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# Topic 4.4
# Effects of endocrine disruptors in aquatic mammals*

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*Abstract*: In the last few decades, various studies have shown that aquatic mammals are sensitive to the toxicological effects of certain xenobiotic compounds, including the large class of endocrine-disrupting chemicals (EDCs). Since some EDCs, particularly organochlorines, tend to bioaccumulate and biomagnify in the aquatic food chain, various aquatic mammals, particularly those high in the food chain, such as pinnipeds, odontocete cetaceans, and polar bears, are potentially "at risk". The main aim of this chapter is to define the state of the art on effects of endocrine disruptors in aquatic mammals, both freshwater and marine. Another aim is to formulate recommendations for future research in this field and finally to define what can be done internationally for hazard/risk assessment and communication of the findings.

## INTRODUCTION

Today there are over 4500 species of mammals on our planet. Aquatic mammals constitute a small percentage of this number, but have an extremely important ecological role in the marine and fresh water environments. Many aquatic mammal species, particularly marine mammals such as pinnipeds and odontocete cetaceans, as top-predators, exercise a dramatic regulatory control at community levels. Their decrease or disappearance in some areas could drastically alter community structure. In the last few decades, various studies have shown that several aquatic mammal species are sensitive to the toxicological effects of certain xenobiotic compounds [1–8], including the large class of endocrine-disrupting chemicals (EDCs). Fish-eating aquatic mammals may be extremely vulnerable to EDCs because of (a) their position in the food chain, (b) dependence on an aquatic/marine food web, (c) they live in areas influenced by industry and agriculture, and (d) their specific reproductive physiology [9]. Since several EDCs and particularly organochlorines, tend to bioaccumulate and biomagnify in the aquatic food chain [10,11], various aquatic mammals, particularly top-predators such as pinnipeds, odontocete cetaceans [12], and polar bears, are potentially "at risk" due to EDC contamination. Indeed, both reproductive and/or nonreproductive toxicities have been found in many fish-eating mammals that live in riverine or coastal areas where contaminant burdens are generally higher than in the open ocean.

EDCs are a structurally diverse group of compounds that may damage the health of humans, wildlife, fisheries, and their progeny, by interaction with the endocrine system [13–16]. They include chemicals used heavily in the past in industry and agriculture, such as polychlorinated biphenyls and organochlorine pesticides, and chemicals under current use, such as plasticizers and surfactants. Many

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known EDCs are estrogenic, affecting reproductive function. Because of the lipophilic persistent nature of most xenobiotic estrogens and their metabolites many bioaccumulate and biomagnify [10,11]; organochlorines are an example.

There are four types of organochlorine endocrine disruptors [17–35] commonly found in aquatic mammals [36–38] (Table 1): environmental estrogens, environmental androgens, antiestrogens, and antiandrogens. These endocrine disruptors act by mimicking steroid sex hormones, both estrogens and androgens, by binding to hormone receptors or influencing cell pathways (environmental estrogens and androgens), or by blocking and altering hormonal binding to hormone receptors (antiestrogens, antiandrogens).

**Table 1** DDT metabolites and the PCB congeners with known estrogenic capacity commonly detected in aquatic mammals as measured in ER binding assays.

| | Activity | Activity references | Potency[1] | aER binding $IC_{50}$ (mM)[2] | ER binding $IC_{50}$ (mM)[4] | ER binding RBA %[5] |
|---|---|---|---|---|---|---|
| DDTs | Estrogen | 17 | | | | |
| *p,p´*-DDT | Estrogen | 23 | | >50[3] | >1000 | |
| | Antiandrogen | 17,20,24 | | | | |
| | ER agonist | 23 | | | | |
| *o,p´*-DDT | Estrogen | 17,20,23,25,26,27 | ++ | 9.1 | 5 | 0.1 |
| | Antiestrogen | 22 | + | >50[3] | >1000 | |
| | Antiandrogen | 22,23 | +++ | | | |
| | ER agonist | 23 | | | | |
| *p,p´*-DDE | Estrogen | 17,20,24,28 | + | | | |
| | Antiestrogen | 22,23 | + | | | |
| | Androgen | 22 | + | | | |
| | Antiandrogen | 22,23,29 | ++ | | | |
| | ER agonist | 23 | | | | |
| | AR agonist | 23 | | | | |
| | AR antagonist | 18,23 | | | | |
| *o,p´*-DDE | Estrogen | 20,26 | | 37.25 | | |
| | ER agonist | 23 | | | | |
| *p,p´*-DDD | ER agonist | 23 | | | >1000 | |
| *o,p´*-DDD | ER agonist | 23 | | 2.26 | | |
| PCBs | | 17,20,23,26,30,31 | | | | |
| Arochlor | Estrogen | 23,27,32 | | | | |
| 1260 | Effect on sexual differentiation | 23,27,32 | | | | |
| | Gonadal abnormalities | 23,27,32 | | | | |
| 95 | Estrogen | 21,33 | + | | | |
| 99 | Estrogen | 21,33 | ++ | | | |
| 101 | Estrogen | 21,34 | | | | <0.001 |
| 118 | Antiestrogen | 21,34 | ++ | | | |
| 153 | Estrogen | 21,35 | +++ | | | 0.004 |

[1]The most potent chemical for each activity was assigned a potency of four plus signs (++++), and the potency of all the chemicals expressed relative to this [21,22].
[2]Inhibitor concentrations necessary for 50 % inhibition ($IC_{50}$) of [$^3$H]17β-estradiol binding to estrogen receptor (aER) in the alligators. The aER binding $IC_{50}$ value for 17β-estradiol was 0.0078 µM.
[3]Compounds that inhibited [$^3$H]17β-estradiol but were insoluble at concentrations necessary to achieve 50 % inhibition [19].
[4]Inhibitor concentrations necessary for 50 % inhibition ($IC_{50}$) of [$^3$H]17β-estradiol binding to ER in the rats. The ER binding $IC_{50}$ value for 17β-estradiol was 0.002 µM [18].
[5]Relative estrogen receptor-binding affinities (RBAs). Competitive binding with estradiol in rat uterine ER preparations [21].

The main aim of this chapter is to define the state of the art on the potential effects of endocrine disruptors, with particular emphasis on organochlorines in aquatic mammals, both freshwater and marine. Another aim is to formulate recommendations for future research in this field and finally to define what can be done internationally for hazard/risk assessment and communication the findings.

