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

Peter J. H. Reijnders
Institute for Forestry and Nature Research
Department of Ecosystem Health
P.O. Box 167, 1790 AD Den Burg, The Netherlands

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

	Where in Process	Species	Location	Mode of Action	Certainty of Mode of Action[a]	Contaminants	Certainty of Contaminants[a]
Reproductive Disorders							
Implantation failure[1]	implantation	harbor seal	Wadden Sea	hormone metabolism enhancement	2	PCBs/-metabolites	2
Failed implantation or fetal development[2]	implantation	beluga whale	St. Lawrence River	unknown	4	organochlorines	4
Stenosis and occlusions[3]	post partum	gray and ringed seals	Baltic Sea	organochlorine induced uterine pathology	3	PCBs/DDE/MSFs	3
Sterility[4]	unknown	gray and ringed seals	Baltic Sea	pathologic sterility	1	PCBs/DDT/-metabolites	3
Premature pupping[5]	late gestation	California sea lion	Southern California Bight	microsomal enzyme induction; steroid mimicking	3	PCBs/DDT	3
Hormonal Disorders							
Low vitamin A /thyroid hormones[6]	all ages	harbor seal	Wadden Sea	binding competition	1	PCBs/-metabolites	2
Reduced testosterone[7]	adults	Dall's porpoise	North Pacific Ocean	unknown	4	DDE/possibly PCBs	4
Lowered estradiol level[8]	implantation	harbor seal	Wadden Sea	enhanced hydroxylation	3	PCBs/-metabolites	2
Morphological Disorders							
Skull lesions (osteoporosis, paradentitis)[9]	predominantly adults	harbor and gray seals	Baltic Sea, Wadden Sea	infection/ hyper- adrenocortical	2	PCBs/DDT/-metabolites	3
Exostosis[10]	all ages	harbor seal	Baltic Sea, west coast of Sweden	unknown	4	PCBs/DDT/-metabolites	3
Testis abnormalities[11]	immatures and adults	minke whale	Southern Ocean	unknown	4	organochlorines	4
Adrenal hyperplasia[12]	unknown	beluga whale	St. Lawrence River	unknown	4	organochlorines	4
Hermaphroditism[13]	fetal	beluga whale	St. Lawrence River	genetic/environmental	4	PCBs/DDT	4

[a] 1 = definite, 2 = probable, 3 = possible, 4 = unknown

[1]Reijnders et al. 1986, Reijnders, this report; [2]Martineau et al. 1994; [3]Helle 1980, Helle et al. 1976; [4]Bergman and Olsson 1985; [5]DeLong et al. 1973; [6]Brouwer et al. 1989; [7]Subramanian et al.1987; [8]Reijnders, this report; [9]Bergman et al. 1992, Stede and Stede 1990; [10]Mortensen et al. 1992; [11]Fujise et al. 1998; [12]Béland et al. 1992; [13]De Guise et al. 1994.

