Reproductive and Developmental Effects of Endocrine-Disrupting Chemicals on Marine Mammals

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Introduction

A large number of xenobiotics with endocrine-disrupting properties have been detected in marine mammal tissue (Wagemann and Muir 1984, Aguilar and Borrell 1995, Colborn and Smolen 1996, Reijnders 1996). Although most of the species known to be contaminated in this way are coastal, considerable concentrations of such compounds have even been detected in at least one cetacean that forages in deep water, the sperm whale (de Boer et al. 1998). Only in a few studies have observed reproductive disorders been found to be associated with certain chlorinated hydrocarbons and their metabolites. Among these studies are those involving ringed and gray seals in the Baltic Sea (Helle 1980, Bergman and Olsson 1985), beluga whales in the St. Lawrence River (Béland et al. 1992), harbor seals in the Wadden Sea (Reijnders 1980), and California sea lions in the eastern Pacific Ocean (DeLong et al. 1973). The findings of these studies, although strongly suggestive, have not been conclusive. The etiology of the observed disorder has usually been uncertain, and proof of a causal relationship between exposure to a specific contaminant and an impact on the reproductive or endocrine system has remained elusive.

This paper discusses the issue from an epidemiological point of view. My focus is on marine mammal species in which disorders in hormone concentrations, reproductive problems, or pathological conditions associated with hormonal imbalance have been observed. An overview of associations between organochlorines and marine mammal reproduction and endocrinology is presented in Table 10. I conclude with some comments on the possibilities of monitoring and evaluating problems related to xenobiotic-induced reproductive impairment and endocrine disruption in marine mammals.

Basic Pharmacology and Physiology of Hormones in Reproduction and Early Development

Hormones are messenger compounds. Their release leads to functional changes in an organism's cells, tissues, and organs. They are produced by endocrine organs and delivered into the bloodstream. A small percentage of hormones circulate freely, but the majority are bound to transport proteins. The free hormones diffuse into the tissues and cells. Target cells possess specific receptor molecules which bind to particular hormones, leading to activation of the receptor. Steroid hormones as well as thyroid hormones play important roles in reproduction and early development. They are discussed separately here.

In vertebrates, sex hormones belong to a group of steroids which are synthesized from cholesterol. Steroids can be divided into four functional groups: the three sex hormone groups (androgens, estrogens, and progesterone) and the glucocorticosteroids. The latter plays a role in regulating metabolism and growth and in osmoregulation.

In mammals, steroid hormones are mainly synthesized in the adrenals, gonads, and placenta. The production of each of the sex hormones is localized in a specific gland such as testosterone in the testes, estrogens and progesterone in the ovaries, and progesterone, estrogens, and testosterone in the adrenals. These organs can, in many species,
## Table 10. Associations between organochlorines and marine mammal reproduction and endocrinology