## STATE OF THE ART OF EDCs IN AQUATIC MAMMALS

High concentrations of polychlorinated biphenyls (PCBs) and DDTs known to be potential EDCs (Table 1) have been detected in different aquatic mammals. Moreover, polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), that for their physicochemical properties are less transportable from terrestrial to marine environmental, are principally accumulated in terrestrial animals [39,40]. Both in vivo and in vitro studies show that these contaminants exhibit a broad spectrum of antiestrogenic responses [17]. The concentration-dependent effects of several PCDD and PCDF congeners as antiestrogens were determined in the aryl hydrocarbon (Ah)-responsive MCF-7 human breast cancer cell lines. For the PCDDs and PCDFs, the order of antiestrogenic potency was 2,3,7,8-tetrachlorodibenzo-*p*-dioxin > 2,3,7,8-tetrachlorodibenzofuran > 2,3,4,7,8-pentachlorodibenzofuran > 1,2,3,7,9-pentachlorodibenzofuran > 1,3,6,8-tetrachlorodibenzofuran [41]. Top predators often acquire large burdens of persistent pollutants through biomagnification of compounds received from contaminated prey [12,42]. Pinnipeds and cetaceans have relatively large amounts of blubber for insulation that readily retain considerable concentrations of highly persistent organochlorine insecticides, PCBs and dioxins (several thousand ppt in toxicity equivalents, TEQs), implying a higher risk from exposure of wildlife to dioxins and related compounds [40]. The estimated TEQ concentrations in the blubber of some cetacean species, such as northern right whale dolphin (*Lissodelphis borealis*) and Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) from the northern North Pacific; Dall's porpoise (*Phocoenoides dalli*) from the Japan Sea; striped dolphin (*Stenella coeruleoalba*) off Sanriku and Fraser's dolphin (*Lagenodelphis hosei*) off Kii Peninsula, Japan; and hump-backed dolphin (*Sousa chinensis*) and finless porpoise (*Neophocaena phocaenoides*) from Hong Kong, exceeded the levels associated with immunosuppression in harbor seals (*Phoca vitulina*) [43]. Many reports have been published on PCDD and PCDF levels in aquatic mammals: Baikal seals [*Phoca (Pusa) siberica Gmel*] from Lake Baikal [44]; harp seals (*Pagophilus groenlandicus*) from Greenland Sea [45]; gray whale (*Eschrichtius robustus*); killer whale (*Orcinus orca*); false killer whale (*Pseudorca crassidens*); Risso's dolphin (*Grampus griseus*) and Dall's porpoise from British Columbia, and harbor porpoises (*Phocoena phocoena*) from British Columbia and central California [46]; beluga whales (*Delphinapterus leucas*) from the St. Lawrence River estuary [47]; dugong (*Dugong dugon*) from the Great Barrier Reef [48], and sea lions (*Otaria flavescens*) from Argentina [39]. The maximum levels were in the ringed seal from the Baltic Sea with 170 pg/g fat of TEQ. In any of these papers, relationships are investigated between PCDD and PCDF levels and endocrine disruptor effects. Some of the best evidence of the relationship between organochlorine chemicals (OCs) and reproductive toxicity comes from semi-field studies on seals [5] and mustelids [49].

## FRESHWATER MAMMALS

Population of mustelids, including mink (*Mustela vison*) and Canadian otter (*Lutra canadiensis*), have declined in areas of the Great Lakes where these species have a high percentage of contaminated fish in the diet [50]. Following outbreaks of reproductive failure in commercial ranching operations, laboratory experiments showed that mink are extremely sensitive to OCs, particularly PCBs and dioxins. Wren [50] tried to test the hypothesis of reproductive dysfunction and population decline in wild mink feeding on Great Lakes fish. However, the evidence was not conclusive. A more recent study using mink demonstrated that exposure to low doses of PCBs over 18 months results in impaired reproduction [51]. Data from Ohio show that the mink harvest between 1982 and 1987 from contaminated counties bordering Lake Erie was consistently lower (380 animals per year) than those from counties far away from Lake Erie (850 animals per year), suggesting an effect of chemicals on mink populations [50]. Preliminary studies from Ontario also suggest that mink harvest is lower in potentially "high PCB exposure areas" compared with lower exposure areas.

Evidence is also available on the effect of EDCs on the harvest otters from four New York State counties adjacent to Lake Ontario and the St. Lawrence River. The harvest data from these four counties show that, between 1960 and early 1970 otter harvest remained stable and then increased. Increased harvest is consistent with improved water quality of Lake Ontario in the past 15 years. It is not possible to draw a causal link between the status of mink and otter populations and exposure to OCs from the Great Lakes without toxicological experiments, so a large amount of research and data analysis needs to be undertaken.

Harding et al. [52] assessed chlorinated hydrocarbon contamination of mink and river otters in Columbia and Fraser River systems of northwestern North America, in relation to their morphological condition. Livers were analyzed for residues of organochlorine pesticides, PCBs, dibenzo-*p*-dioxins, and dibenzofurans. Contaminant levels were relatively low compared to those documented in other North American populations, although they ranged higher than those detected during an earlier survey of these regional populations. Although a few individual animals with gross abnormalities of the reproductive system did not show high levels of contamination, there was a significant negative correlation between total PCB concentrations (such as Aroclor 1260) and baculum length in juvenile mink ($r = 0.707$; $p = 0.033$; $n = 8$).

In ranch-reared mink, used as a model in an experimental trial to investigate the potential effects of exposure to two petroleum products on sea otters (*Enhydra lutris*), females exposed to bunker C fuel oil in the diet had significantly reduced reproductive success (3.4 kits/female) although their offspring's only exposure to the petroleum products was in utero or during nursing. Sea otter populations consuming contaminated food or colonizing previously oiled habitats may therefore have reduced reproductive success [53]. In addition, the European otter (*Lutra lutra*) population in Europe declined dramatically in 1960s to 1980s, and exposure to PCBs leading to impaired reproduction has been considered a major cause of this decline [54]. A study of otter populations and levels of organochlorine pesticide residues and PCBs in otter feces (spraints) was made on rivers in East Anglia, England. Population and contaminant levels were compared against target values, the first based on the index value of a stable population of otters on the River Severn (Wales) and the second (4 mg/kg) taken as the "no effect level" for all individual contaminants [55]. Forty-four % of samples had concentrations of contaminants exceeding the "level of concern". Contamination, especially by PCBs, is therefore regarded as a factor affecting otter populations, which may not be viable in East Anglia without repeated releases of captive-bred animals [36].