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), whichstimulates 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 17ß-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 Sangalang 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.
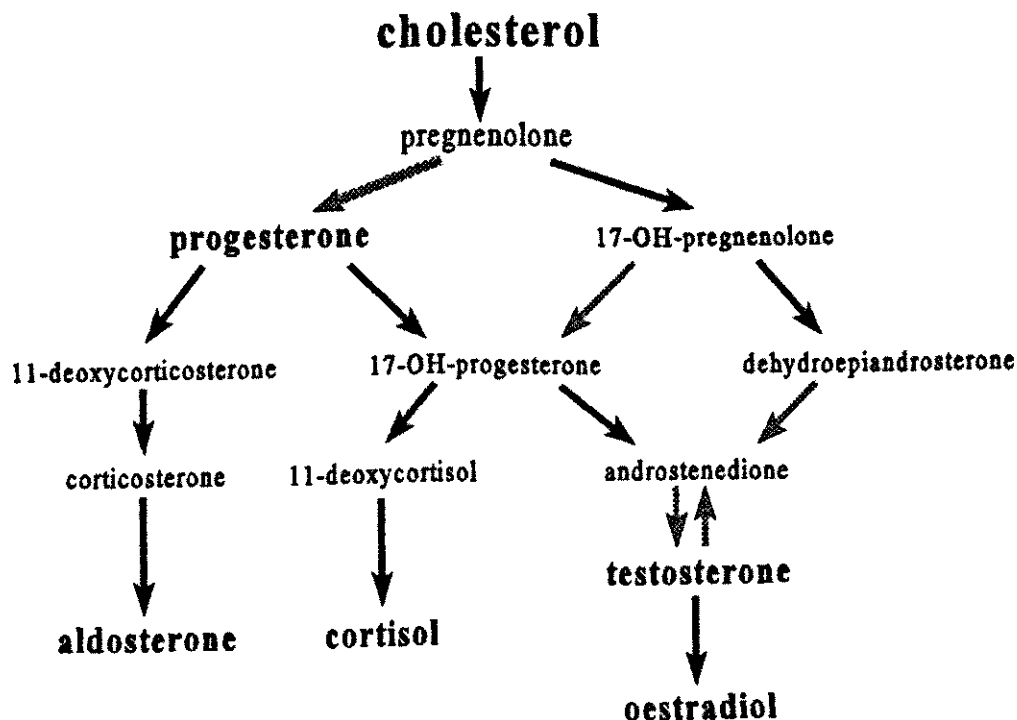

Figure 5.   Major steroidogenic pathways in mammalian endocrine tissues. Black arrows indicate P450-mediated conversions.

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 congener-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

Aguilar, A., and A. Borrell. 1995. Pollution and harbour porpoises in the eastern North Atlantic: a review. Report of the International Whaling Commission (Special Issue 16):231–242.

Béland, P., S. De Guise, and R. Plante. 1992. Toxicology and pathology of St. Lawrence marine mammals. Report to World Wildlife Fund–Canada by National Institute of Ecotoxicology, Montreal. 95 pp.

Bergman, A., and M. Olsson. 1985. Pathology of Baltic ringed seal and grey seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? Finnish Game Research 44:47–62.

Bergman, A., M. Olsson, and S. Reiland. 1992. Skullbone lesions in the Baltic gray seal (*Halichoerus grypus*). Ambio 21:517–519.

Boer, J. de, P. G. Wester, H. J. C. Klamer, W. E. Lewis, and J. P. Boon. 1998. Do flame retardants threaten ocean life. Nature 394:28–29.

Boon, J. P., P. J. H. Reijnders, J. Dols, P. Wensvoort, and M. T. J. Hillebrand. 1987. The kinetics of individual polychlorinated biphenyl congeners in female harbour seals (*Phoca vitulina*), with evidence for structure-related metabolism. Aquatic Toxicology 10:307–324.

Boon, J. P., E. van Arnhem, S. Jansen, N. Kannan, G. Petrick, J. C. Duinker, P. J. H. Reijnders, and A. Goksöyr. 1992. The toxicokinetics of PCBs in marine mammals with special reference to possible interactions of individual congeners with the cytochrome P450-dependent monooxygenase system: an overview. Pages 119–159 *in* C. H. Walker and D. R. Livingstone (eds.), Persistent Pollutants in Marine Ecosystems. Pergamon, Oxford.

Boon, J. P., J. van der Meer, C. R. Allchin, R. J. Law, J. Klungsöyr, P. E. G. Leonards, H. Splidd, E. Storr-Hansen, C. McKenzie, and D. E. Wells. 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. Archives of Environmental Contamination and Toxicology 33:298–311.

Boon, J. P., H. M. Sleiderink, M. S. Helle, M. Dekker, A. van Schanke, E. Roex, M. T. J. Hillebrand, H. J. C. Klamer, B. Govers, D. Pastor, D. C. Morse, P. G. Wester, and J. de Boer. 1998. The use of a microsomal *in vitro* assay to study phase I biotransformation of chlorobornanes (toxaphene) in marine mammals and birds: possible consequences of biotransfor-

mation for bioaccumulation and genotoxicity. Comparative Biochemistry and Physiology (C) 121:385–403.