<table>
<thead>
<tr>
<th>Reproductive Disorders</th>
<th>Species</th>
<th>Location</th>
<th>Mode of Action</th>
<th>Certainty of Mode of Action</th>
<th>Contaminants</th>
<th>Certainty of Contaminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation failure&lt;sup&gt;1&lt;/sup&gt;</td>
<td>harbor seal</td>
<td>Wadden Sea</td>
<td>hormone metabolism enhancement</td>
<td>2</td>
<td>PCBs/metabolites</td>
<td>2</td>
</tr>
<tr>
<td>Failed implantation or fetal development&lt;sup&gt;1&lt;/sup&gt;</td>
<td>beluga whale</td>
<td>St. Lawrence River</td>
<td>unknown</td>
<td>4</td>
<td>organochlorines</td>
<td>4</td>
</tr>
<tr>
<td>Sterility&lt;sup&gt;2&lt;/sup&gt;</td>
<td>gray and ringed</td>
<td>Baltic Sea</td>
<td>organochlorine induced uterine pathology</td>
<td>3</td>
<td>PCBs/DDE/MSFs</td>
<td>3</td>
</tr>
<tr>
<td>Premature pupping&lt;sup&gt;3&lt;/sup&gt;</td>
<td>California sea lion</td>
<td>Southern California Bight</td>
<td>microsomal enzyme induction; steroid mimicking</td>
<td>3</td>
<td>PCBs/DDT</td>
<td>3</td>
</tr>
<tr>
<td>Hormonal Disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low vitamin A thyroid hormones&lt;sup&gt;3&lt;/sup&gt;</td>
<td>harbor seal</td>
<td>Wadden Sea</td>
<td>binding competition</td>
<td>1</td>
<td>PCBs/metabolites</td>
<td>2</td>
</tr>
<tr>
<td>Reduced testosterone&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Dall's porpoise</td>
<td>North Pacific Ocean</td>
<td>unknown</td>
<td>4</td>
<td>DDE/possibly PCBs</td>
<td>4</td>
</tr>
<tr>
<td>Lowered estradiol level&lt;sup&gt;6&lt;/sup&gt;</td>
<td>implantation</td>
<td>Wadden Sea</td>
<td>enhanced hydroxylation</td>
<td>3</td>
<td>PCBs/metabolites</td>
<td>2</td>
</tr>
<tr>
<td>Morphological Disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skull lesions (osteoporosis, paradentitis)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>harbor seal</td>
<td>Baltic Sea, Wadden Sea</td>
<td>infection/hyper-adrenocortical</td>
<td>2</td>
<td>PCBs/DDT/metabolites</td>
<td>3</td>
</tr>
<tr>
<td>Exostosis&lt;sup&gt;7&lt;/sup&gt;</td>
<td>harbor seal</td>
<td>Baltic Sea, west coast of Sweden</td>
<td>unknown</td>
<td>4</td>
<td>PCBs/DDT/metabolites</td>
<td>3</td>
</tr>
<tr>
<td>Testis abnormalities&lt;sup&gt;8&lt;/sup&gt;</td>
<td>minke whale</td>
<td>Southern Ocean</td>
<td>unknown</td>
<td>4</td>
<td>organochlorines</td>
<td>4</td>
</tr>
<tr>
<td>Adrenal hyperplasia&lt;sup&gt;9&lt;/sup&gt;</td>
<td>beluga whale</td>
<td>St. Lawrence River</td>
<td>unknown</td>
<td>4</td>
<td>organochlorines</td>
<td>4</td>
</tr>
<tr>
<td>Hermaphroditism&lt;sup&gt;10&lt;/sup&gt;</td>
<td>beluga whale</td>
<td>St. Lawrence River</td>
<td>genetic/environmental</td>
<td>4</td>
<td>PCBs/DDT</td>
<td>4</td>
</tr>
</tbody>
</table>


Also produce small quantities of the other sex hormones. The specific pathway of biosynthesis in mammals is in the following order: cholesterol → pregnenolone → progesterone → androstenedione → testosterone → estradiol (Fig. 5).

The hypothalamus and pituitary are responsible in most vertebrates for the regulation of hormone concentrations and the timing of reproduction and sexual development. The hypothalamus produces a peptide, gonadotropic releasing hormone (GnRH), which stimulates the pituitary to synthesize follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones regulate the synthesis of progesterone, estradiol, and testosterone. Through the production of FSH and LH, the synthesis (positive and negative feedback) of sex hormones and gonadal development are regulated.
Thyroid hormones are important in the structural and functional development of sex organs and the brain, both intrauterine and postnatal. They are synthesized by the thyroid gland under stimulation through the thyroid stimulating hormone produced by the pituitary. The form that is most important as a biological parameter is thyroxine (T₄, tetraiodothyroxine).

**Disorders in Hormone Concentrations**

A negative correlation has been observed between testosterone levels and tissue concentrations of DDE and possibly PCBs in Dall's porpoises (Subramanian et al. 1987). In a semi-field experiment with harbor seals, Reijnders (1986, 1990) found that lower levels of 17β-estradiol occurred around the time of implantation. A possible explanation for the observed lower hormone levels in both Dall's porpoises and harbor seals would be an increased breakdown of steroids as a consequence of PCB- or PCB metabolite-induced enzyme activity. Enhanced steroidogenesis caused by some metals and a PCB mixture (Arochlor 1254) has been demonstrated in vitro for gray seals (Freeman and Sanganlang 1977). Furthermore, increased metabolism of PCBs as a result of P450-enzyme induction has already been demonstrated in marine mammals (Tanabe et al. 1988, Boon et al. 1992). A second explanation might be that PCB and DDE, or metabolites thereof, bind to hormone carrier proteins and/or hormone receptors. If such binding were to occur, either tissue metabolism of steroids would be hindered or binding of steroids to receptor proteins in target tissue would be impeded. It is conceivable that both mechanisms — increased steroid breakdown and the binding of xenobiotic compounds to carrier proteins or hormone receptors — could operate in tandem.