## MARINE MAMMALS

The marine environment is exposed to a variety of EDCs by an array of sources, for example, industrial effluent and agricultural run-off. The result is the potential exposure of marine mammals to EDCs, not only cetaceans and pinnipeds, but also mammals such as the polar bears that feed on marine organisms.

### Polar bears

The polar bear (*Ursus maritimus*) is a top predator of the Arctic marine ecosystem. Polar bears prey primarily on ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*), which live exclusively on the sea ice. They also consume larger prey such as walruses (*Odobenus rosmarus*) and white whales.

Pseudo-hermaphroditism observed in polar bears is thought to be an effect of EDCs. Two female polar bears at Svalbard (Norway) had both female and male genitals [56]. The two pseudo-hermaphrodites were genetically females but also had small ponise in front of their vagina. On different occasions in another two bears, aberrant genitalia morphology with a high degree of chloral hypertrophy has also been reported and classified as female pseudo-hermaphroditism. The observed rate of female pseudo-hermaphroditism in the Svalbard area was 1.5 % (4/269). The authors believe that the pseudo-hermaphroditism observed could be a result of endocrine disruption by OCs, especially PCBs, which concentrate in fat to very high levels.

Skaare et al. [57] found an association between organochlorine contaminants and retinol and thyroid hormones $T_4$ and triiodothyronine ($T_3$) in blood plasma of polar bears caught at Svalbard. Retinol concentration and the ratio of total $T_4$ ($TT_4$) to free $T_4$ ($FT_4$) decreased linearly with increasing concentrations of PCBs and HCB. The consequences of these associations for individuals and populations is unknown. Reduced plasma retinol concentration may be indicative of incipient retinol deficiency which manifests as impaired growth and development of epithelial tissue and reduced immune system and reproductive function. Low cub survival was reported in polar bears from Svalbard [58]. Immunoglobulin G (IgG) levels and OCs were determined in blood plasma of these polar bears [8]. IgG concentrations increased with age and were significantly higher in males than females. IgG was correlated negatively with PCB levels and with three PCB congeners (IUPAC numbers 99, 194, and 206). HCB was also correlated negatively with IgG, suggesting an immunotoxic effect.

A new compound, 4-hydroxyheptachlorostyrene (4-OH-HpCS) was identified as a major component in the chlorinated phenolic compound fraction of plasma of polar bears captured in the Nunavut Territory (Canada). Levels of 4-OH-HpCS ranged from 2.89 to 22.9 ng/g wet weight (w.w.) in polar bear plasma ($n = 30$) representing 2.89–24.8 % of total quantified chlorinated phenolic compounds [59]. Transthyretin (TTR) is assumed to be the main plasma protein responsible for specific binding of phenolic compounds in plasma, because most of the compounds, including 4-OH-HpCS, have a similar structure to the natural ligand, thyroxine ($T_4$). The study of Sandau et al. [59] indicated that the phenolic metabolites of relatively minor contaminants have the capacity to bind to circulating proteins, and their significance as potential endocrine-disrupting agents. The extent to which they disrupt thyroid hormone and retinol homeostasis cannot be underestimated.

## Pinnipeds

Populations of harbor seals from the Dutch Wadden Sea had low reproductive success and declining population numbers that were attributed to the impact of PCBs [60]. Other studies showed that female harbor seals fed fish from the polluted Wadden Sea had a lower reproductive success (50 %) than seals fed less-contaminated fish. Implantation failure was found to be associated with lower levels of 17β-estradiol [61], induced by EDCs.

There is ample evidence that populations of Baltic ringed seals (*Phoca hispida bothnica*) and grey seals (*Halichoerus grypus*) have declined markedly over the past 100 years [62]. Overhunting and habitat destruction might have been the contributing factors, however, it is generally accepted that persistent pollutants (PCBs, DDTs), which adversely affect reproductive performance, was the major cause. Earlier studies showed that seals in the Baltic Sea (of which the Bay of Bothnia is the northernmost part) carry very high burdens of DDT and PCBs. Pregnant seals are reported [62] to have a much lower content of these compounds than nonpregnant animals. Females with over 70 mg PCB/kg of extractable fat are apparently unable to reproduce. Only 19 % of the grey seals in the Baltic proper have less than 70 mg PCB compared with 25 % in the Bay of Bothnia. In ringed seals, the values ranged from 39 to 54 % in the northernmost part of the Bay of Bothnia [63,64].

Most studies reporting concentrations of organochlorines in pinnipeds have investigated ringed, grey, and harbor seals [65]. Very few studies have been carried out on pinnipeds from the southern hemisphere. Before 1980, the highest mean wet-weight blubber concentrations of DDT and related metabolites (911 +/– 582 μg/g) were recorded in sea lions from California. The highest pre-1980 blubber concentrations of PCBs (1470 +/– 922 μg/g) were recorded in harbor seals from the Netherlands. Since 1980, the highest blubber concentrations of PCBs have been recorded in grey seals of the Dee estuary, UK [46.79 (10.17–116.68) μg/g]. The highest DDT concentrations measured in individual pinnipeds are in the 1–15 μg/g range recorded in ringed, grey, and harbor seals and Australian fur seals. Reported effects on pinnipeds suggested to result from organochlorine contamination, include skeletal deformities and impact on reproduction, such as uterine occlusion.

## Cetaceans

Research indicates that some cetaceans, particularly odontocetes, have detectable and sometimes extremely high levels of substances known or suspected to be EDCs such as PCBs, DDTs, chlorinated pesticides, brominated flame retardants, and tributyltin (TBT). Biologists have reported a range of effects of these chemicals on cetaceans including immunosuppression, cancer, skin lesions, secondary infections and diseases, sporadic die-offs, and reduced reproductive success. There are several examples suggesting that exposure to OC insecticides and PCBs affected endocrine function and reproduction in marine mammals. For example, transformation of epididymal and testicular tissue has been observed in North Pacific minke whales (*Balaenoptera acutorostrata*) [66].

