive functioning of wildlife is very sensitive to many pesticides, e.g., PCBs; DDT and organic mercurials. Consequently, there may be a reduction of the reproductive rate long before more easily detected effects become manifest. Therefore, there is a need for rapid and inexpensive methods for evaluating the reproductive hazards of the increasing number of potentially toxic chemicals. The two techniques, placental perfusion and the modified embryotoxic studies of embryonated hens' eggs described above, fulfil these demands and may thus become useful tools for a rapid and inexpensive appraisal of potential reproductive failures in wildlife.

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