Figure 5. Major steroidogenic pathways in mammalian endocrine tissues. Black arrows indicate P450-mediated conversions.
With respect to the first mechanism, Troisi and Mason (1998) found experimentally that rates of progesterone and testosterone metabolism in harbor seals were negatively correlated with both PCB concentrations and the level of P450-enzyme induction. Applying the findings by Troisi and Mason (1998) to Dall's porpoise, the lowered levels of testosterone reported by Subramanian et al. (1987) could be explained by a hindrance in the transformation of precursors for testosterone by PCB or DDE metabolites. This postulated explanation obviously needs further testing.

Concerning the second mechanism, it is known that PCB metabolites, in particular PCB-methyl sulfones, bind to uteroglobin (Patnode and Curtis 1994). Since we did not find any receptor interference in in vitro pilot experiments with harbor seal blood, this mechanism is not considered a likely explanation for the observed reproductive failure in this species. The finding by Troisi and Mason (1998) of decreasing metabolism of progesterone and testosterone in liver microsomes does not allow a conclusion about a possible impediment of the transformation of progesterone and testosterone, that might in turn have led to the lower estradiol levels observed in harbor seals. This is because those transformations occur in the reproductive organs. However, it has been found by Funae and Imaoka (1993) that sex-dependent cytochrome P-450 isoenzyme patterns exist. It is known that induction of CYP1A(2) causes increased hydroxylation of estradiol, leading to enhanced excretion and hence lower levels of estradiol. It has been demonstrated that induction of CYP1A is significant in harbor seals (Boon et al. 1987). I postulate that enhanced breakdown of estradiol, through enzyme-induced metabolism by organochlorines, is a plausible mode of action to explain the lower levels of estradiol observed in harbor seals. Estradiol has a priming effect on the proliferation of the endometrium, in effect preceding proliferation of the luminal and glandular epithelium under the influence of progesterone. The lower levels of estradiol could have impaired endometrial receptivity and prevented successful implantation of the blastocyst. Further studies are planned to investigate this possibility.

Decreased levels of thyroid hormones have been found in harbor seals (Brouwer et al. 1989) as a consequence of competition between a hydroxylated metabolite of PCB-77 and thyroid hormones for binding to a transport protein, transthyretin (TTR). Such a hypothyroid condition can have significant effects on early development and later reproductive performance. Fetal accumulation of hydroxylated PCB-metabolites has been found to occur in experimental animals and may also occur in seals (Brouwer et al. 1998). Given that thyroids are involved in the development of Sertoli and Leydig cells (spermatogenesis), brain development, and early development of the sex organs, further research in this area is warranted.

Green et al. (1996) and Green (1997) provide additional information on lactational transfer of PCB-methyl sulfones (PCB-MSFs) from gray seals to their offspring. They report that the summed concentrations of PCB-MSFs (in lipid) are approximately 5% of the total PCB concentration. A similar ratio was found in seal milk. The uptake of PCB-MSFs is therefore quantitatively important. Moreover, the pups excrete only approximately 0.5% from the amount they ingest. In contrast to the mobilization of PCB-congeners from maternal blubber to milk, which is negatively correlated with congeners-lipophilicity, the metabolization of PCB-MSFs is independent from the degree of chlorination as well as the chlorination pattern. The ratio PCB-MSFs/total PCBs is therefore higher in milk than in blubber. This metabolization process, offering a certain protection against the more lipophilic PCBs, obviously does not work in the case of the PCB-MSFs. The significance of this finding remains unclear.

Reproductive Disorders

Clear cases of hermaphroditism were observed in about two out of 120 examined beluga whales in the St. Lawrence River (De Guise et al. 1994, P. Béland, pers. comm.). This condition has been
attributed to hormonal disturbance in early pregnancy, whereby normal differentiation of male and female organs was disrupted. Research is ongoing to test that hypothesis and to acquire information on the underlying mechanism.

In examined mature female Baltic seals, 30% of the gray and 70% of the ringed seals exhibited partial or complete sterility, caused by stenosis and occlusions. Recent studies suggest that PCB- and DDE-methyl sulfones are the toxic compounds responsible for these abnormalities (Olsson et al. 1994). A plausible hypothesis is that early pregnancy is interrupted, perhaps via decreased uteroglobin binding due to the methyl sulfone-occupation or low hormone levels, followed by development of pathological disorders. Toxic effects in several steps in the brain-hypothalamic-hypophyseal-adrenal-placental axis could be involved in the latter stage (Reijnders and Brasseur 1992). Further investigations are needed to elucidate this phenomenon. DeLong et al. (1973) found premature pupping in California sea lions to be associated with high PCB and DDE levels. The concurrent finding in the animals of pathogens with known potential to interfere with pregnancy rendered it impossible to attribute causation specifically to either of the organochlorines.