Another example is the endangered beluga whales of the St. Lawrence estuary (SLE), now amongst the most contaminated animals on earth, with tumors and reproductive problems [67,68]. A population of approximately 650 beluga inhabits a short segment of the SLE. Over 17 years (1983–1999), Martineau et al. [68] have examined 129 (or 49 %) of 263 SLE beluga carcasses reported stranded. De Guise et al. [69] reported in these animals a true hermaphrodite *Delphinapterus leucas.* This animal had two testicles, two separate ovaries, and the complete ducts of each sex; cervix, vagina, and vulva were absent. Mature spermatozoa were found in the lumen of seminiferous tubules in the testicles, and numerous involuted corpora lutea were recognized in the ovaries.

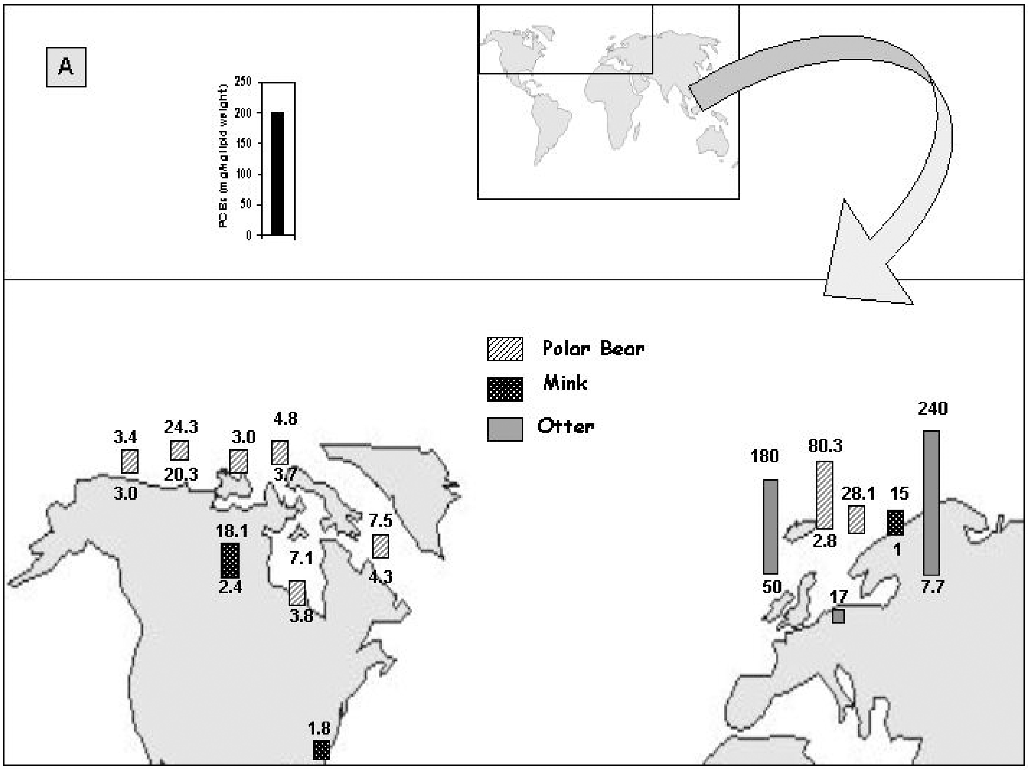
Increasing residue levels of PCBs and DDE in the blubber of Dall's porpoises were found to have a negative association with testosterone levels in blood. Testosterone levels decreased in a statistically significant way with increasing DDE concentrations. These results suggest that current levels of environmental contamination by persistent organochlorines can cause an imbalance in sex hormones and subsequent reproductive abnormalities in the wild. The other hormone measured, aldosterone, which has no sexual function, showed levels that were independent of the effects of PCBs and DDE [70].

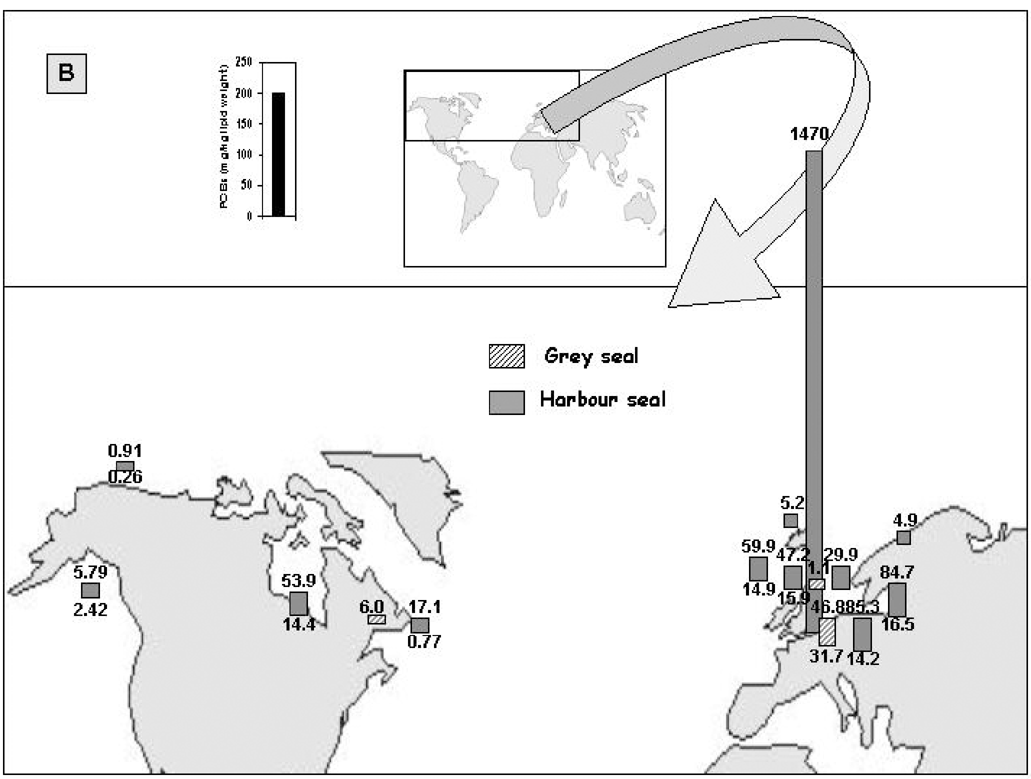
In the endangered bowhead whale (*Balaena mysticetus*), not all sexual activity leads to conception. Pseudo-hermaphroditism has been reported in at least two males with testicular feminization [71], a relatively high incidence, as only 76 bowheads were closely examined between 1980 and 1989 [72]. Concentrations of most organochlorine contaminants in biological material of 20 bowhead whales from Barrow (Alaska, USA) were low compared to those in tissues of other cetaceans, especially odontocetes, but it is impossible to exclude the possibility that pseudo-hermaphroditism is independent of these contaminants.