Brouwer, A., P. J. H. Reijnders, and J. H. Koeman. 1989. Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal *Phoca vitulina*. Aquatic Toxicology 15:99–106.

Brouwer, A., D. C. Morse, M. C. Lans, G. Schuur, A. J. Murk, E. Klason-Wehler, A. Bergman, and T. J. Visser. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicology and Industrial Health 14 (1/2):59–84.

Colborn, T., and M. J. Smolen. 1996. Epidemiological analysis of persistent organochlorine contaminants in cetaceans. Reviews of Environmental Contamination and Toxicology 146:91–171.

Colborn, T., F. S. vom Saal, and A. M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives 101:378–384.

De Guise, S., A. Lagacé, and P. Béland. 1994. True hermaphroditism in a St. Lawrence beluga whale (*Delphinapterus leucas*). Journal of Wildlife Diseases 30:287–290.

DeLong, R. L., W. G. Gilmartin, and J. G. Simpson. 1973. Premature births in Californian sea lions: association with high organochlorine pollutant residue levels. Science 181:1168–1170.

Fossi, M. C. 1994. Nondestructive biomarkers in ecotoxicology. Environmental Health Perspectives 102:49–54.

Freeman, H. C., and G. B. Sangalang. 1977. A study on the effects of methylmercury, cadmium, arsenic, selenium, and a PCB, (Arochlor 1254) on adrenal and testicular steroidogeneses *in vitro*, by the gray seal *Halichoerus grypus*. Archives of Environmental Contamination and Toxicology 5:369–383.

Fujise, Y., R. Zenati, and H. Kato. 1998. An examination of the W-stock hypothesis for North Pacific minke whales, with special reference to some biological parameters using data collected from JARPA surveys from 1994 to 1997. Paper SC/50/RMP12 presented to the IWC Scientific Committee, Oman. 14 pp.

Funae, Y., and S. Imaoka. 1993. Cytochrome P450 in rodents. *In* J. B. Schenkman and H. Greim (eds.), Cytochrome P450. Springer Verlag, Berlin, Germany.

Green, N. J. L. 1997. A study of polychlorinated biphenyls in the lactating grey seal. PhD thesis, Lancaster University, Lancaster, U.K. 175 pp.

Green, N. J. L., P. van Raat, K. Jones, and P. de Voogt. 1996. PCBs and their methyl sulfonyl metabolites in the maternal blubber, milk, pup blubber and faeces of grey seals. Organohalogen Compounds 29:453–457.

Grossman, C. J. 1985. Interactions between the gonadal steroids and the immune system. Science 227:257–261.

Helle, E. 1980. Lowered reproductive capacity in female ringed seals (*Phoca hispida*) in the Bothnian Bay, northern Baltic Sea, with special reference to uterine occlusions. Ann. Zoologica Fennica 17:147–158.

Helle, E., M. Olsson, and S. Jensen. 1976. PCB levels correlated with pathological changes in seal uteri. Ambio 5:261–263.

Letcher, R., W. Lewis, M. van den Berg, W. Seinen, and J. Boon. 1998. Biotransformation of coplanar PCBs, PCDDs, and PCDFs and specific cytochrome P450 isoenzyme activities in harbour seal (*Phoca vitulina*): selective inhibition of *in vitro* metabolism in hepatic microsomes. Organohalogen Compounds. In press.

Martineau, D., S. De Guise, M. Fournier, L. Shugart, C. Girard, A. Lagacé, and P. Béland. 1994. Pathology and toxicology of beluga whales from the St. Lawrence estuary, Québec, Canada. Past, present and future. The Science of the Total Environment 154:201–215.