Abnormal testes — transformation of epididymal and testicular tissue — have been observed in North Pacific minke whales (Fujise et al. 1998). A possible relationship with levels of organochlorines has been postulated. Further histological examinations and pathology assessments are being conducted to investigate this phenomenon.

Other Hormone-Related Disorders

Besides sterility, a suite of pathologies and disorders have been observed in Baltic seals. These include exostosis in harbor seal skulls (Mortensen et al. 1992) and osteoporosis in gray seal skulls (Olsson et al. 1994). Similar disorders have been found in harbor seals in the Wadden Sea (Stede and Stede 1990). This disease complex is characterized as hyperadrenocorticism. It is unclear whether hyperadrenocorticism is manifested in early development.

The research field between reproductive biology and immunology is in an early stage of development. It is known that both the humoral (antibodies) and cellular (lymphocytes) aspects of the immune system are regulated by estrogens and androgens (Grossman 1985). Disruption in steroid hormone balance might therefore lead to malfunctioning of the immune system. Of relevance in the present context is the role of progesterone and estradiol in preventing the maternal-fetal rejection response. This relationship could help explain the observed problems of harbor seals in the Wadden Sea as these problems occurred at around the time of implantation. Also, corticosteroids (Wilckens and de Rijk 1997) and thyroid hormones (Brouwer et al. 1989, 1998) are involved in immune functioning. The possible effects of xenobiotic-caused thyroid and corticosteroid hormone imbalances on early development and reproduction are insufficiently known.

Monitoring and Evaluation of Effects and Related Research Needs

There are serious impediments to monitoring and evaluating hormone-related xenobiotic effects. Firstly, the majority of the present tests do not measure transgenerational influence, yet several disorders occur only in the adult stage or offspring. Secondly, gene expression is affected, not gene constitution. Therefore, mutagenicity endpoint tests are not particularly relevant. Thirdly, many tests are in vitro, and this complicates investigations of disruption in neurobehavioral function and reproductive morphological development. Finally, some xeno-estrogens only become biologically active after in vivo metabolism (e.g., methoxychlor).

Development of hormone-responsive cell cultures as biomarkers could provide a partial solution (see Colborn et al. 1993). Biomarkers are available to measure exposure to xenobiotics,
including xeno-estrogens (Fossi 1994). A series of recent studies describe techniques to investigate metabolism of PCBs, PCDDs, PCDFs, and toxaphenes in marine mammals (Troisi and Mason 1997, Boon et al. 1997, 1998, Letcher et al. 1998). These provide opportunities to measure exposure of marine mammals to, for example, xeno-estrogens and other endocrine-disrupting contaminants. However, preparatory research has to be carried out to adapt existing biomarker protocols for use in marine mammal studies. This includes sampling tissue, particularly of neonatal and juvenile animals, to analyze for (1) thyroid hormones and vitamin A (important in cell differentiation) in blood and brain; (2) thyroid hormones and vitamin A in brain and liver; (3) estrogen-receptor binding capacity of ovarian, brain, and liver tissue; (4) glial fibrillary acidic protein and synaptophysins in brain; (5) P450-enzyme induction (i.e., CYP-1A) in liver, brain, and uterus; and (6) levels of xeno-estrogens in blubber, blood, liver, and brain.

To enhance evaluation of the results, in terms of biological significance, it is important to carry out investigations and compare trends in animals from relatively unpolluted as well as highly polluted areas. Initially, a choice has to be made for model compounds as well as for model species occurring over a gradient of pollution. Studies in relatively clean areas serve mainly to obtain reference values for exposure to contaminants and indicators for the status (functioning) of the studied population. Studies in highly polluted areas make it possible to carry out research on pathology in neonatal and juvenile animals. In combination with analyses for contaminants and associated physiological and pathological responses, these studies will facilitate development of a multiple response concept as described by Reijnders (1994) and Reijnders and de Ruiter-Dijkman (1995). The development of techniques to extrapolate observed individual responses, and thereby evaluate population-level and possibly ecosystem-level effects, is equally important.

References


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