## Hot spots: The case study of Mediterranean cetaceans

Man-made EDCs range across all continents and oceans. Some geographic areas are potentially more threatened than others (Fig. 1); one of these is the Mediterranean Sea. This basin has limited exchange of water with the Atlantic Ocean, and is surrounded by some of the most heavily populated and industrialized countries in the world. Levels of some xenobiotics are therefore much higher here than in other seas and oceans [73,74]. In this peculiar environment, top predators (such as large pelagic fish and marine mammals) tend to accumulate large quantities of polyhalogenated aromatic hydrocarbons (PHAHs) and toxic metals [37,42]. Levels of PHAHs in a top predator of the Mediterranean, the striped dolphin, are 1–2 orders of magnitude higher than in Atlantic and Pacific individuals of the same species [75] (Fig. 1).

Nondestructive studies of Mediterranean cetaceans have revealed the hazard to which odontocete species are exposed in relation to EDCs in blubber [76]. Significant differences in total levels of

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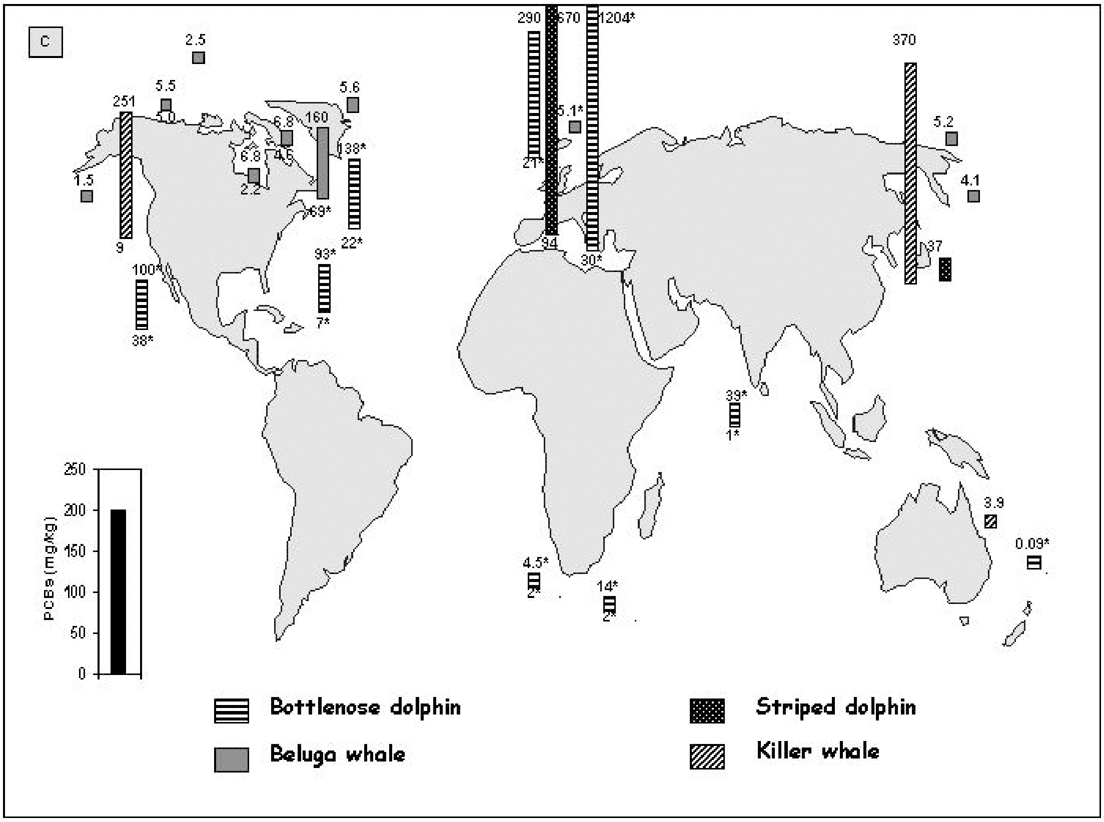


**Fig. 1** Minimum and maximum mean levels of total PCBs (mg/kg lipid weight) in marine fish-eating mammals from various part of the world. **A** = freshwater mammals and polar bears; **B** = pinnipeds; **C** = cetaceans. Numbers with * in C are in mg/kg lipid weight, the others in mg/kg wet weight. Data in this figure were taken from the references listed in this paper.

organochlorine EDCs were found between odontocetes and mysticetes. Highest mean levels were found in striped dolphins [OC-EDCs = 40.0 µg/g fresh weight (f.w.)], followed by bottlenose dolphins (OC-EDCs = 24.3 µg/g f.w.) and common dolphins (OC-EDCs = 15.0 µg/g f.w.). Differences in organochlorine bioaccumulation and consequent potential risk due to endocrine disruptors, are primarily related to different positions in the marine food chain. Of the Mediterranean cetaceans investigated, the species with the highest levels of OCs and the highest biomarker response seems to be the striped dolphin. Interspecies differences in susceptibility to EDCs must be considered because high levels of contaminants and high biomarker responses do not necessarily mean high risk for the species.

Some cetaceans, such as the common dolphin, have almost completely disappeared from the Mediterranean Sea. Using skin biopsy as an ecotoxicological tool to study intraspecific susceptibility to contaminants, a high statistical correlation was found between benzo(a)pyrene monoxigenase (BPMO) activity and total DDTs, *p,p′*-DDE, *o,p′*-DDT, total PCBs and 153 congener (Pearson correlations significant at $p < 0.05$) in male specimens of common dolphin. This suggests that EDCs may be a major stress factor for common dolphin populations in the Mediterranean Sea. Similar correlation (Spearman rank correlations significant at $p < 0.05$) was obtained in fin whales sampled in the Ligurian Sea from 1992 to 1995 [77] between BPMO activity and organochlorine levels in skin biopsy specimens from males, but not females or males and females together. Future studies are needed to explore the role of detoxification enzymes and estrogen receptors (ERs) in interspecies susceptibility to EDC contaminants using fibroblast cell cultures of different species [78].