Mortensen, P., A. Bergman, A. Bignert, H.-J. Hansen, T. Härkönen, T., and M. Olsson. 1992. Prevalence of skull lesions in harbour seals (*Phoca vitulina*) in Swedish and Danish museum collections: 1835–1988. Ambio 21:520–524.

Olsson, M., B. Karlsson, and E. Ahnland. 1994. Diseases and environmental contaminants in seals from the Baltic and the Swedish west coast. The Science of the Total Environment 154:217–227.

Patnode, K. A., and L. R. Curtis. 1994. 2,2',4,4',5,5'- and 3,3',4,4',5,5'- hexachlorobiphenyl alteration of uterine progesterone and estrogen receptors coincides with embryotoxicity in mink (*Mustela vison*). Toxicology and Applied Pharmacology 127:9–18.

Reijnders, P. J. H. 1980. Organochlorine and heavy metal residues in harbour seals from the Wadden Sea

and their possible effects on reproduction. Netherlands Journal of Sea Research 14:30–65.

Reijnders, P. J. H. 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324:456–457.

Reijnders, P. J. H. 1990. Progesterone and oestradiol-17ß concentration profiles throughout the reproductive cycle in harbour seals (*Phoca vitulina*). Journal of Reproduction and Fertility 90:403–409.

Reijnders, P. J. H. 1994. Toxicokinetics of chlorobiphenyls and associated physiological responses in marine mammal, with particular reference to their potential for ecotoxicological risk assessment. The Science of the Total Environment 154:229–236.

Reijnders, P. J. H. 1996. Organohalogen and heavy metal contamination in cetaceans: observed effects, potential impact and future prospects. Pages 205–217 in M. P. Simmonds and J. D. Hutchinson (eds.), The Conservation of Whales and Dolphins. John Wiley and Sons, Chichester, U.K.

Reijnders P. J. H., and S. M. J. M. Brasseur. 1992. Xenobiotic induced hormonal and associated developmental disorders in marine organisms and related effects in humans. Pages 131–146 in T. Colborn and C. Clement (eds.), Chemically Induced Alterations in Sexual and Functional Development: the Wildlife/Human Connection. Princeton Scientific Publishing Company, Inc., Princeton, N.J.

Reijnders, P. J. H., and E. M. de Ruiter-Dijkman. 1995. Toxicological and epidemiological significance of pollutants in marine mammals. Pages 575–587 in A. S. Blix, L. Wallöe, and O. Ulltang (eds.), Whales, Seals, Fish and Man. Elsevier Science BV., Amsterdam.

Stede, G., and M. Stede. 1990. Orientierende Untersuchungen von Seehundschädeln auf pathalogische Knochenveränderungen. Pages 31–53 in Zoologische und Ethologische Untersuchungen zum Robbensterben. Inst. f. Haustierkunde, Kiel, Germany.

Subramanian, A. N., S. Tanabe, R. Tatsukawa, S. Saito, and N. Miyazaki. 1987. Reductions in the testosterone levels by PCBs and DDE in Dall's porpoises of northwestern North Pacific. Marine Pollution Bulletin 18:643–646.

Tanabe, S., S. Watanabe, H. Kan, and R. Tatsukawa. 1988. Capacity and mode of PCB metabolism in small cetaceans. Marine Mammal Science 4:103–124.

Troisi, G. M., and C. F. Mason. 1997. Cytochromes P450, P420 and mixed-function oxidases as biomarkers of polychlorinated biphenyl (PCB) exposure in harbour seals (*Phoca vitulina*). Chemosphere 35:1933–1946.

Troisi, G. M., and C. F. Mason. 1998. Steroid hormone metabolism in harbour seals (*Phoca vitulina*) exposed to polychlorinated biphenyls (PCBs). Marine Environmental Research. In press.

Wagemann, R., and D. G. C. Muir. 1984. Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1297. 97 pp.

Wilckens, T., and R. de Rijk. 1997. Glucocorticoids and immune function: unknown dimensions and new frontiers. Immunology Today 18:418–424.