## FUTURE RESEARCH NEEDS

The above review demonstrates that research is necessary to resolve uncertainties that remain in this field of study. Strengthening international collaboration in the following broad research areas is a step in this direction. Some specific recommendations for future research are described below.

### Endocrine-mediated effects in aquatic mammals

Basic knowledge on endocrine systems of aquatic mammals and the range of mechanisms by which endocrine disruptors, especially organochlorines, interfere with reproductive success, immune function, and neurobehavioral parameters at key stages of life cycles, will provide the platform for understanding differences in the response of different species, both in a qualitative and quantitative sense. It is important to investigate interspecies susceptibility to EDCs, particularly in species living in hot spot areas (e.g., polar bears in Svalbard, Baltic ringed seals in the Baltic Sea, common dolphin in the Mediterranean Sea—see Fig. 1).

### New methodology: Nondestructive techniques in the study of aquatic mammals

Development of a series of nondestructive techniques to evaluate residue levels and biomarker responses is recommended, in place of the lethal approach, for hazard assessment and conservation of endangered species of aquatic mammals [79]. In the last 10 years, much interest has been shown internationally [80] toward skin biopsies as a sensitive nonlethal technique for hazard assessment of free-ranging cetaceans exposed to EDCs [42,81,82].

The biopsy dart method has been used successfully on a range of cetacean species (at least 30) in oceans and the Mediterranean Sea. The response of fin whales to the dart was no change in behavior in 80 % of the cases. Skin biopsy is a powerful tool for toxicological studies for the following reasons: (a) it allows collection of a large number of samples across a wide geographic range; (b) it allows collection of sequential samples from the same animal if identified by photo identification or other techniques; (c) it is suitable for residue analysis of PHAHs, including dioxin group chemicals (suitable for calculation of TEQs), heavy metals, and polycyclic aromatic hydrocarbons (PAHs); (d) it is suitable for biomarker analysis: induction of MFO by enzyme assay (BPMO) and immunohistochemical assay (CYP1A1); DNA damage; fibroblast cell culture.

In conclusion, a number of successful studies show that cetacean skin biopsies are a powerful nonlethal tool for assessing ecotoxicological risk in marine mammals and aspects of feeding ecology and food preferences. In this context, it is also essential to develop more specific and sensitive biomarkers for detecting endocrine-mediated effects in individuals and populations.

### Monitoring hot spots and species/populations at risk

Marine mammals are at the top of the food chain, and much monitoring data has been collected in the last few decades on tissue levels of persistent organic pollutants (POPs) from various species in different parts of the world (Fig. 1). It is essential to expand basic long-term monitoring programs of "sentinel" aquatic mammal species, using nonlethal techniques, particularly in "hot spots". This work can provide baseline data on population status in relation to EDC exposure. It is essential to improve international collaboration and cooperative research (e.g., Pollution 2000+) to assess exposure and effects of EDCs on a more global basis, particularly PCBs chosen as model compounds in selected species such as bottlenose dolphins and harbor porpoises. For conservation of biodiversity, it is important to focus on population/subgroups most likely to be susceptible to EDCs.

### Future management

Management is essential at international level in order to define the main lines of hazard/risk assessment and communication to follow.

### Causal criteria for assessing EDCs in aquatic mammals: A proposed framework

The UNEP volume entitled *Global Assessment of the State-of-the-Science of Endocrine Disruptors* [83] proposed a powerful framework to examine the hypothesis that chemicals with endocrine activity are causing adverse effects in wildlife populations. The framework that can be applied to hazard assessment of aquatic mammals begins with a clear statement of "hypothesis", which contains two distinct elements. First, the outcome of concern (e.g., decreased reproductive function in Baltic seals) is linked to a putative stressor (e.g., PCBs) that is affecting populations. Second, exposure to EDC stressors is postulated to result in endocrine-mediated events that ultimately lead to the outcome of concern. In order to evaluate the scientific evidence regarding their potential relationship we use scientific evidence on five aspects: (1) ***Temporality*** (e.g., high levels of PCBs are strongly correlated with reduced reproductive success in Baltic seal populations); (2) ***Strength of the association*** (e.g., the link between adverse reproductive outcomes and chemical etiology is weak); (3) ***Consistency of the observations*** (e.g., observation on a global scale provides evidence that the reproductive system of Baltic seals may be susceptible); (4) ***Biological plausibility*** (e.g., results of semi-field studies with Baltic seals provide general links with OCs exposure and reproductive outcome); and (5) ***Evidence of recovery*** (e.g., reproduction improves as levels of contamination decline).

## MANAGEMENT, HAZARD/RISK ASSESSMENT, AND RISK COMMUNICATION

The proposed framework could enable identification of "aquatic mammal populations potentially at risk" and "hot spots" in which management of hazard/risk assessment and risk communication can be applied (see "hot spot areas" in Fig. 1). It is necessary to strengthen international collaboration in the following broad activities:

- Continuous monitoring of aquatic mammal populations in "hot spots" compared to the same species in control areas. Hazard assessment of "populations potentially at risk" in particular "hot spot" areas.
- International agreement for the reduction of input of new EDCs into the freshwater environment and coastal areas.
- International agreement for scientific research on a global scale focused on monitoring, conservation, and protection of endangered species of aquatic mammals.
- Identification of human populations "potentially at risk" due to consumption of cetacean-based whale products (identification of the "maximum daily input of EDCs").
- Transfer of scientific information to regulatory agencies at regional and international level for regulation and conservation.

## CONCLUSION

Current knowledge makes it sufficiently evident that some aquatic mammal species, such as certain seal populations, have been adversely affected by environmental contaminants in the EDC category, but evidences are mounting to show at least indirectly, that the chemicals grouped as EDCs are affecting several aquatic mammals populations through some endocrine-dependent mechanisms. Research has largely focused on compounds that persist and bioaccumulate in organisms. Future research on endocrine-mediated effects in aquatic mammals, the development of new nondestructive monitoring methodologies, and validation of hazard/risk assessment procedures in endangered species/populations could enable better future protection and conservation of these ecologically essential animals.

## REFERENCES


1. A. Bernhoft, J. U. Skaare, O. Wiig, A. E. Derocher, H. J. S. Larsen. *J. Toxicol. Environ. Health Part A* **59** (7), 561–574 (2000).
2. UNEP. *UNEP/POPS/CONF/4*, 22, Stockholm, Sweden (2001).
3. T. Colborn. *Environ. Toxicol. Chem*. **17** (1), 1–2 (1998).
4. A. Arukke, F. R. Knudsen, A. Goksoyr. *Environ. Health Perspect.* **105**, 418–422 (1997).
5. S. Tanabe, S. Watanabe, H. Kan, R. Tatsukawa. *Mar. Mamm. Sci.* **4**, 103–124 (1988).
6. T. Colborn, F. S. vom Saal, A. M. Soto. *Environ. Health Perspect.* **101**, 378–384 (1993).
7. T. Colborn, D. Dumanoski, J. P. Myers. *Our Stolen Future,* Dutton, Penguin Books, New York (1996).
8. T. Colborn, M. J. Smolen, R. Rolland. *Toxicol. Ind. Health* **14** (1/2), 9–23 (1998).
9. B. Gillesby and T. Zacharewski. *Environ. Toxicol. Chem.* **17** (1), 3–14 (1998).
10. H. O. Adami, L. Lipworth, L. Titus-Ernstoff, H. Chung-cheng, A. Hamberg, U. Anhlborg, J. Baron, D. Trichopoulos. *Cancer Cause Control* **6**, 551–566 (1995).
11. W. R. Kelce, C. R. Stone, S. C. Laws, L. E. Gray. *Nature* **375**, 581–586 (1995).
12. P. M. Vonier, D. A. Crain, J. A. McLachlan, L. J. Guillette Jr., F. A. Steven. *Environ. Health Perspect.* **104**, 1318–1322 (1996).
13. P. W. Wong and I. N. Pessah. *Mol. Pharmacol.* **49**, 740–751 (1996).
14. G. Hansen Larry. *Environ. Health Perspect.* **106** (1), 171–189 (1998).
15. P. Sohoni and J. P. Sumpter. *J. Endocrinol.* **158**, 327–339 (1998).
16. K. Hilscherova, M. Machala, K. Kannan, A. L. Blankenship, J. P. Giesy. *Environ. Sci. Pollut. Res.* **7** (3), 159–171 (2000).
17. K. Nesaretnam, D. Corcoran, R. R. Dils, P. Darbre. *Mol. Endocrinol.* **10**, 923–936 (1996).
18. J. A. Nelson. *Biochem. Pharmacol.* **23**, 447–451 (1974).
19. S. Safe, B. Astroff, M. Harris, T. Zacharewski, R. Dickerson, R. Romkes, I. Biegel. *Pharmacol. Toxicol.* **69**, 400–409 (1991).
20. A. M. Soto, C. Sonneschein, K. L. Chung, M. F. Fernandez, N. Olea, F. O. Serrano. *Environ. Health Perspect.* **103** (7), 113–122 (1995).
21. L. R. Cooper and R. J. Kavlock. *J. Endocrinol*. **152**, 159–166 (1997).
22. S. H. Safe. *Environ. Health Perspect.* **108**, 487–493 (2000).
23. M. Bergeron, J. Crews, J. A. McLachlan. *Environ. Health Perspect.* **102**, 780–781 (1994).
24. S. H. Safe. *Crit. Rev. Toxicol.* **24** (2), 87–149 (1994).
25. M. B. Matta, C. Cairncross, R. M. Kocan. *Environ. Toxicol. Chem*. **17**, 99–115 (1998).
26. T. M. Sajjd, Masters Thesis, University of Illinois at Urbana-Champaign, Urbana, IL (1996).
27. M. S. Wolf, D. Camann, M. Gammon, S. D. Stellman. *Environ. Health Perspect.* **105**, 13–14 (1997).
28. M. H. Li, Y. D. Zhao, L. G. Hansen. *Bull. Environ. Contam. Toxicol.* **53**, 583–590 (1994).
29. C. F. Mason and S. M. Macdonald. *Sci. Total Environ.* **138** (1–3), 147–160 (1993).
30. L. Marsili. *IJEP* **13**, 416–452 (2000).
31. A. Aguilar, A. Borrell, P. J. Reijnders. *Mar. Environ. Res.* **53** (5), 425–452 (2002).
32. B. Jimenez, M. J. Gonzalez, L. M. Hernandez, E. Eljarrat, J. Rivera, M. C. Fossi. *Chemosphere* **38** (3), 507–515 (1999).
33. S. Tanabe. *Mar. Poll. Bull.* **45**, 69–77 (2002).

34. V. Krishnan and S. Safe. *Toxicol. Appl. Pharmacol.* **120** (1), 55–61 (1993).
35. M. C. Fossi, L. Marsili, G. Neri, S. Casini, G. Bearzi, E. Politi, M. Zanardelli, S. Panigada. *Mar. Environ. Res.* **50** (1–5), 643–647 (2000).
36. T. B. Minh, H. Nakata, M. Watanabe, S. Tanabe, N. Miyazaki, T. A. Jefferson, M. Prudente, A. Subramanian. *Arch. Environ. Contam. Toxicol.* **39** (3), 398–410 (2000).
37. E. N. Tarasova, A. A. Mamontov, E. A. Mamontova, J. Klasmeier, M. S. McLachlan. *Chemosphere* **34** (11), 2419–2427 (1997).
38. M. Oehme, M. Schlabach, K. Hummert, B. Luckas, E. S. Nordoy. *Sci. Total Environ.* **162** (2–3), 75–91 (1995).
39. W. M. Jarman, R. J. Norstrom, D. C. G. Muir, B. Rosenberg, M. Simon, R. W. Baird. *Mar. Pollut. Bull.* **32** (5), 426–436 (1996).
40. D. C. G. Muir, C. A. Ford, B. Rosenberg, R. J. Norstrom, M. Simon, P. Béland. *Environ. Pollut.* **93** (2), 219–234 (1996).
41. D. Haynes, J. F. Muller, M. S. McLachlan. *Chemosphere* **38** (2), 255–262 (1999).
42. P. E. G. Leonards. Ph.D. thesis, Free University of Amsterdam (1997).
43. C. D. Wren. *J. Toxicol. Environ. Health* **33** (4), 549–85 (1991).
44. B. Brunstrom, B. O. Lund, A. Bergman, L. Asplund, I. Athanassiadis, M. Athanasiadou, S. Jensen, J. Ordberg. *Environ. Toxicol. Chem.* **20**, 2836–2840 (2001).
45. L. E. Harding, M. L. Harris, C. R. Stephen, J. E. Elliott. *Environ. Health Perspect.* **107** (2), 141–147 (1999).
46. J. A. Mazet, I. A. Gardner, D. A. Jessup, L. J. Lowenstine. *J. Wildl. Dis.* **37** (4), 686–692 (2001).
47. A. Roos, E. Greyerz, M. Olsson, F. Sandegren. *Environ. Pollut.* **111**, 457–469 (2001).
48. C. F. Mason and S. M. Macdonald. *Sci. Total Environ.* **138** (1–3), 127–145 (1993).
49. Ø. Wiig, A. E. Derocher, M. M. Cronin, J. U. Skaare. *J. Wildl. Dis.* **34** (4), 792–796 (1998).
50. J. U. Skaare, A. Bernhoft, Ø. Wiig, K. R. Norum, E. Haug, D. M. Eide, A. E Derocher. *J. Toxicol. Environ. Health Part A* **62** (4), 227–241 (2001).
51. Ø. Wiig. *Ursus.* **10**, 25–32 (1998).
52. C. D. Sandau, I. A. T. Meerts, R. J. Letcher, A. J. Mcalees, B. Chittim, A. Brouwer, R. J. Norstrom. *Environ. Sci. Technol.* **34** (18), 3871–3877 (2000).
53. P. J. H. Reijnders. *Neth. J. Sea Res.* **14**, 30–65 (1980).
54. P. J. H. Reijnders. *J. Reprod. Fert.* **90**, 403–409 (1990).
55. ICES. *ICES CM 1993/N:3* (1992).
56. E. Helle, M. Olsson, S. Jensen. *Fauna Flora (Stockholm)* **71** (2), 41–48 (1976).
57. J. G. Vos, E. Dybing, H. A. Greim, O. Ladefoged, C. Lambré, J. V. Tarazona, I. Brandt, A. D. Vethaak. *Crit. Rev. Toxicol.* **30** (1), 71–133 (2000).
58. J. D. Hutchinson and M. P. Simmonds. *Rev. Environ. Contam. Toxicol.* **136**, 123–167 (1994).
59. Y. Fujise, R. Zenati, H. Kato. *SC/50/RMP12 IWC Scientific Committee*, p. 14, Oman (1998).
60. S. De Guise, D. Martineau, P. Béland, M. Fournier. *Environ. Health Perspect.* **103** (4), 73–77 (1995).
61. D. Martineau, K. Lemberger, A. Dallaire, P. Labelle, T. P. Lipscomb, P. Michel, I. Mikaelian. *Environ. Health Perspect.* **110** (3), 285–292 (2002).
62. S. De Guise, A. Bisaillon, B. Seguin, A. Lagace. *Anat. Histol. Embryol.* **23** (3), 207–216 (1994).
63. A. Subramanian, S. Tanabe, R. Tatsukawa, S. Saito, N. Miyazaki. *Mar. Pollut. Bull.* **18** (12), 643–646 (1987).
64. R. J. Tarpley, G. H. Jarrell, J. C. George, J. Cubbage, G. G. Stott. *J. Mamm.* **76**, 1267–1275 (1995).
65. L. M. Philo, Jr., E. B. Shotts, J. C. George. In *The Bowhead Whale,* J. J. Burns, J. J. Montague, C. J. Cowles (Eds.), pp. 275–312, Society for Marine Mammalogy, Special Publication No. 2, Allen Press, Lawrence, KS (1993).

66. G. T. Ankley, E. Mihaich, R. Stahl, D. Tillitt, T. Colborn, S. McMaster, R. Miller, J. Bantle, P. Campbell, N. Denslow, R. Dickerson, L. Folmar, M. Fry, J. Geisy, L. E. Gray, P. Guiney, T. Hutchinson, S. Kennedy, V. Kramer, G. LeBlanc, M. Mayes, A. Nimrod, R. Patino, R. Peterson, R. Purdy, T. Ringer, L. Touart, G. Van Der Kraak, T. Zacharewski. *Environ. Toxicol. Chem.* **17**, 68–87 (1998).
67. A. Bernard, C. Hermans, F. Broeckaert, G. De Poorter, A. De Cock, G. Houins. *Nature* **401**, 231–232 (1999).
68. L. Marsili and S. Focardi. *Environ. Poll.* **91** (1), 1–9 (1996).
69. M. C. Fossi, S. Casini, L. Marsili. *Chemosphere* **39** (8), 1273–1285 (1999).
70. L. Marsili, M. C. Fossi, G. Notarbartolo Di Sciara, M. Zanardelli, B. Nani, S. Panigada, S. Focardi. *Chemosphere* **37** (8), 1501–1510 (1998).
71. L. Marsili, M. C. Fossi, G. Neri, S. Casini, C. Gardi, S. Palmeri, E. Tarquini, S. Panigada. *Mar. Environ. Res.* **50** (1–5), 649–652 (2000).
72. M. C. Fossi and L. Marsili. *Biomarkers* **2**, 205–216 (1997).
73. M. C. Fossi, G. Lauriano, M. Simmonds, F. Borsani. *J. Cet. Res. Manage.* **3**, 333–335 (2001).
74. M. C. Fossi, L. Marsili, C. Leonzio, G. Notarbartolo Di Sciara, M. Zanardelli, S. Focardi. *Mar. Poll. Bull.* **24** (9), 459–461 (1992).
75. M. C. Fossi, C. Savelli, L. Marsili, S. Casini, B. Jimenez, M. Junin, H. Castello, J. A. Lorenzani. *Chemosphere* **35** (8), 1623–1635 (1997).
76. WHO/UNEP/ILO. International Programme on Chemical Safety (IPCS) T. Damstra, S. Barlow, A. Bergman, R. Kavlock, G. Van Der Kraak (Eds.) (2001).